Virtual Screening of a Series of Phytocompounds from Lagenaria Siceraria for the Identification of Potential Antidiabetic Drug Candidates, in Silico Study and Drug Design Approaches

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Article

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Abstract: Background: Currently, limited number of therapeutic options are available to treat Diabetes mellitus, and to find a potential candidate, laboratory work takes time and also needs animal studies. So, the aim of this study was to determine the efficacy of some natural phytocompounds with the aid of molecular docking, bioinformatics and *in silico* drug design approaches. Method: Two proteins (Human CYP3A4 linked to metformin and Human dipeptidyl peptidase-IV) were selected and molecular docking studies were conducted using Pymol, AutoDock Vina, PyRx, and Discovery Studio. Different important pharmacokinetic parameters like ADME and toxicity data were obtained from online databases SwissADME and pkCSM program. Results: It was found that human dipeptidyl peptidase-IV (PDB ID: 4A5S) has exhibited a maximal affinity of -9.7 Kcal/mol for bryonolic acid and hesperidin, but only -6.7 kcal/mol for metformin hydrochloride. Similarly, the highest affinity of hesperidin for human CYP3A4 bound to metformin (PDB ID: 5G5J) is -10.7 Kcal/mol compared to metformin hydrochlor (-6.3 Kcal/mol). Besides, all the compounds have been documented outstanding ADMET profile, and accepted by drug-likeness or Lipinski rule. Conclusions: The present study suggested that these compounds can be further investigated *in vitro* and *in vivo* to establish them as lead compounds against Diabetes mellitus..

Keywords: *Lagenaria siceraria*; *In silico* study; Drug design; Diabetes mellitus; Molecular docking; ADMET

1. Introduction

Diabetes mellitus type 2 is one of the major health concerns of the 21st century in the globe. According to data, the prevalence of diabetes has been doubled in both developed and developing countries in the last two decades [1]. By 2030, more than a half billion people are anticipated to have diabetes worldwide [2]. Diabetes can hinder productivity by causing illness and death at an early age, and its impacts are likely to get worse as the disease starts to affect younger people [3]. Edwin Gale referred to diabetes as "the diabetes apocalypse" due to its prominence on the public health agenda as a global epidemic and a danger to human health and the global economy [4]. The worldwide expenditure on diabetes was at least USD 376 billion in 2010, and is projected to reach USD 490 billion by 2030 [5]. The diabetes epidemic and its effects are caused by complex genetic and epigenetic systems which interact with a system that is made up of many behavioral and environmental factors [1].

Diabetes mellitus is caused by a deficit or lower efficacy of endogenous insulin or the inappropriate use of insulin by target cells, and therefore. is characterized by an abnormal

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metabolism of carbohydrates, hypertension, and vasculature-affecting complications [6]. It can directly influence blood lipid levels, resulting in diabetes dyslipidaemia [7]. The basic characteristics of diabetic dyslipidemia include elevated blood levels of triglyceride (TG), decreased high density lipoprotein (HDL), and elevated levels of low-density lipoprotein (LDL) [8,9].

Currently, insulin and several oral antidiabetic medicines such as sulfonylureas, thiazolidinediones, and glucosidase inhibitors are being used to treat diabetes. To improve glycemic control, these medications can be used alone or in combination [10,11]. Good glycemic control slows the onset or progression of diabetes complications, but does not eliminate them altogether. In addition to the limitations described above, each of the aforementioned antidiabetic medicines is linked with a variety of major side effects [12,13]. Consequently, antidiabetic discovery has moved its attention to medicinal plants in order to provide innovative, promising, and cost-effective medications with minimal side effects [14]. There are more than one thousand plant species that are utilized as traditional medicines for diabetes mellitus all over the world [15]. The Cucurbitaceae family has long been a source of therapeutic compounds in research. Several plant components, particularly fruits, from this family have been studied for their pharmacological activities [16].

