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(RESEARCH ARTICLE)

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Citrullus lanatus natural product library: A hoard of viable potential inhibitor candidates for diabetes mellitus type II therapeutic target enzymes

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Abstract

The ethnomedicinal function of several cucurbit species (cucumber, squash, melon, gourd, etc.) on diseases (i.e. diabetes, microbial infections, cancer, etc.), and as free radical scavengers have been reported. However, there remains infinitesimal record on citron watermelon broad use, which reflects the reason for wide oversight of underutilization, from lack of recognition for functional food crop development and commercialization. The objective of this study focus towards identifying the natural phytochemicals of *C. lanatus* on water melon molecules online database for insilico computational approach to screened for suitable antidiabetic lead, and construction of phylogenetic relatedness of target Type 2 Diabetes Mellitus proteins was conducted to predict reoccurrence of insilico analysis output with other homologous target proteins. The T2DM protein targets, phytochemical and standard inhibitory drugs are mined from different databases. Insilico docking analysis, and adme/toxicity profiling was virtually screened for the best fit. In additionally, phylogenetic relationship of the target proteins was aligned and construction with other homologous protein targets to predict the reoccurrence confidence of results on compounds. Seventeen phytochemicals out of the nineteen potential drug candidates substantially passed the profiling test. Also, homologous protein targets with > 90% bootstrap confidence are likely to produce a reoccurring insilico result. Thus, these phytochemicals fulfil all the enlisted criteria and it is suggestively determined to be suitable for the development of potent antidiabetic drugs. It is evident that phytochemicals from *Citrullus lanatus* produced satisfactory insilico output, and this reflect it to be a probable reservoir containing other potential therapeutic drug candidates for antidiabetic drug discovery and development. Additionally, derivatives can be developed for further effective screening result.

Keywords: Ethnomedicinal; Natural Products; Type II Diabetes Mellitus (T2DM); Phylogenetic; Therapeutic Target; Insilico Profiling

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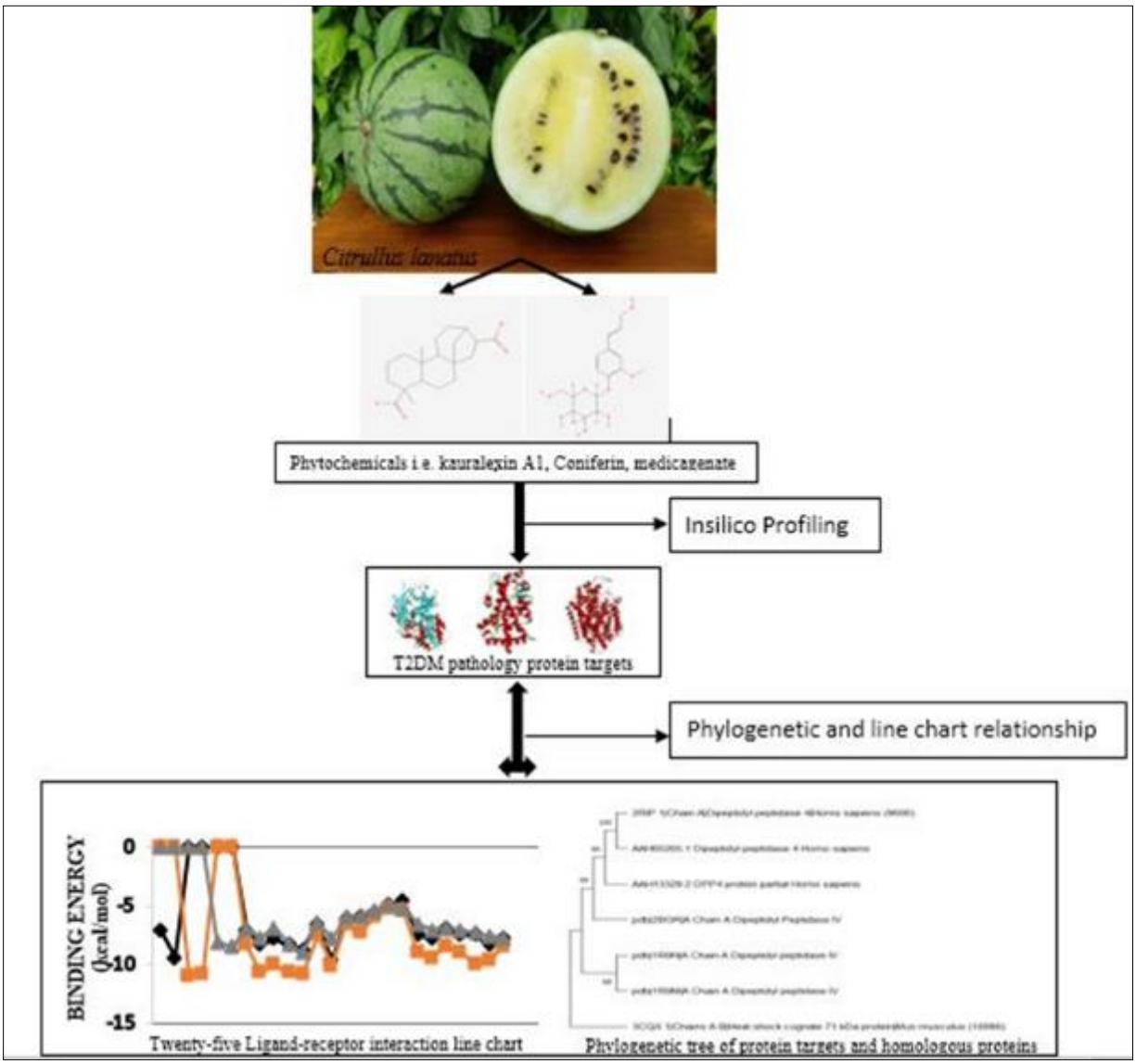
Graphical abstract



1. Introduction

The collective effort to raise the standard of treatments against the world deadliest diseases (communicable and non-communicable) has brought a conscious cognizance of drug discovery, design and development pertinence into scientific studies and practices. Subsequently, recent reports on the increasing statistic of some of the common disease, has raised an alarming threat on the quality of life and wellness of a fraction of human population (usually quite large) around the globe. A typical example to recon is diabetes mellitus (DM). Diabetes mellitus (DM) can be an inherited or acquired metabolic deficiency, and it has affected a global population scale of about 5%, with various forms of both short/long-term defies among diabetic patients, thus, becoming one of the medical system menace [1].

Diabetes Mellitus type II (T2DM) is a noninsulin independent metabolic disease, often known to occur as a consequence of increased hyperglycaemia (excess blood glucose), due to resistance to secreted insulin and dysfunctional β-cell. Notably, theories on the vascular complications pathophysiology consisting the increase of polyol (sorbitol) pathway metabolic flux, induction of non-enzymatic glycosylation of biomolecules by the elevated blood glucose and subsequently accumulates the advanced glycation end-products (AGEs) above threshold [2], together with the activation of protein kinase C, unifies to causing an induction of oxidative stress [3]. This processes unification

determines the factor for initiation and progression of T2DM complications. It is of record that DM can likely progress into several complications such as cardiovascular diseases, retinopathy, neuropathy, nephropathy, stroke, obesity, ischemic heart disease, peripheral vascular disease, foot ulcer, and a variety of heterogeneous diseases, dependently on the relative metabolic pathways abnormality [4-6], with T2DM causing over 95% of the diseases comorbidity [5].

According to International Diabetes Federation speculations are by 2040, over 642 million of the world populations would be suffering from diabetes all over the world [7]. Also, from Nigeria alone, a direct expenditure on diabetes related management/treatment are estimated to range from $1.071 billion to $1.639 billion annually, therefore constituting its economic burden [8]. The individual and general communal health problem of diabetes is continuously escalating exponentially (2.6% annually) throughout the world [9] and according to World Health Organization, diabetes will be the seventh leading cause of death in 2030 [10]. This rising concerns has led to several Governmental and NGOs awareness campaigns to educated people on the causes, symptoms and appropriate management options for diabetes and it complications. While various types of oral hyperglycemic drugs (acarbose, miglitol, voglibose, sulfonylureas, etc.) are available for diabetes treatment [2], these approved synthetic drugs have not been able to establish long term glycaemic control or comorbidity progression reversal, and are usually downplay by adverse demerits, based on their lack of specificity and actions, coupled with the cost which are especially challenging to access for the poor in developing and underdeveloped countries. This has therefore created the urgency for alternative therapeutic development, with limited associating shortcomings [3].

Exploiting the inhibitory cue of biomolecules (carbohydrates, lipids) hydrolyzing enzymes by natural inhibitors are identified as the therapeutic tactics that can aid in efficient and effective target specific management/prevention of diabetes pathophysiology, to discover and develop potential novel natural bioactive leads [11, 12]. As a consequence, natural products have become widely recognized as the future of therapeutic agent source, due to the enormous invaluable extracts, and are broadly accepted in the aid for conventional therapy [13]. Meanwhile, in comparison to synthetic source therapy, extracts from natural source are considered safe, easy to locate and access, reasonably cheap and with low incidence of adverse effects, thus, facilitating the surging diversion into phytomedicine [14, 15].

Historically, traditional herbal medicine and their relative natural products have in practice been extensively tested in the treatments for Alzheimer's disease, diabetes mellitus, gynaecological and neurological illness, and several others disease, via the dietary consumption of the leaves, fruits, other body parts, and also the crude extracts from common plants in Nigeria [15, 16]. Also, from perusal of literature, it is known that over 400 identified plants from ethnobotanical library have traditionally been employed in diabetes and it progressive abnormality treatment.

Hence, this has offered an unparalleled structural variety for promising new leads [15]. More so, the rich antioxidant phytoconstituents of plants can shield β-cells from reactive oxygen species (ROS) (a precursor for diabetes induction) [17]. The scavenging attribute of plant phytochemicals on a wide range of ROS is an evidence suggesting their antioxidant activities [18]. While majority of plant are good source of this phytochemicals compounds, one famous underutilized fruit highly rich in them is citron watermelon (*Citrullus lanatus* var. citroides), but lacks well defined dedicated research, development and well defined value chains for the crop.

Citron watermelon (*Citrullus lanatus* var. citroides, 2n=2x=22) is a hardy crop that belong to *Cucurbitaceae* family. It characteristic tolerance to biotic and abiotic stress (drought, pest, heat stress, bacterial and viral infection) make it an important multi-purpose crop that can thrive under restricted production input in arid and semi-arid environment. It is considered a crop of choice for cultivation under harsh conditions, farmed in small scale in sub-Saharan Africa (SSA), and is often used as a rootstock for sweet dessert watermelon (*C. lanatus* var. lanatus). In Africa, the fresh and dried leaves, fruit and seeds serves as food, feed and the ingredient for the pharmaceutical sectors [19]. Citron watermelon seed is rich in oil, protein and unsaturated fatty acids (i.e., linoleic, oleic, palmitic and stearic), the fruit; in low total soluble solids (TSS) and natural sugars (i.e., fructose, sucrose and glucose) and high organic acids and carotenoids content especially β-carotene, and the leaves; mainly contains cucurbitacins and their glycosides derivatives with natural pharmacological and therapeutic values [19-21]. In several parts of Africa, the leaves and fruit of citron watermelon are used for hypertension treatment, while the roasted seed is consumed as appetizer and as remedy for constipation respectively [22, 23].

Although, upon the reports on the ethnomedicinal function of other cucurbit species (cucumber, squash, melon, gourd, etc.) on diseases (i.e. diabetes), there remains infinitesimal record on citron watermelon broad use, which reflects the reason for wide oversight of underutilization, from lack of recognition for functional food crop development and commercialization [19]. Over 1000 natural products have been detected in the various *Citrullus* spp. however, majority are with little or no appropriate quantification [24]. Hence, there are poor documentation of the natural therapeutic

components of watermelon mode of action on common diseases except via traditional application, which are still much mysterious.

Therefore, the objective of this study would be driven towards identifying the natural phytochemicals of *C. lanatus* on watermelon molecule online database for insilico computational approach to screened for suitable antidiabetic lead. Accordingly, three known therapeutic molecular targets were selected, based on previously reported activities against T2DM, viz., Dipeptidyl peptidase IV inhibitors (DPP4), Sodium-glucose co-transporter type 2 inhibitors (SGLT2) and Peroxisome proliferator-activated receptor-gamma (PPAR-γ).