Bottle gourd (*Lagenaria siceraria* (Molina) Standl.), commonly known as white-flowered gourd or calabash, is a diploid species (2n) of the genus Lagenaria of the Cucurbitaceae family [17,18]. The genus Lagenaria includes six species: *L. siceraria* (Molina) Standl., *L. abyssinica* (Hook f.) Jeffrey, *L. guineensis* (G. Don) Jeffrey, *L. ruffa* (Gilg.) Jeffrey, *L. breviflora* (Benth.) Roberty, and *L. sphaerica* (Sonder) Naudin. All six species are found in Africa, which is regarded to be the center of the genetic diversity of *L. siceraria* [19]. It can be differentiated from other types of pumpkin by its white blooms, distinctive fruit, seed, and leaf forms [20]. *Lagenaria siceraria* is the only economically valuable species grown globally for a variety of purposes, including food, medicinal, adornment, and the production of household goods, and musical instruments. This fruit is found in two varieties: sweet and bitter. The sweet type is typically eaten as a vegetable, but the bitter, wild species is favored for medicinal purposes [21].

Bottle gourd has been linked to a variety of health advantages, including cardioprotective, diuretic, anti-cancer, aphrodisiac, general tonic, antidote to some poisons and scorpion stings, alternate purgative, and cooling properties. Fresh bottle gourd fruit juice is used to treat a variety of ailments, including diabetes, hypertension, flatulence, liver problems, and as a diuretic [22–24]. Syrup made from the succulent fruits can be used to treat a variety of respiratory conditions, as well as pain, ulcers, fever, asthma, and other bronchial problems. The fruit's pulp has several medicinal uses, including as a cough remedy, an antidote to poisons, and a cooling diuretic [23,25].

Numerous chemical substances, such as sterols, terpenoids, flavonoids, and saponins, have been identified from this species. The fruit is said to include the triterepenoide cucurbitacins B, D, G, and H, two sterols, fucosterol and campesterol, aerpene bryonolic acid, flavone-C glycosides (a ribosome inactivating protein), and lagenin [26]. These chemical components are known for giving anti-diabetic properties. Therefore, the primary purpose of this study was to develop a useful pharmaceutical agent derived from the natural source. In light of this, the aim of the study is to treat diabetic mellitus using chemical components derived from *Lagenaria siceraria* applying computational tools like molecular docking, ADMET characteristics, Lipinski Rules, etc. This new technique ushers in a new age for constructing effective drugs and designing novel biological substances in a short amount of time and at a reduced cost [27]. This study, therefore, can open a new door for the development of a cost-effective diabetes treatment with fewer side effects.

2. Results

2.1. Structure optimization

Optimized chemical structure provides an essential visual representation of a chemical element, which is helpful for molecule stability [28]. Table 1 displays the optimized chemical structures.

Table 1. Optimized chemical structures of phytoconstituents reported from *Lagenaria siceraria*.

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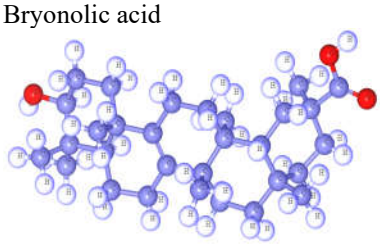
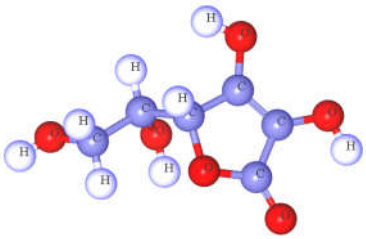
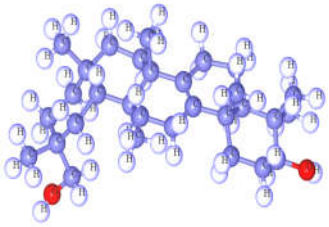
Bryonolic acid



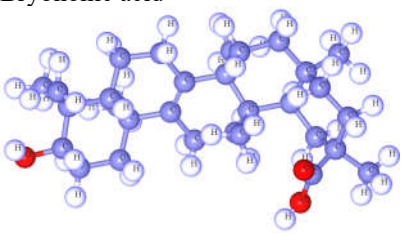
L-ascorbic acid



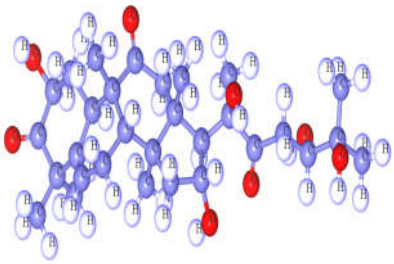
Bryonolol



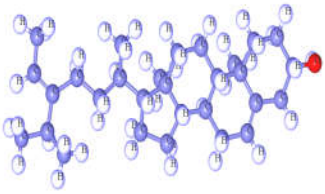
Bryononic acid



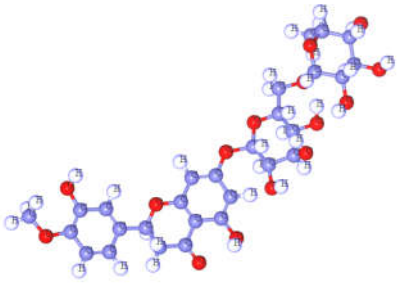
Cucurbitacin G



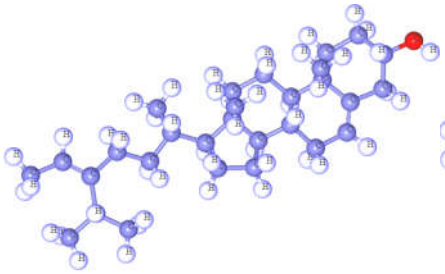
Fucosterol



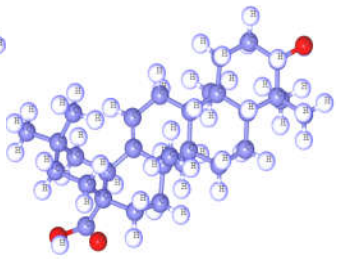
Hesperidin



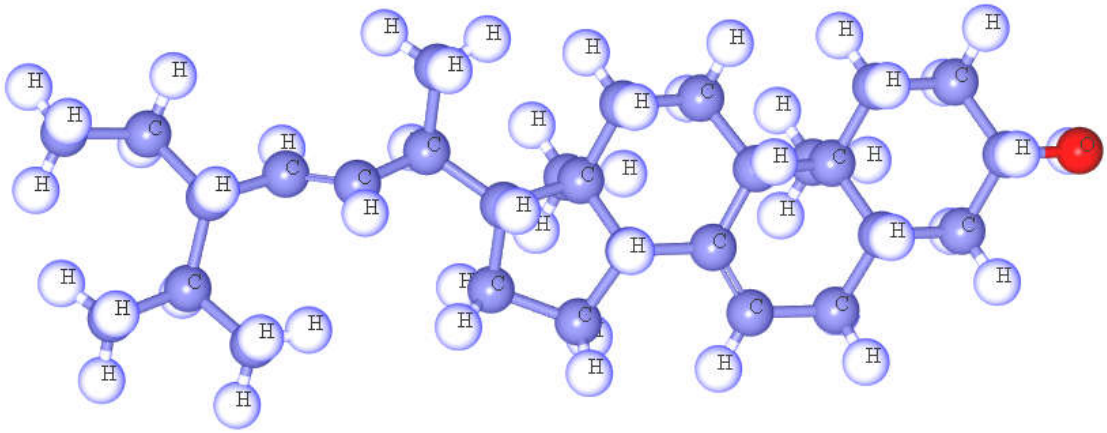
Isofucosterol



Oleanolic acid



Spinasterol



2.2. Lipinski rule, Pharmacokinetics and Drug likeness

The data related to Lipinski rule are predicted using the online data set SwissADME, and the derived values are displayed in Table 2. The acquired values revealed that the molecular weight ranges from 176.12 to 610.56 Dalton, the Hydrogen bond acceptor ranges from 01 to 15 and the Hydrogen bond donor ranges from 01 to 8. The bioavailability index of the ligands is found to range from 0.17 to 0.85. Hence, according to the gathered data, with the exception of ligand 7, all other ligands obey the Lipinski rule. As nearly all of the compounds have accepted the Lipinski value, they can be recommended for oral medications and additional investigations, such as molecular docking, ADMET, and other relevant analyses.