2. Material and methods

2.1. Software and Webservers used

AdmetSAR, Discovery Studio (DS) version 21.1, OpenBabel, PyMol 1.3, PyRx, Ligplot+, Mega 11, PubChem, Protein data bank, SwissADME and NCBI were used to carry out whole work design.

2.2. Retrieval and Preparation of the Three Dimensional Structure of the Target Proteins

The X-ray crystallographic structures of the human known target proteins DPP4 (PDB: 2RIP), SGLT2 (PDB: 7VSI), PPAR-γ (PDB: 4EMA) (Figure 1), were downloaded from RCSB protein Data Bank (www.pdb.org), and prepared for molecular docking simulation using DS v. 21.1. The standard drugs for each are Aloglipton, Canagliflozin, and Rosiglitazone respectively.

2.3. Curation of Watermelon Phytochemical Library

The natural compounds previously identified from *Citrullus lanatus* in traces without appropriate quantification are mined via the watermelon molecules online database (watermelon.naturalproducts.net) [24]. The randomly picked nineteen phytochemicals includes: Allantoin, Apigenin, Coniferin, Fraxidin, Isorhamnetin, Isobazzanene, Kauralexin A1, Kauralexin A2, Kauralexin A3, Petivericin, Medicagenate, Pelargonidin, Petiveriin, Pipecolic acid, Quinic acid, Spermidine, Spermine, Taxifolin, Vestitone.

2.4. Ligand Preparation for Docking

The structure of the downloaded phytochemicals, native ligands and standard drugs were converted to suitable format (pdb format), and minimized using uniform force fields for docking software readability (pdbqt format) studies. The energy minimized ligand molecules were docked into refined humans' specific diabetic proteins as mentioned above.

2.5. Molecular Docking Simulation

Virtual screening by molecular docking was performed according to Sharma et al. protocol [12]. At first, the active binding sites of the molecular targets were mapped out using DS v. 21.1 via identification of the native ligand (NL) interaction with the amino acids that comes in contact at the search space (centre of 2OLE; x=-1.3082, y=5.7109, z=-2.109, centre of 2RIP; x=62.1675, y=54.8921, z=86,4364, centre of 4EMA; x=16.6384, y=6.2880, z=42.3678, centre 7VSI; x=37.6229, y=49.1391, z=46.1741), while other parameters were as the default setting. Molecular docking was performed by using AutoDock Vina software [25] in PyRx platform (GUI version 0.8). The best analyzed conformations with the lowest binding energy (kcal/mol) were further used for 2D interactions of the complex protein-ligand structure, inclusive of the determination of other parameters using Ligplot+ v.1.4.5 software and PyMol software.

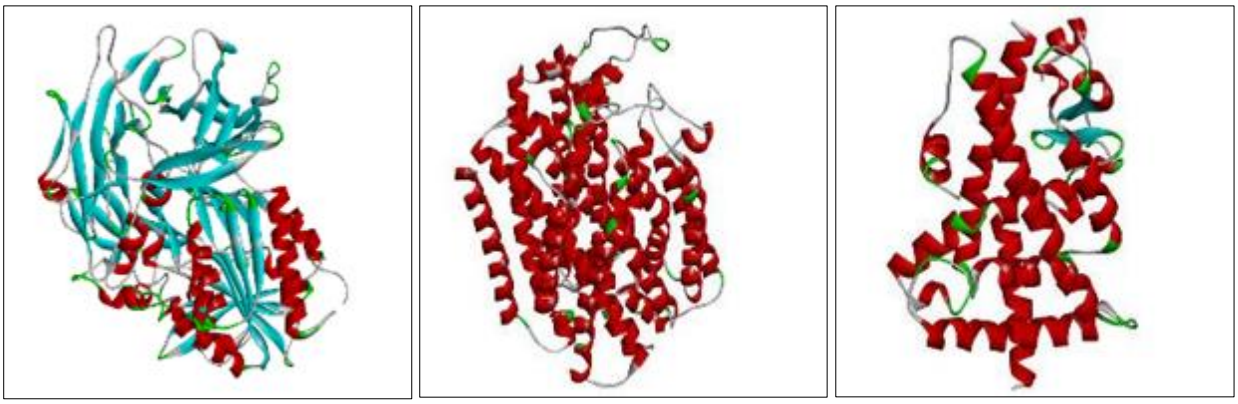
2.6. Pharmacokinetics and ADME/Toxicity Profiling

The pharmacokinetic properties such as the Absorption, Distribution, Metabolism, Excretion, and Toxic behavior of ligands to human body are screened using the SwissADME (http://:www.swissadme.ch/index.php) and admetSAR prediction tool webserver (http://lmmd.ecust.edu.cn/admetsar2). This plays a significant role in proffering the "drug-likeness", "medicinal chemistry" and "lead likeness" and toxicity potential of new drugs, phytochemicals, food additives and industrial chemicals candidates. It serves as a pre-requite establishment for a valid complementary method before in-vivo/in-vitro analysis [12, 26].

2.7. Phylogenetic analysis

The genetic relatedness of the three target protein sequences under study was compared with other similar human protein sequences models. Five DPPIV protein sequences (AAH65265.1, AAH13329.2, 2BGR, 1R9N, 1R9M), five SGLT2

protein sequences (P31639.1, CAB81772.1, 7FEN, NP_003032.1, XP_006721135.3, 3CQX_1) and eight PPAR-γ protein sequences (3ADX, 3ADW, 3ADV, 3ADU, 3ADT, 3ADS, 3B3K, 3R8I) were retrieved from RCSB protein Data Bank (www.pdb.org) and National center for biotechnology information (https://www.ncbi.nlm.nih.gov/) database in a fasta format. An outgroup sequence of heat shock protein from *Mus musculus* was used.

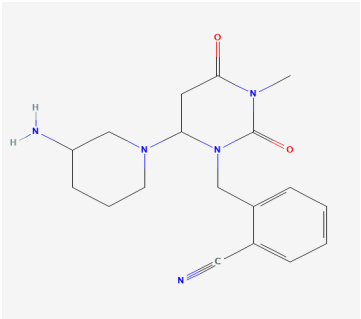
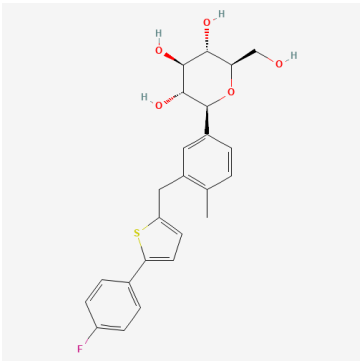


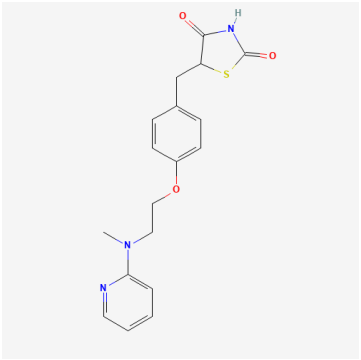
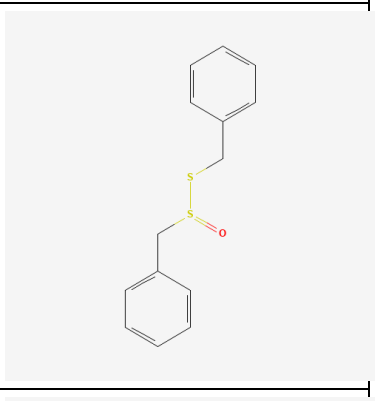
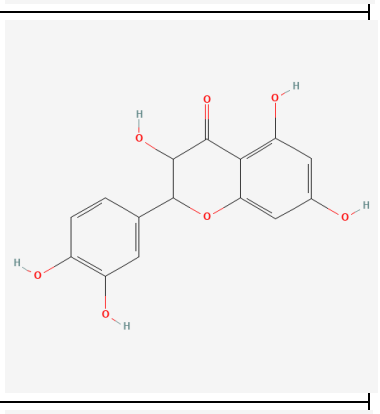
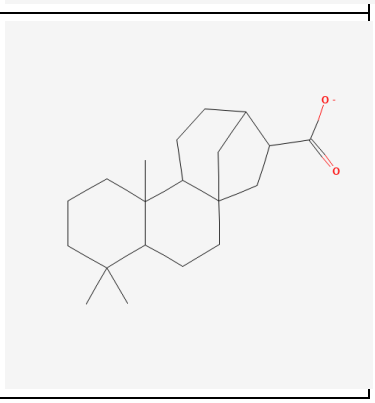
(A) **(B)** **(C)**

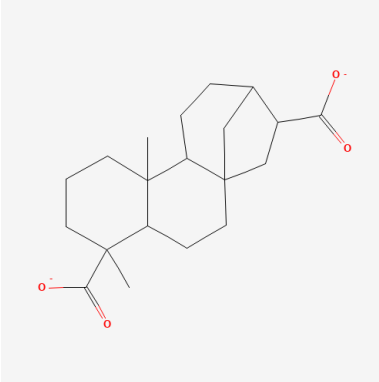
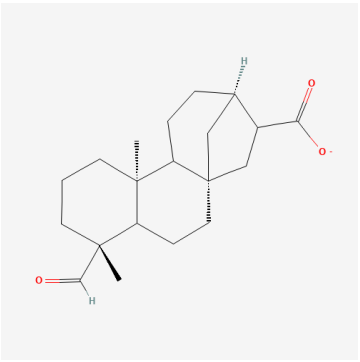
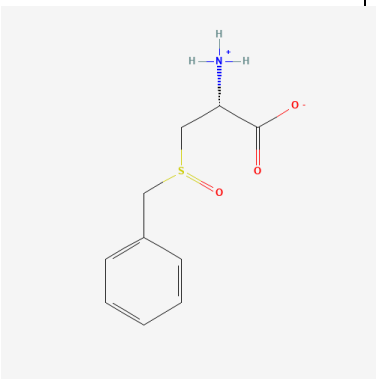
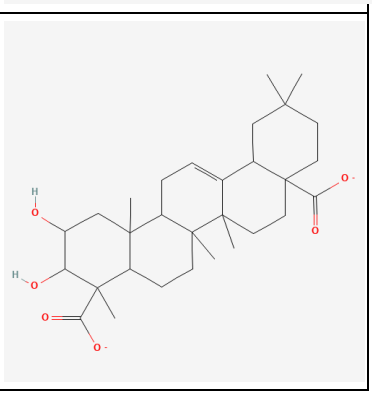
Figure 1 3D X-ray crystallographic structure conformation of T2DM targets; A- Dipeptidyl peptidase IV, B- SGLT2-Sodium-glucose co-transporter type 2, C- PPAR-γ- Peroxisome proliferator-activated receptor gamma

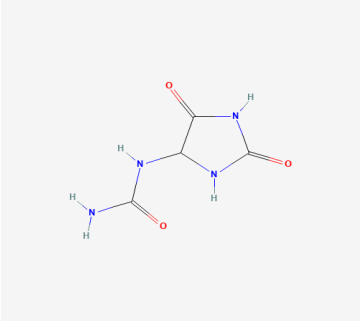
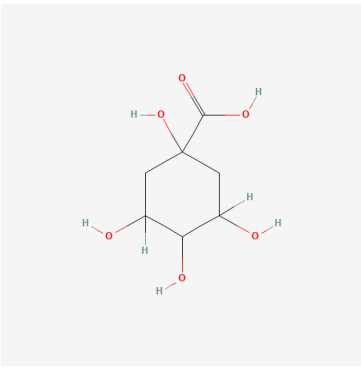
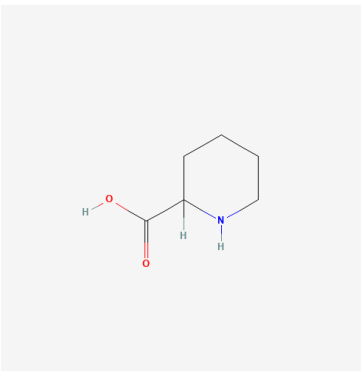
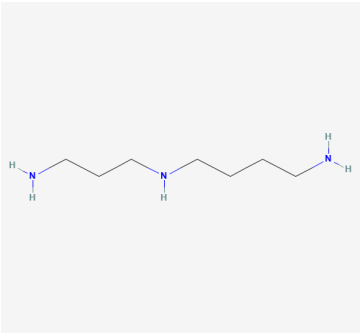
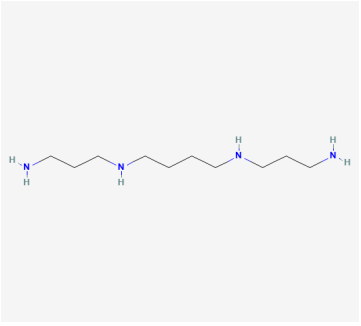
The sequences were aligned using the ClusterW algorithm, and phylogenetic tree was constructed using the Neighbor-joining statistical method of Mega 11 software [27].

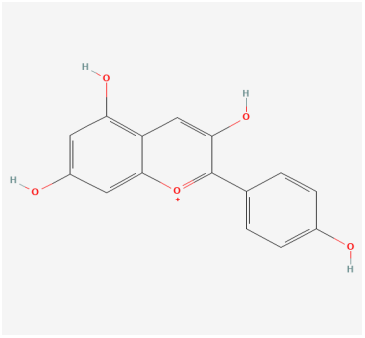
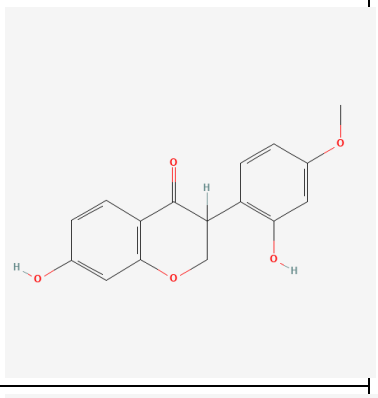
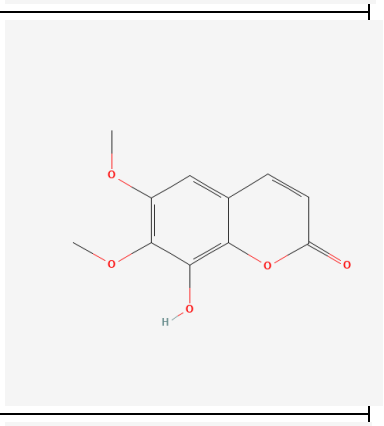
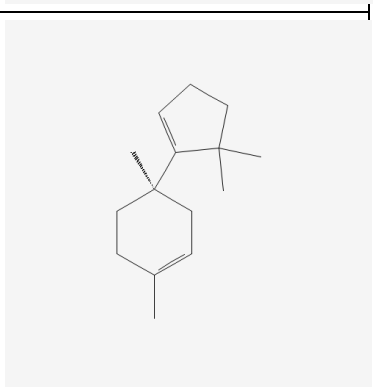
Table 1 Properties of *Citrullus lanatus* Phytochemicals and Standard Drug Candidates

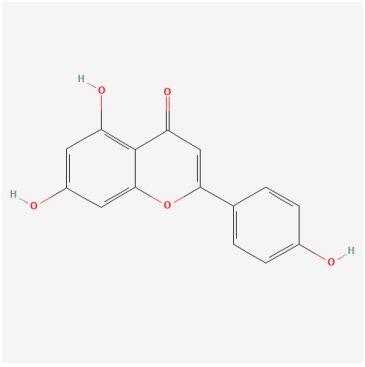
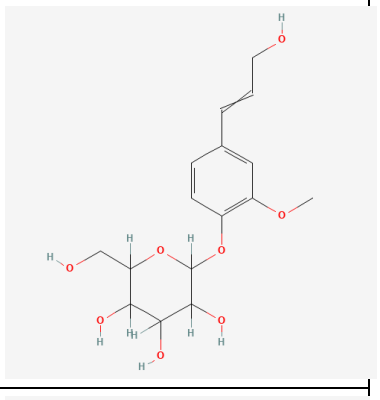
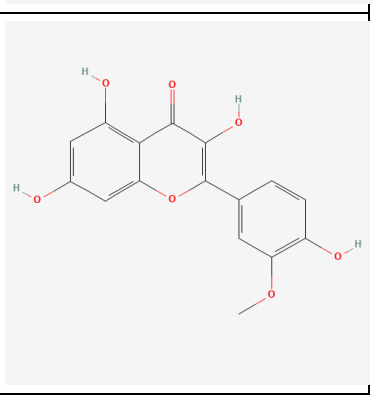
Sr. No.	Ligands (class)	PubChem/ AFC Id	Chemical formula	Molecular weight (g/mol)	2D Structural formula
1	Aloglipton	72791033	C18H23N502	341.4	
2.	Canagliflozin	24812758	C24H25FO5S	444.5	

3.	Rosiglitazone	77999	C18H19N303S	357.4	
4.	Petivericin (flavonoid)	10244468/ AFC001150	C14H14OS2	262.39	
5	Taxifolin (flavonoid)	471/AFC00 1943	[C15H11O7]	304.25	
6	Kauralexin A1 (terpenoid)	90657333/ AFC001483	[C20H31O2]-	303.46	

7	Kauralexin A2 (terpenoid)	75826609/ AFC001473	[C20H28O4]2-	332.43	
8	Kauralexin A3 (terpenoid)	90657502/ AFC001485	[C20H29O3]-	317.44	
9	Petiveriin (unknown)	91820550/ AFC001507	C10H13NO3S	227.28	
10	Medicagenate (terpenoid)	74427715/ AFC001546	[C30H44O6]2-	500.67	

11	Allantoin (alkaloid)	204/AFC000027	C4H6N4O3	158.12	
12	Quinic acid (Phenolic acid)	1064/AFC000262	C7H12O6	192.17	
13	Pipecolic acid (small peptide)	849/AFC001846	C6H11NO2	129.16	
14	Spermidine (alkaloid)	1102/AFC000176	C7H19N3	145.25	
15	Spermine (alkaloid)	1103/AFC000177	C10H26N4	202.34	

16	Pelargonidin (unknown)	440832/AFC000722	[C15H11O5]+	271.25	
17	Vestitone (isoflavonoid)	171769/AFC000599	C16H14O5	286.28	
18	Fraxidin (coumarin)	3083616/AFC000857	C11H10O5	222.19	
19	Isobazzanene (terpenoid)	14830703/AFC001228	C15H24	204.35	

20	Apigenin (flavonoid)	5280443/AFC000881	C15H10O5	270.24	
21	Coniferin (phenylpropanoid)	3496897/AFC000873	C16H22O8	342.34	
22	Isorhamnetin (flavonoid)	5281654/AFC000950	C16H7O7	316.26	

3. Results and discussion

3.1. *Citrullus lanatus* Library Information

Citrullus lanatus library hoard over a thousand of natural products with little or no known therapeutic record. The characteristic information about the nineteen phytochemicals were retrieved from watermelon molecules online and crosschecked on PubChem database as listed in Table 1.

3.2. Molecular Docking Analysis Output

Meng et al. [28] described molecular Docking as a trusted structure-based-drug-design (SBDD) with a substantial degree of predictive accuracy for the structural conformation of ligand-receptor target binding site.

The Docking analysis output of the twenty-five candidates consisting; nineteen *C. lanatus* phytochemicals against the three therapeutic molecular targets i.e. DPPIV, SGLT2, and PPAR-y showed some potential candidates with good binding affinity and better binding modes as compared to the three co-crystalized native ligands (NL 1; pyrrolidin, NL 2; Empagliflozin, NL 3; 2,4-Thiazolidiinedione) and three standard inhibitor drugs (Aloglipton, Canagliflozin, Rosiglitazone) employed (Table 2). Figure 2. Represents the binding energy line chart of the three therapeutic molecular targets with the twenty-five candidates. The characteristic energy fluctuation of the phytochemical candidates to each

protein targets active sites indicates the variability of molecular interaction of bonds between the complexes residues, and this is given by the binding constant and the Gibbs free energy [29].

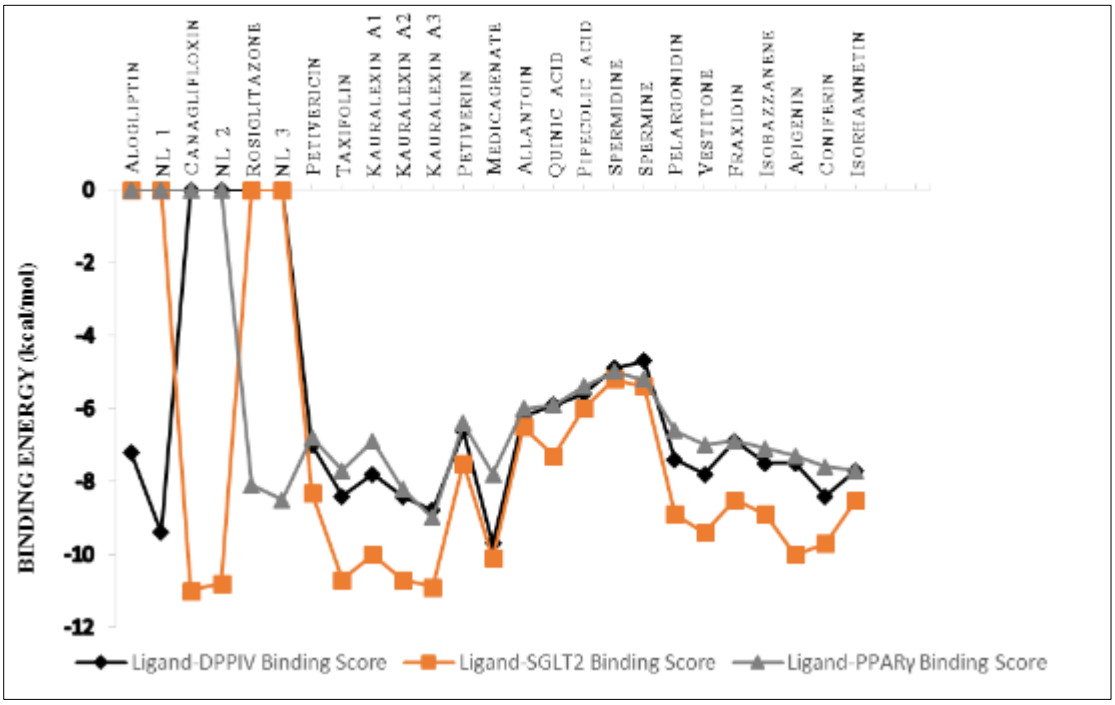


Figure 2 A line chart representing the binding energy of three therapeutic molecular targets i.e. DPPIV, SGLT2 and PPAR-y with nineteen phytochemicals, three native ligands and three standard drugs. NL 1- Pyrrolidin, NL 2- Empagliflozin, NL 3- 2,4-Thiazolidiinedione

Table 2 The Binding energy of *Citrullus lanatus* phytochemical, native ligands and standard drugs against therapeutic targets of Type 2 Diabetes mellitus

Sr. No.	Ligands	Docking score (Kcal/mol)		
		DPPIV	SGLT2	PPAR-γ
1	Alogliptin (SD)	-7.2	-	-
2	NL 1	-9.4	-	-
3	Canagliflozin (SD)	-	-11	-
4	NL 2	-	-10.8	-
5	Rosiglitazone (SD)	-	-	-8.1
6	NL 3	-	-	-8.5
7	Petivericin	-7	-8.3	-6.8
8	Taxifolin	-8.4*	-10.7*	-7.7
9	Kauralexin A1	-7.8*	-10	-6.9
10	Kauralexin A2	-8.4*	-10.7*	-8.2*
11	Kauralexin A3	-8.8*	-10.9*	-9*
12	Petiveriin	-6.6	-7.5	-6.4
13	Medicagenate	-9.7*	-10.1	-7.8
14	Allantoin	-6.2	-6.5	-6

15	Quinic acid	-5.9	-7.3	-5.9
16	Pipecolic acid	-5.6	-6	-5.4
17	Spermidine	-4.9	-5.2	-5
18	Spermine	-4.7	-5.4	-5.2
19	Pelargonidin	-7.4*	-8.9	-6.6
20	Vestitone	-7.8*	-9.4	-7
21	Fraxidin	-6.9	-8.5	-6.9
22	Isobazzanene	-7.5*	-8.9	-7.1
23	Apigenin	-7.5*	-10	-7.3
24	Coniferin	-8.4*	-9.7	-7.6
25	Isorhamnetin	-7.7*	-8.5	-7.7

DPPIV, Dipeptidyl peptidase IV; SGLT2, Sodium-glucose co-transporter type 2; PPAR-γ, Peroxisome proliferator-activated receptor gamma; NL 1, Pyrrolidin; NL 2, Empagliflozin; NL 3, 2,4-Thiazolidiinedione; SD, Standard Drug; *, best fit potential candidate.

In table 3, the binding scores of Ligand-DPPIV complexes ranges between -4.7 (Kcal/mol) in spermine to -9.7 (Kcal/mol) in Medicagenate, with Medicagenate possessing the best binding score of -9.7 (Kcal/mol) than the native ligand and standard inhibitor drug. while Taxifolin, Kauralexin A1, Kauralexin A2, Kauralexin A3, Medicagenate, Pelargonidin, Vestitone, Isobazzanene, Apigenin, Coniferin, Isorhamnetin having good binding score than the reference standard (Aloglipton) of -7.2 Kcal/mol. Also, the eleven candidates previously mention possess a better molecular hydrophobic interaction which make the scoring fit much better that the standard drug (Aloglipton). While vestitone have one hydrogen bonding with amino acid Asn 710, thirteen hydrophobic bonding interaction with the amino acid Arg 125, His 126, Glu 205, Glu 206, Ser 209, Tyr 547, Ser 630, Tyr 631, Val 656, Trp 659, Tyr 662, Tyr 666 and Val 711 residues, and Coniferin having three hydrogen bonding with amino acid Arg 125, Tyr 662, and Asn 710, plus twelve hydrophobic bonding interaction with amino acids Glu 205, Glu 206, Val 207, Ser 209, Phe 357, Tyr 547, Ser 630, Tyr 631, Trp 659, Tyr 662, Val 711, His 740 residue, the other nine phytochemical candidates poses hydrophobic bond interaction between six and ten amino acid residues compared to the standard inhibitor drug with five amino acid residues. Although, the native ligand (pyrrolidin) possess four hydrogen bonding interaction with the amino acid Glu 205, Glu 206, Tyr 662 residues, phytochemicals such as Kauralexin A1, Kauralexin A3, pelargonidin and Isorhamnetin however shows a relatively strong hydrogen bonding interaction with two amino acid residues clearly represented in the 2D and 3D structural conformation in Figure 3. The absence of visible hydrogen bonding interaction between Kauralexin A2, Isobazzanene and Apigenin against the amino acids residues of DPPIV protein target, underlines the significant influence of hydrophobic surface interaction (i.e. pi-pi, pi-sigma, Akyl, pi-Akyl, etc.) on the high binding score than the standard inhibitor drug.

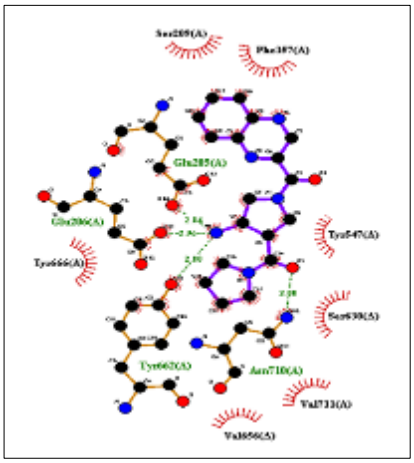
Table 3 Ligand report showing best binding information with DPPIV Protein (2RIP)

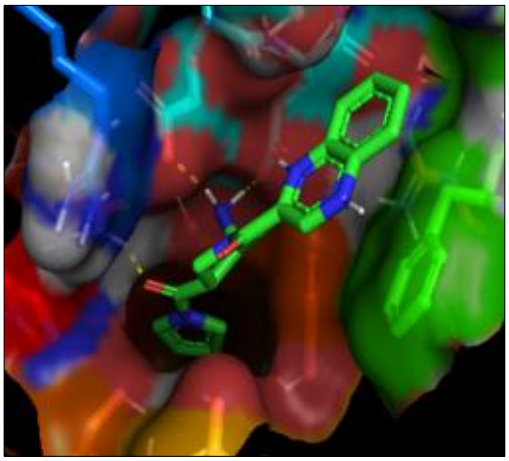
Sr. No.	**Compounds/ Ligands**	**Docking score (Kcal/mol)**	**H- bond**	**H-bond Distance (Å)**	**Hydrophobic interaction**
1	Alogliptin	-7.2	Tyr 585	3.04	Ser 209, Arg 356, Phe 357, Pro 359, Ser 360
2	NL 1	-9.4	Glu 205 Glu 206 Tyr 662 Asn 710	2.84 2.96 2.80 3.00	Ser 209, Phe 357, Tyr 547, Ser 630, Val 656, Tyr 666, Val 711
3	Taxifolin	-8.4	Arg 125	2.93	Glu 205, Glu 206, Ser 209, Phe 357, Tyr 547, Ser 630, Tyr 662, Tyr 666, Asn 710, His 740

4	Kauralexin A1	-7.8	Glu 206 Arg 669	2.98, 3.05 3.08, 3.13	Glu 205, Glu 206, Ser209, Phe 357, Tyr 666, Asn 710
5	Kauralexin A2	-8.4	-	-	Glu 206, Val 207, Ser 209, Phe 357, Arg 358, Tyr 547, Tyr 666
6	Kauralexin A3	-8.8	Glu 205 Tyr 662	2.73 3.08	Glu 206, Val 207, Ser 209, Phe 357, Arg 358, Tyr 547, Tyr 666, Arg 669
7	Medicagenate	-9.7	Asn 710	2.86	Arg 125, Glu 205, Glu 206, Ser 209, Phe 357, Arg 358, Tyr 547, Ser 630, Tyr 662, Tyr 666
8	Pelargonidin	-7.4	Glu 206 Tyr 662	2.98 2.74	Glu 205, Phe 357, Tyr 547, Ser 630, Tyr 631, Val 656, Trp 659, Asn 710, Val 711
9	Vestitone	-7.8	Asn 710	2.94	Arg 125, His 126, Glu 205, Glu 206, Ser 209, Tyr 547, Ser 630, Tyr 631, Val 656, Trp 659, Tyr 662, Tyr 666, Val 711
10	Isobazzanene	-7.5	-	-	Glu 206, Val 207, Ser 209, Phe 357, Arg 358, Tyr 547, Tyr 666
11	Apigenin	-7.5	-	-	Glu 205, Glu 206, Val 207, Ser 209, Phe 357, Arg 358, Tyr 547, Tyr 662, Tyr 666
12	Coniferin	-8.4	Arg 125 Tyr 666 Asn 710	2.73 2.98 2.80, 2.90	Glu 205, Glu 206, Val 207, Ser 209, Phe 357, Tyr 547, Ser 630, Tyr 631, Trp 659, Tyr 662, Val 711, His 740
13	Isorhamnetin	-7.7	Arg 125 Ser 209	3.00 2.83	Glu 205, Glu 206, Phe 357, Tyr 547, Ser 630, Tyr 662, Tyr 666

DPPIV, Dipeptidyl peptidase IV; NL 1, Pyrrolidin.

(A)





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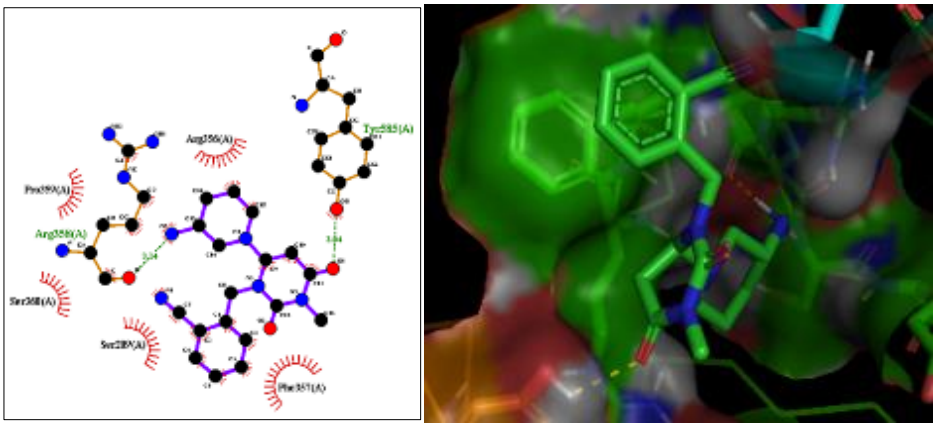
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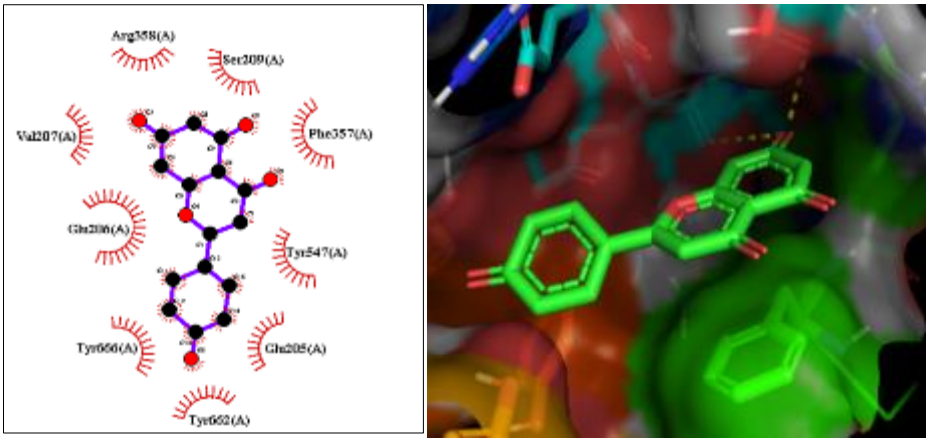
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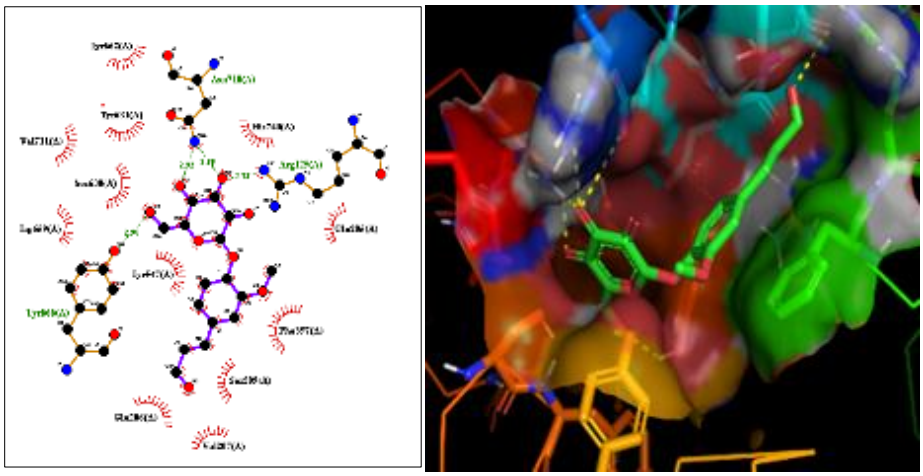

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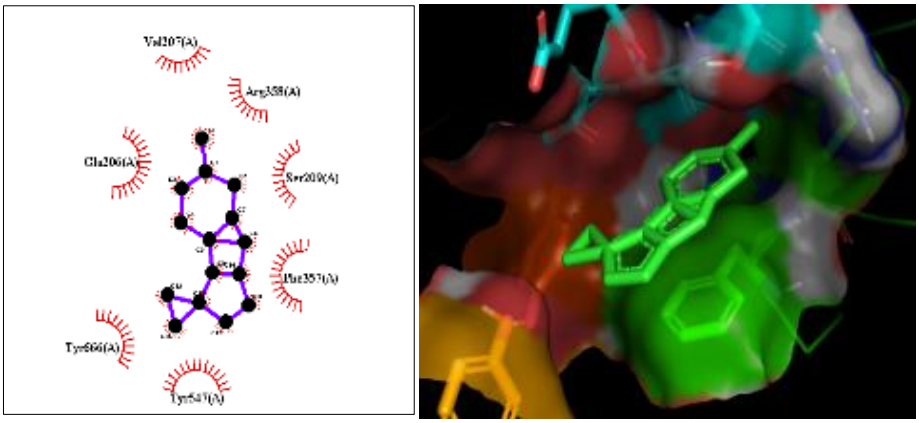
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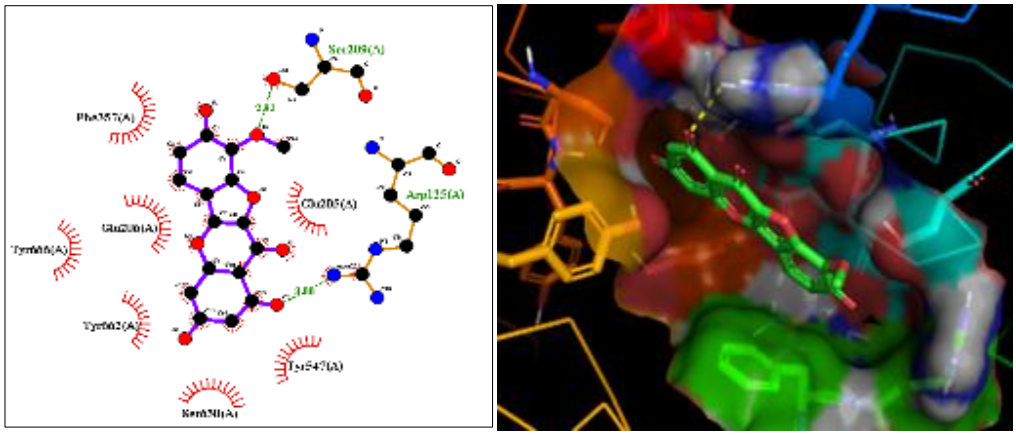
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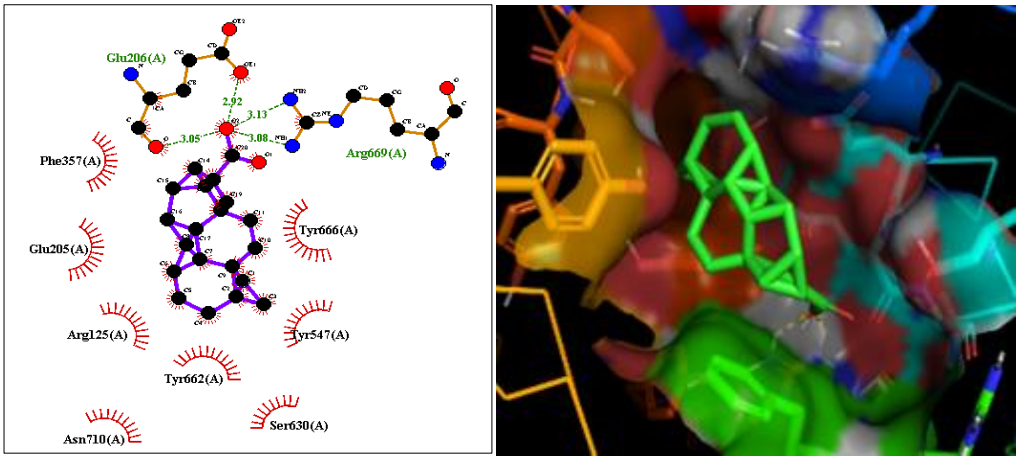
(G)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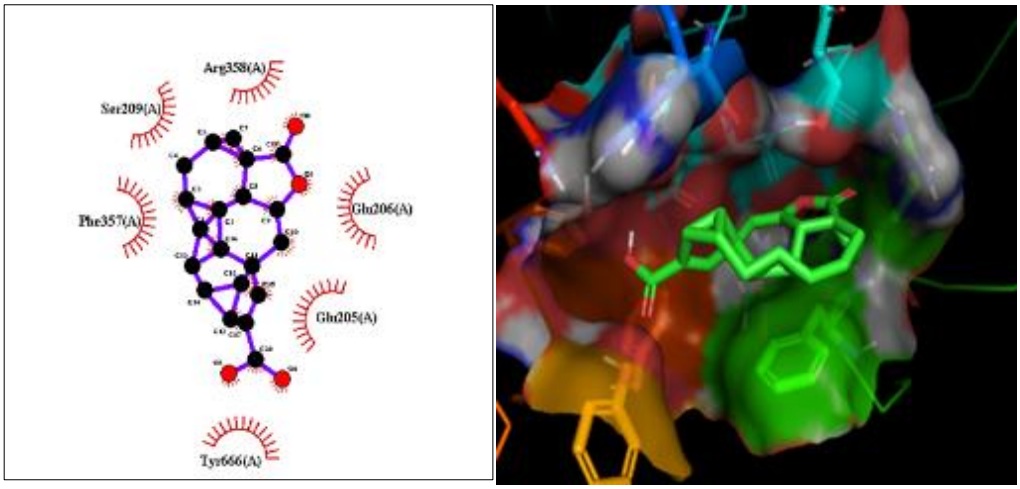
(H)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Phe357(A)
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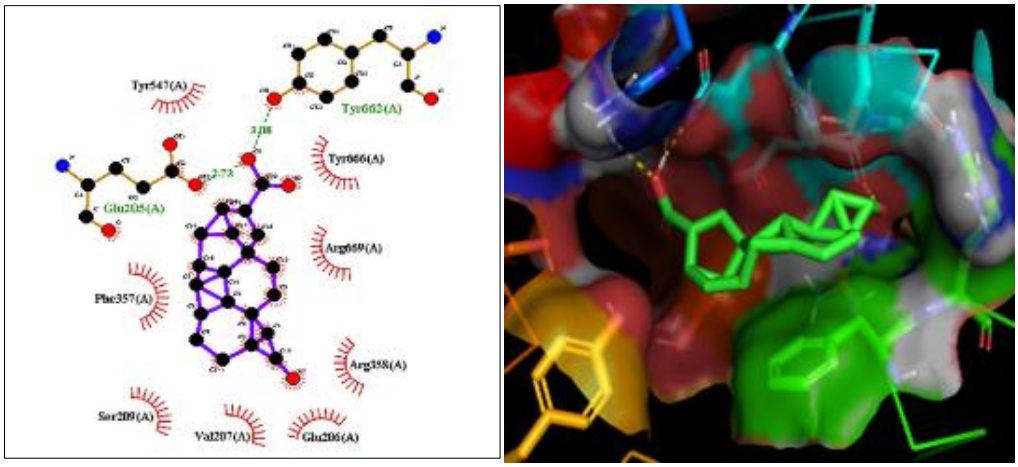
Glu205(A)
Tyr666(A)

(I)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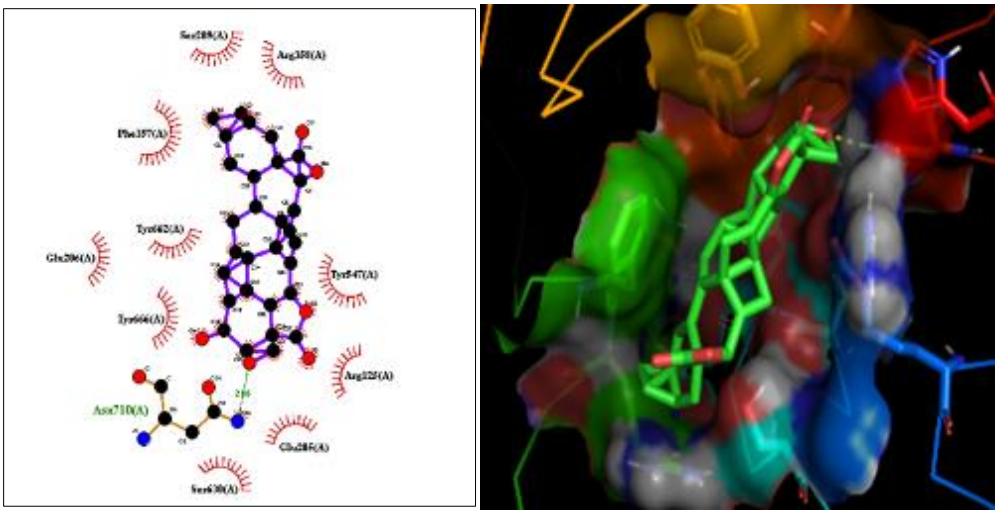
(J)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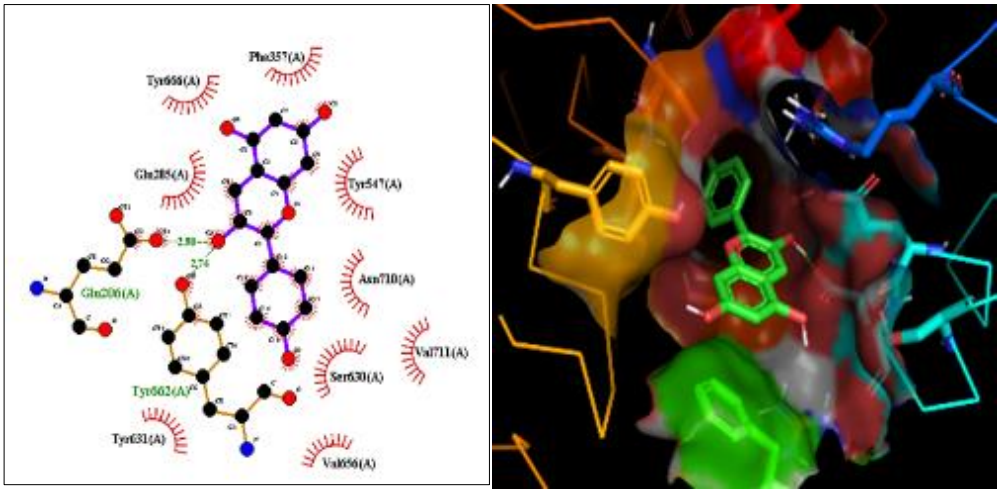
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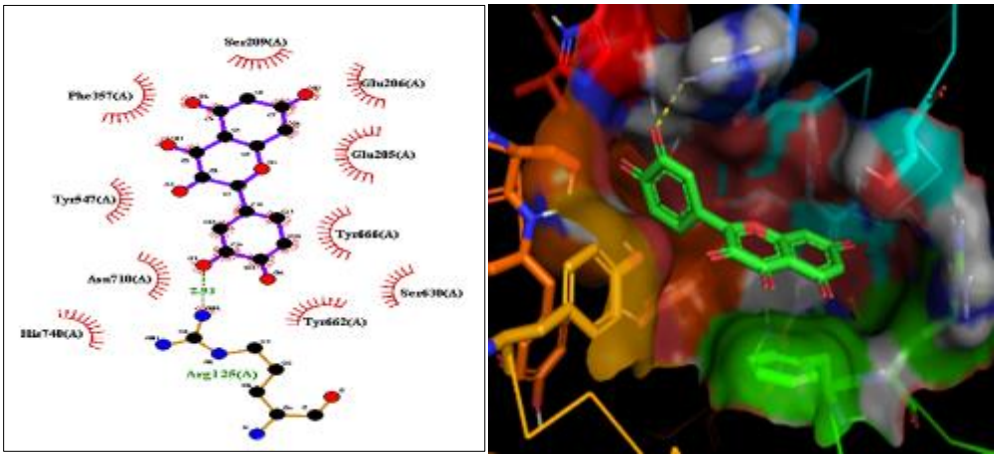
(L)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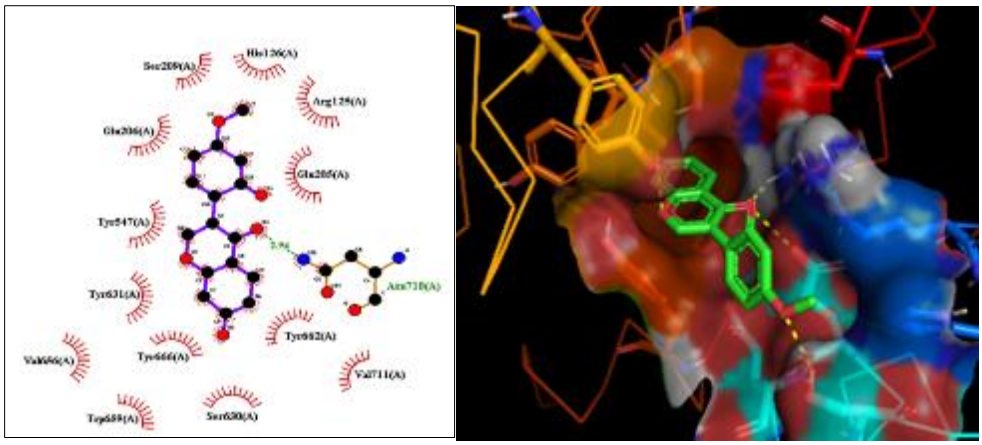
(M) 

Figure 3 A 2D and 3D molecular interaction surface between the best complexes amino acid residues; A- NL 1-DPPIV, B-Aloglipton-DPPIV, C- Apigenin-DPPIV, D- Coniferin-DPPIV, E- Isobazzanene-DPPIV, F- Isorhamnetin-DPPIV, G- Kauralexin A1-DPPIV, H- Kauralexin A2-DPPIV, I- Kauralexin A3-DPPIV, J- Medicagenate-DPPIV, K- Pelargonidin-DPPIV, L- Taxifolin-DPPIV, M- Vestitone-DPPIV

In Table 4, only three potential candidate have a preferable binding scores between the Ligand-SGLT 2 complexes ranges between -10.7 (Kcal/mol) in Taxifolin and Kauralexin A2 to -10.9 (Kcal/mol) in Kauralexin A3, of which is closer in binding score to the standard inhibitor drug (Canagliflozin), but better than the native ligand (Empagliflozin). The hydrogen bond interaction between the standard inhibitor drug, native ligand against the SGLT 2 protein are attached to six amino acids residues (five similar, one different), Asn 75, Phe 98, Glu 99, Ser 287, Lys 321, and Trp 291/Gln 457 respectively. This justifies the characteristic high binding affinity between the complexes. Also, the three potential phytochemical candidates hold a maximum of two hydrogen bond interaction in Taxifolin with the amino acids residues Trp 291, Lys 321, and one hydrogen bond interaction with the other two candidates (Kauralexin A2, A3) to the amino acid residues Thr 87 and Trp 291 respectively. The hydrophobic bond interaction between the three phytochemical candidate with the receptor complexes are at least eleven amino acids residues in Kauralexin A2 and maximum of thirteen in Taxifolin. This interaction with the residual amino acids are similar between them by Asn 75, Gly 79, His 80, Phe 98, Val 157, Tyr 290, Phe 453, thus emphasize there good binding energy to the protein target. These molecular interactions are clearly represented in the 2D and 3D structural conformation in Figure 4.

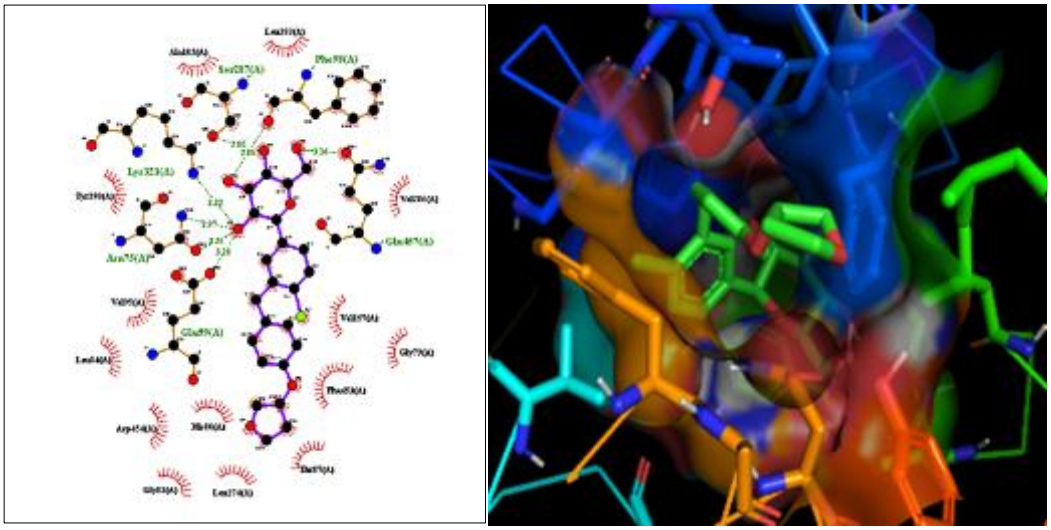
Table 4 Ligand report showing binding information with SGLT2 Protein (7VSI)

Sr. No.	Compounds/ Ligands	Docking score (Kcal/mol)	H- bond	H-bond Distance (Å)	Hydrophobic interaction
1	Canagliflozin	-11	Asn 75 Phe 98 Glu 99 Ser 287 Trp 291 Lys 321	2.88 3.07 3.18 2.51 3.31 3.25, 3.32	Gly 79, His 80, Gly 83, Leu 84, Val 95, Ala 102, Val 157, Leu 274, Val 286, Tyr 290, Phe 453, Asp 454, Gln 457, Tyr 526
2	NL 2	-10.8	Asn 75 Phe 98 Glu 99 Ser 287 Lys 321 Gln 457	2.97, 3.26 2.88 3.28 2.86 3.32 3.16	Gly 79, His 80, Thr 87, Val 95, Phe 98, Ala 102, Thr 153, Val 157, Leu 283, Val 286, Tyr 290, Phe 453, Asp 454, Gln 457
3	Taxifolin	-10.7	Trp 291 Lys 321	2.81 3.17	Asn 75, Gly 79, His 80, Gly 83, Leu 84, Val 95, Phe 98,

					Glu 99, Ala 102, Val 157, Ser 287, Tyr 290, Phe 453
4	Kauralexin A2	-10.7	Thr 87	2.73	Asn 75, Gly 79, His 80, Gly 83, Leu 84, Phe 98, Glu 99, Val 157, Tyr 290, Phe 453, Gln 457
5	Kauralexin A3	-10.9	Trp 291	3.35	Asn 75, Gly 79, His 80, Phe 98, Ala 102, Thr 153, Val 157, Val 286, Ser 287, Tyr 290, Phe 453, Gln 457

SGLT2; Sodium-glucose co-transporter type 2, NL 2; Empagliflozin

(A)



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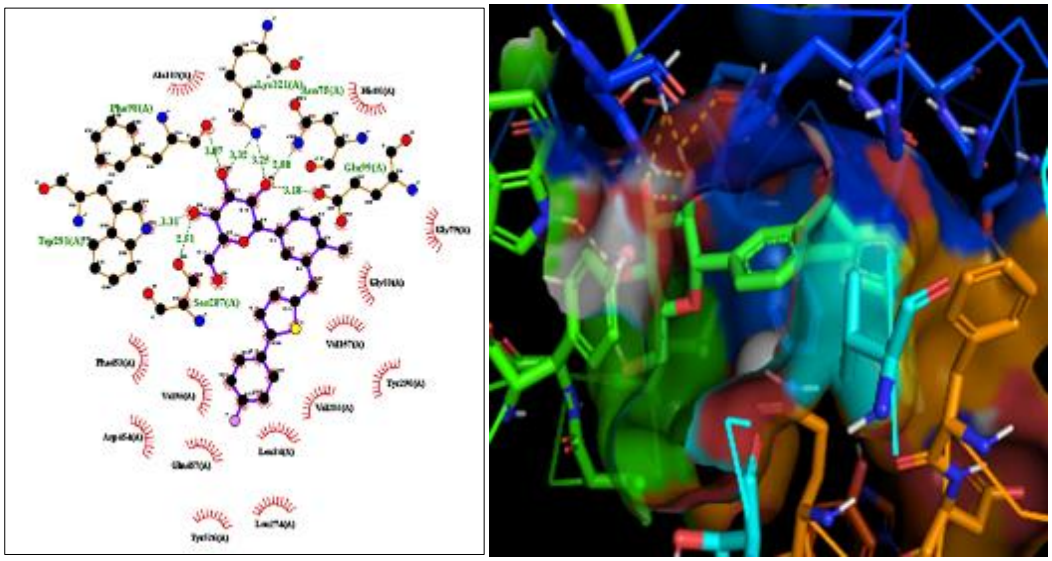


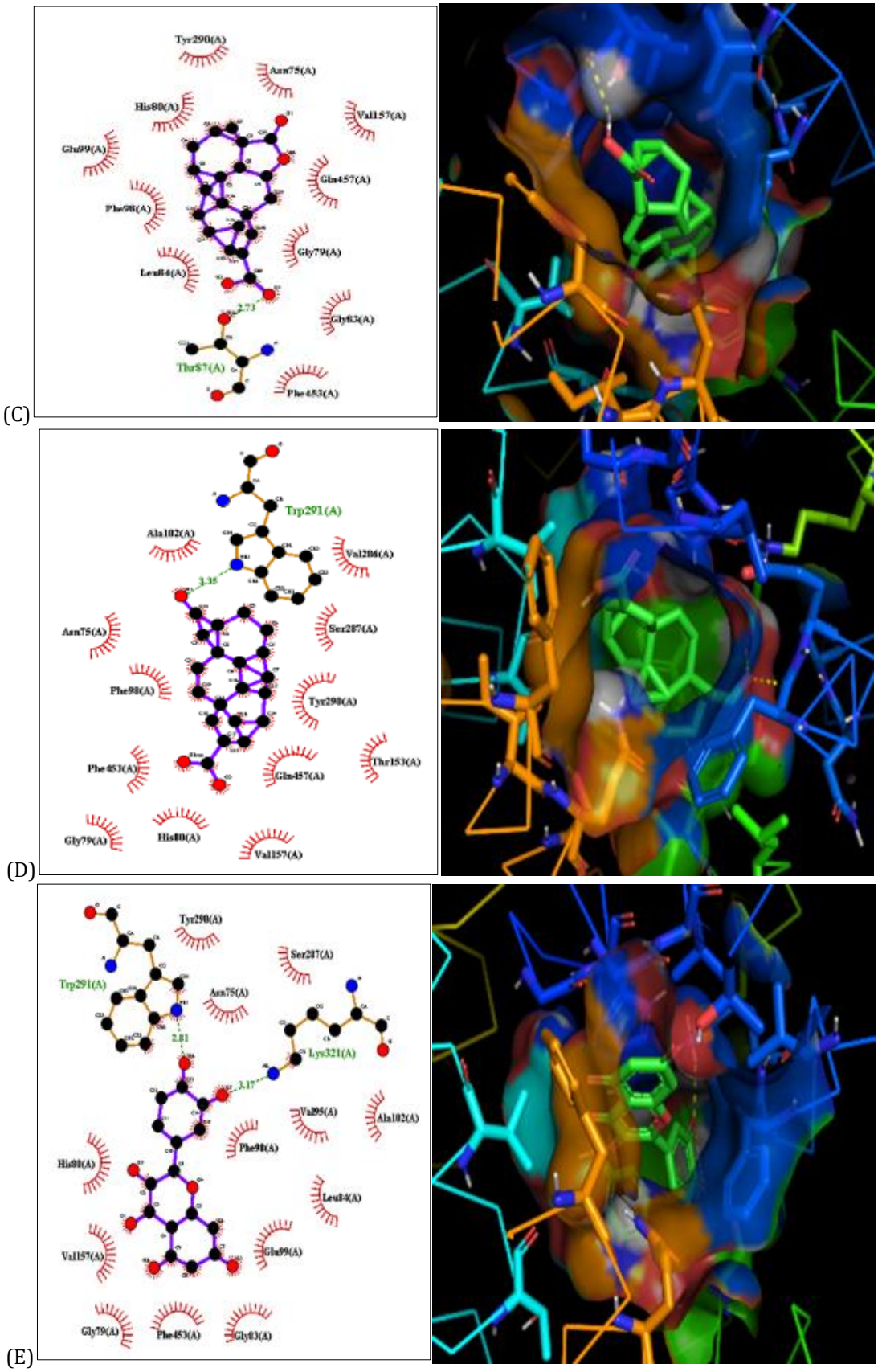


Figure 4 A 2D and 3D molecular interaction surface between the best complexes amino acid residues; A- NL 2-SGLT2, B-Canaglifloxin-SGLT2, C- Kauralexin A2-SGLT2, D- Kauralexin A3-SGLT2, E- Taxifolin-SGLT2

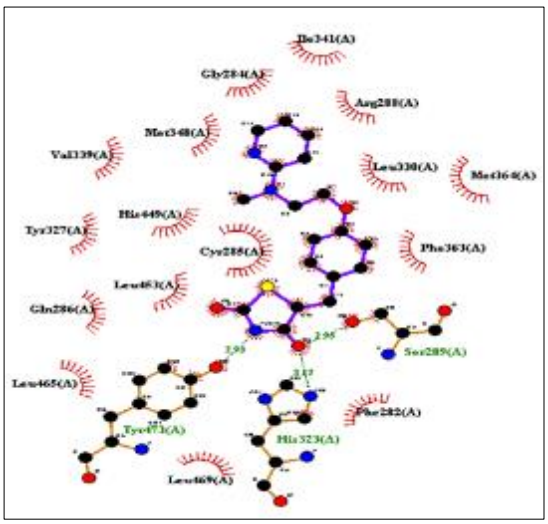
The best molecular interaction affinity with the PPAR-γ binding pocket occurs with the two phytochemical candidates. Kauralexin A3 shows the best binding scores of -9 (Kcal/mol) with its PPAR-γ protein complex, while Kauralexin A2 have a score of -8.2 (Kcal/mol) which are both better than the standard inhibiting drug (Rosiglitazone) of -8.1 (Kcal/mol) as shown in table 5. Although, the native ligand (2,4-Thiazolidiinedione) holds a relatively higher binding affinity than Kauralexin A2; a function that was contributed from the hydrogen bonding interaction that exist with the three amino acids residues, Ser 289, His 323, Tyr 473, and absence of visible hydrogen bond interaction with the Kauralexin A2. The molecular hydrophobic bonding surface from the two potential phytochemical candidates also aids in the good binding score reported. While the standard inhibiting drug and the native ligand possess thirteen similar interactions with the residual amino acids (Phe 282, Gly 284, Cys 285, Gln 286, Arg 288, Try 327, Leu 330, Val 339, Ile 341, Met 348, Phe 363, Met 364, His 449), the residue at Leu 453, Leu 465, Leu 469 provided the native ligand a better binding affinity, as compared to the Ser 289 and Ile 326 amino acids residues on the standard drug binding pocket. These molecular interactions are clearly represented in the 2D and 3D structural conformation in Figure 5.

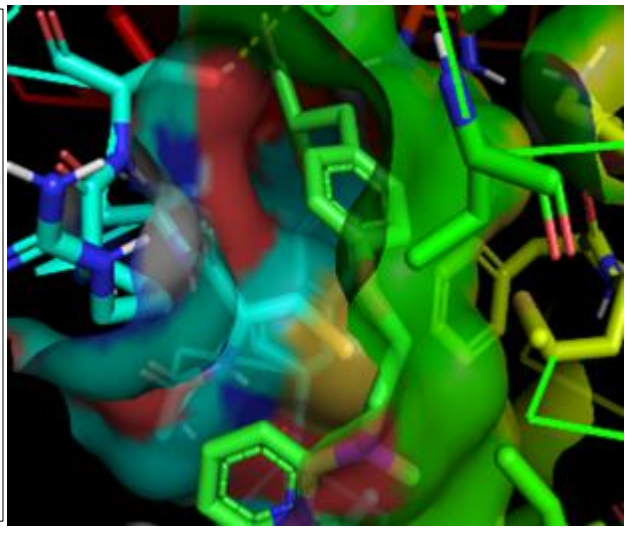
Table 5 Ligand report showing best binding information with PPAR-γ Protein (4EMA)

Sr. No.	Compound/ Ligands	Docking score (Kcal/mol)	H- bond	H-bond Distance (Å)	Hydrophobic interaction
1	Rosiglitazone	-8.1	His 323 Tyr 473	3.05 2.95	Phe 282, Gly 284, Cys 285, Gln 286, Arg 288, Ser 289, Ile 326, Try 327, Leu 330, Val 339, Ile 341, Met 348, Phe 363, Met 364, His 449
2	NL 3	-8.5	Ser 289 His 323 Tyr 473	2.95 3.12 2.93	Phe 282, Gly 284, Cys 285, Gln 286, Arg 288, Try 327, Leu 330, Val 339, Ile 341, Met 348, Phe 363, Met 364, His 449, Leu 453, Leu 465, Leu 469,
3	Kauralexin A2	-8.2	-	-	Gly 284, Cys 285, Phe 287, Arg 288, Leu 330, Ile 333, Leu 340, Ile 341, Ser 342
4	Kauralexin A3	-9	Ser 342	3.23	Gly 284, Cys 285, Arg 288, Leu 330, Val 339, Ile 341, Met 348, Met 364

PPAR-γ; Peroxisome proliferator-activated receptor gamma, NL 3; 2,4-Thiazolidiinedione

(A)





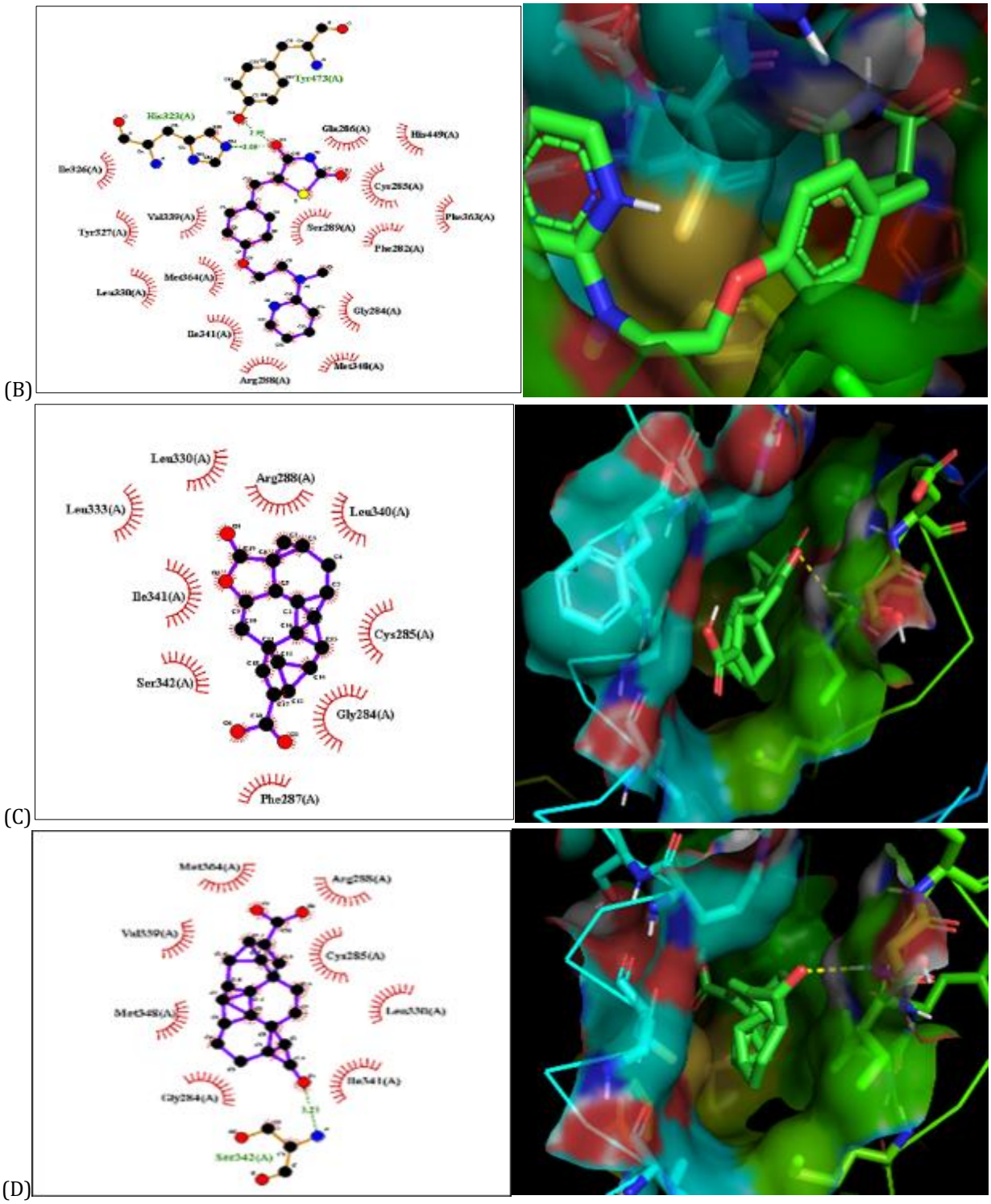


Figure 5 A 2D and 3D molecular interaction surface between the best complexes amino acid residues; A- NL 3-PPAR-γ, B- Rosiglitazone- PPAR-γ, C- Kauralexin A2- PPAR-γ, D- Kauralexin A3- PPAR-γ

The insilico ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) profiling of the phytochemicals depicts the probability of the potential drug candidates to undergo a profitable interaction with specific protein targets for successful drug discovery and development. Hence, profiling of the candidates must pass some certain established pharmacokinetics, druglikeness and medicinal chemistry rules [12, 29], which can serve as a first-hand filter in discovery and development phase of the drug to avoid costly preclinical and clinical catastrophe.

Table 6 Insilico ADME/toxicity profile

Sr. No.	Ligands	Lipinski Rule Violation	Leadlikeness Violation	Solubility LogS	Human Intestinal Absorption	Human Oral Bioavailability	Blood-Brain Barrier	$CaCo_2$ Perme-ability	Acute Oral Tox. log(1mol/kg)	Carcino-genic	CYP-2C9	CYP-2D6	CYP-1A2	CYP-2C19
1	Aloglipton	Nil	Nil	-3.041	HIA+	HOB-	BBB+	$CaCo_2$ +	III (1.879)	-	-	-	-	-
2	Canagliflozin	Nil	1	-2.877	HIA+	HOB-	BBB+	$CaCo_2$ -	III (2.613)	-	-	-	-	-
3	Rosiglitazone	Nil	1	-3.239	HIA+	HOB+	BBB+	$CaCo_2$ -	III (2.418)	-	+	-	+	+
4	Petivericin	Nil	Nil	-2.654	HIA+	HOB+	BBB+	$CaCo_2$ +	III (2.133)	+	-	-	-	+
5	Taxifolin	Nil	Nil	-2.999	HIA+	HOB-	BBB-	$CaCo_2$ -	II (2.146)	-	-	-	+	-
6	Kauralexin A1	1	1	-4.707	HIA+	HOB+	BBB+	$CaCo_2$ +	III (2.285)	-	-	-	-	-
7	Kauralexin A2	Nil	1	-4.276	HIA+	HOB-	BBB+	$CaCo_2$ +	III (1.793)	-	-	-	-	-
8	Kauralexin A3	Nil	1	-4.415	HIA+	HOB-	BBB+	$CaCo_2$ +	III (1.817)	-	-	-	-	-
9	Petiveriin	Nil	1	-1.800	HIA-	HOB-	BBB+	$CaCo_2$ -	III (1.857)	-	-	-	-	-
10	Medicagenate	1	2	-4.140	HIA+	HOB+	BBB+	$CaCo_2$ -	I (3.544)	-	-	-	-	-
11	Allantoin	Nil	1	-1.551	HIA+	HOB+	BBB+	$CaCo_2$ -	III (1.857)	-	-	-	-	-
12	Quinic acid	Nil	1	-0.390	HIA+	HOB+	BBB-	$CaCo_2$ -	III (2.207)	-	-	-	-	-
13	Pipecolic acid	Nil	1	-0.734	HIA-	HOB+	BBB+	$CaCo_2$ -	III (1.480)	-	-	-	-	-
14	Spermidine	Nil	1	-0.500	HIA+	HOB+	BBB+	$CaCo_2$ +	III (2.116)	-	-	-	+	-
15	Spermine	Nil	2	-0.500	HIA+	HOB+	BBB+	$CaCo_2$ +	III (2.290)	-	-	-	+	-
16	Pelargonidin	Nil	Nil	-3.212	HIA+	HOB-	BBB-	$CaCo_2$ -	II (0.982)	-	+	-	+	+
17	Vestitone	Nil	Nil	-3.026	HIA+	HOB-	BBB-	$CaCo_2$ +	III (2.394)	-	+	-	+	+
18	Fraxidin	Nil	1	-3.385	HIA+	HOB+	BBB-	$CaCo_2$ +	II (1.403)	-	-	-	+	-
19	Isobazzanene	1	2	-4.543	HIA+	HOB-	BBB+	$CaCo_2$ +	III (2.065)	+	-	-	-	-
20	Apigenin	Nil	Nil	-2.777	HIA+	HOB-	BBB-	$CaCo_2$ +	III (1.484)	-	+	-	+	+
21	Coniferin	Nil	Nil	-1.203	HIA-	HOB-	BBB-	$CaCo_2$ -	III (2.032)	-	-	-	-	-
22	Isorhamnetin	Nil	Nil	-3.222	HIA+	HOB-	BBB-	$CaCo_2$ -	III (1.664)	-	+	-	+	+

+; Positive; -; Negative; Solubility normal range; −6.5 to 0.5, HIA% < 30%= HIA-; HIA% > 30%= HIA+; Acute Oral Toxicity; I- extremely toxic; II-moderately toxic; III-slightly toxic; IV-non-toxic; CYP-2C9 inhibitor; CYP-2D6 inhibitor; CYP- 1A2 inhibitor; CYP-2C19 inhibitor

The relationship between a potential drug pharmacokinetics and physicochemical parameters must be figure out by Lipinski rule-of-five [30]. From his highlights, to consider a drug orally feasible, it must not violate more than one of such rules; not greater than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms), not greater than 10 hydrogen bond acceptors (nitrogen or oxygen atoms), a molecular mass less than 500 Daltons and an octanol-water partition coefficient log P not greater than 5 [31]. All candidates in this study fall within the acceptable range of oral drug candidates with few violations by Medicagenate, spermine and isobazzanene, which violate two leadlikness parameter. The blood-brain barrier (BBB) interference are attributed with some neurological dysfunction like amyotrophic lateral sclerosis (ALS), epilepsy, oedema, brain traumas, and Parkinson's disease [32]. Therefore, drug candidates reflecting this BBB crossing with a TPSA < 79 $Å^2$ and WLogP less than 6 as reported by Ishola et al. [31] will be suitably important in the development of CNS-acting therapeutics (Table 6 and 7).

Table 7 Insilico ADME/toxicity profile continuation

Sr. No.	Ligands	TPSA ($Å^2$)	WLogP	Hepatotoxicity
1	Aloglipton	93.67	-0.20	-
2	Canagliflozin	118.39	3.06	+
3	Rosiglitazone	96.83	2.11	+
4	Petivericin	61.58	4.35	-
5	Taxifolin	127.45	0.86	+
6	Kauralexin A1	40.13	3.79	-
7	Kauralexin A2	80.26	1.52	-
8	Kauralexin A3	57.20	2.96	-
9	Petiveriin	104.05	-0.99	-
10	Medicagenate	120.72	2.60	-
11	Allantoin	113.32	-2.94	-
12	Quinic acid	118.22	-2.32	-
13	Pipecolic acid	49.33	-0.17	-
14	Spermidine	64.07	-0.34	-
15	Spermine	76.10	-0.36	-
16	Pelargonidin	94.06	3.20	+
17	Vestitone	75.99	2.47	+
18	Fraxidin	68.90	1.52	+
19	Isobazzanene	0.00	4.87	-
20	Apigenin	90.90	2.58	+
21	Coniferin	128.84	-1.23	-
22	Isorhamnetin	120.36	2.29	+

TPSA- Topology polar surface area

Thus, out of the nineteen phytochemical candidates used in this study, Kauralexin A1, Kauralexin A2, Kauralexin A3, Petiveriin, Medicagenate, Allantoin, Pipecolic acid, Spermidine, Spermine, Petivericin, and Isobazzanene can transverse the BBB, however, the last two candidates exhibit carcinogenic properties. This barricade is essential for restricted CNS microenvironment in/outflux, for adequate neuronal function [31]. While the Kauralexin A2, and Kauralexin A3 possess a considerable binding affinity for the three antidiabetic protein target, its significant BBB transversion could be employed for anti-neurodegenerative diseases drugs development.

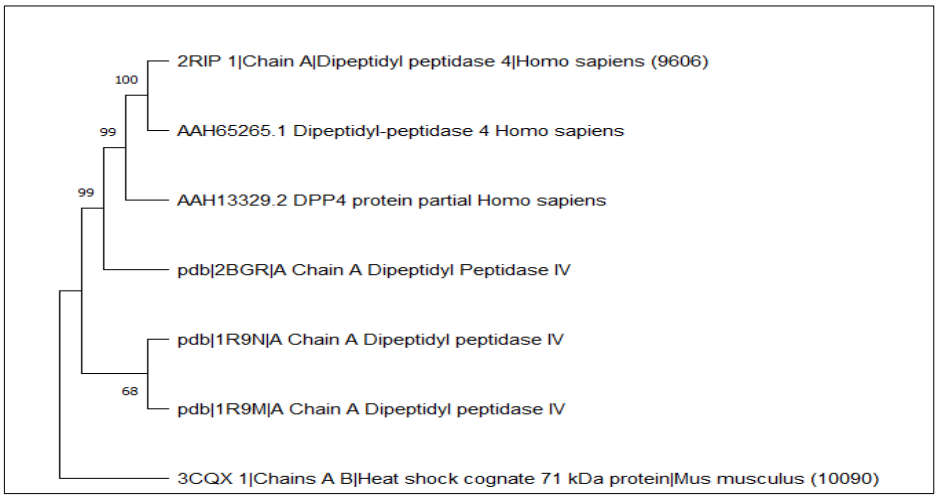
The acute oral toxicity profile of the drug candidates shows they are within the category II to III (Moderate-slightly toxic), except Medicagenate which is in the category I (extremely toxic). Also, all candidates are readily absorbed into the intestine except petivericin, pipecolic acid and Coniferin which are negative. Accessibility of the drug candidates through the membrane are determined by their $Caco_2$ permeation. Hence, this attribute is better in the case of Petivericin, Kauralexin A1, Kauralexin A2, Kauralexin A3, Spermidine, Spermine, Pelargonidin, Vestitone, Fraxidin, Isobazzanene and Apigenin.

Seventeen phytochemicals out of the nineteen potential drug candidates substantially passed the profiling test as shown in table 6 and table 7. Thus, these phytochemicals fulfil all the enlisted criteria similar to Sharma et al. [12] findings, and it is suggestively determined to be suitable for the development of potent antidiabetic drugs. Also, the ability of Kauralexin A2 and Kauralexin A3 to bind effectively to the three protein targets (DPPIV, SGLT2 and PPAR-γ), Taxifolin to bind effectively with two protein targets (DPPIV and SGLT2), and Medicagenate, pelargonidin, vestitone, isobazzanene, Apigenin, Coniferin and Isorhamnetin to one protein target (DPPIV) could be pivotal in the treatment of T2DM diseases, coupled with the link ability of the phytochemical candidates to interact strongly than the standard inhibiting drugs to individual receptors. A therapeutic drug can be inferred from this collection.

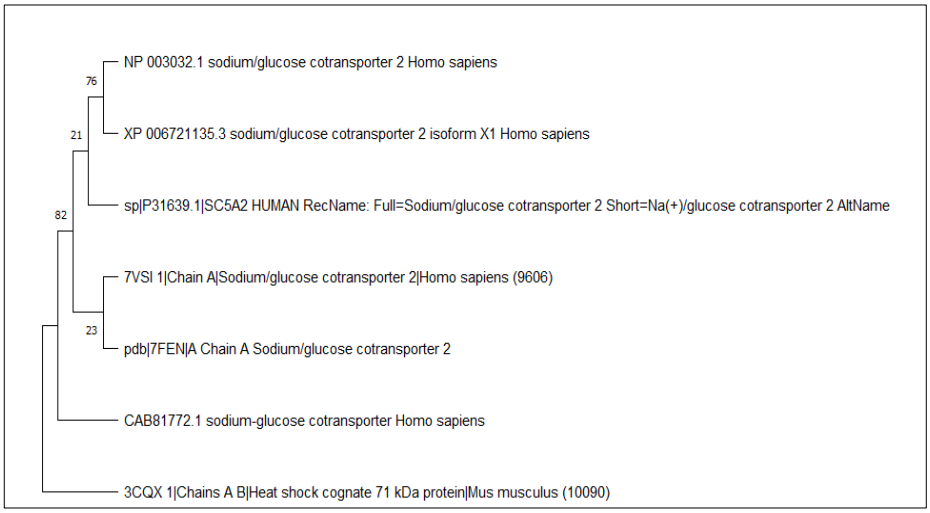
3.3. Phylogenetic Relationship

The phylogenetic relationship between the protein target sequences and the corresponding selected homologs for DPPIV, infers that a likely comparable result from the docking bond affinity can be generated with AAH65265.1, AAH13329.2 and 2BGR based on the high bootstrap confidence of >90% and might not be similar for 1R9N, 1R9M protein target due to the relatively low < 90% bootstrap confidence. Similarly, PPAR-γ protein target sequences phylogenetic relationship to 3ADX, 3ADW and 3ADV is likely be produce reoccurring result of the insilico profiling, with dissimilarity to 3ADU, 3ADT, 3ADS, 3B3K, 3R8I protein targets. From the SGLT2 protein sequences phylogenetic tree, the bootstrap confidence < 90% suggests that none of the protein targets (P31639.1, CAB81772.1, 7FEN, NP_003032.1, XP_006721135.3, 3CQX_1) can produce a reoccurring insilico result (Figure 6).

(A)



(B)



(C)

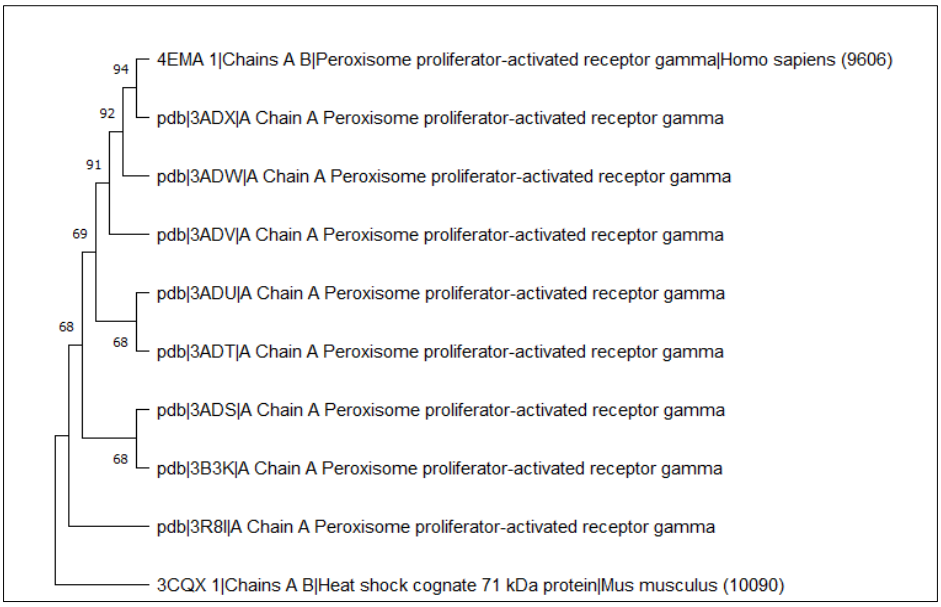


Figure 6 Phylogenetic relationship between the test protein target sequence, other homologous sequences and an outgroup for: A- DPPIV, Dipeptidyl peptidase IV; B- SGLT2, Sodium-glucose co-transporter type 2; C- PPAR-γ, Peroxisome proliferator-activated receptor gamma

3.4. Abbreviations

- AGEs: Advanced Glycation End-products.
- BBB: Blood-Brain Barrier.
- DM: Diabetes Mellitus.
- DPPIV: Dipeptidyl peptidase IV.
- DS, Discovery Studio.
- NGO: Non-Governmental Organizations.
- NL: Native Ligand
- PPAR-γ: Peroxisome Proliferator-activated Receptor-gamma.
- RCSB: Research Collaboratory for Structural Bioinformatics.
- ROS: Reactive Oxygen Species.
- SBDD: structure-based-drug-design.
- SGLT2: Sodium-glucose co-transporter type 2.
- SSA: Sub-Saharan Africa.
- T2DM: Type II Diabetes Mellitus.
- TSS: Total Soluble Solids
- TPSA: Topological Polar Surface Area.

4. Conclusion

The insilico study of citron watermelon selected phytochemicals into the binding cavity of DPPIV, SGLT2 and PPAR-γ showed a favourable interaction than the standard inhibitory drugs. Also, the phylogenetic relatedness confidence infers the reoccurrence of results with other similar protein targets can be obtained. These potential phytochemicals (Taxifolin, Kauralexin A1, Kauralexin A2, Kauralexin A3, Petiveriin, Medicagenate, Allantoin, Quinic acid, Pipecolic acid, Spermidine, Spermine, Pelargonidin, Vestitone, Fraxidin, Apigenin, Coniferin, Isorhamnetin) can serve as novel drug candidate for T2DM management and treatment. Additionally, derivatives can be developed for further screening. All of the insilico parameters are satisfactory, and reflect *Citrullus lanatus* as a probable reservoir containing potential therapeutic drug candidates.

Hence, the findings of this study will be beneficial for researchers to focus more on underutilized plants to develop new and effective formulations to manage problems of diabetics' patients having T2DM.

Compliance with ethical standards

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Disclosure of conflict of interest

The Authors declares that there is no competing interests regarding the publication of this document.

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