Table 2. Data of Lipinski rule, Pharmacokinetics and Drug likeness.

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Ligand No.	Parameters of Lipinski rule, Pharmacokinetics and Drug likeness							
	PubChem ID	Molecular weight (Dalton)	Hydrogen bond acceptor	Hydrogen bond donor	Topological polar surface area (Å²)	Lipinski rule		Bioavailability Score
						Result	violation	
01	159970	456.7	3	2	57.53	Yes	1	0.85
02	54670067	176.12	6	4	107.22	Yes	0	0.55
03	15756408	442.72	2	2	40.46	Yes	1	0.55
04	472768	456.7	3	2	57.53	Yes	1	0.85
05	441818	534.68	8	5	152.36	Yes	1	0.55
06	5281328	412.69	1	1	20.23	Yes	1	0.55
07	10621	610.56	15	8	234.29	No	3	0.17
08	5281326	412.69	1	1	20.23	Yes	1	0.55
09	10494	456.7	3	2	57.53	Yes	1	0.85
10	5281331	412.69	1	1	20.23	Yes	1	0.55

2.3. Molecular docking

Ten targeted compounds and standard (Metformin hydrochloride) have been incorporated into molecular docking studies against two receptor-binding proteins, Human CYP3A4 bound to metformin (PDB ID: 5G5J) and Human dipeptidyl peptidase-IV (PDB ID: 4A5S). Human dipeptidyl peptidase-IV exhibited a maximal affinity of -9.7 Kcal/mol for bryonolic acid and hesperidin, but only -6.7 Kcal/mol for Metformin hydrochloride. Similarly, the highest affinity of hesperidin for Human CYP3A4 bound to metformin (PDB ID: 5G5J) was found to be -10.7 Kcal/mol. In contrast, the conventional Metformin hydrochloride achieved a value of -6,3 Kcal/mol. The results have been shown in Table 3.

Table 3. Summary of molecular docking.

No	Name	PubChem CID	Human CYP3A4 bound to metformin (PDB ID: 5G5J)	Human dipeptidyl peptidase-IV (PDB ID: 4A5S)

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			Binding affinity (Kcal/mol)	Binding affinity (Kcal/mol)
01	Bryonolic acid	159970	-9.3	-9.7
02	L-ascorbic acid	54670067	-9.9	-9.1
03	Bryonolol	15756408	-8.3	-9.5
04	Bryononic acid	472768	-9.7	-9.2
05	Cucurbitacin G	441818	-9.6	-9.0
06	Fucosterol	5281328	-9.9	-9.1
07	Hesperidin	10621	-10.7	-9.7
08	Isofucosterol	5281326	-9.2	-9.3
09	Oleanolic acid	10494	-9.1	-9.1
10	Spinasterol	5281331	-9.5	-9.2
Metformin hydrochloride		14219	-6.3	-6.7

2.4. Ligand-protein interaction and molecular docking poses

The interaction between protein and ligand has been visualized by Biovia discovery studio 2021. After molecular docking studies, this section describes how a targeted protein interacts to phytomolecules and how many active sites can be detected. Typically, hydrogen bonds and hydrophobic bonds account for binding energy [29]. In **Figure 1**, bryonolic acid has been shown to interact with Human dipeptidyl peptidase-IV. In the 2D structure, it is clearly seen that hydrogen and hydrophobic bonds are present which are bonded with ligand and they are responsible for the pharmacological efficacy. The most potential active amino acid residues present in Human dipeptidyl peptidase-IV (PDB ID: 4A5S) with bryonolic acid are ASP A:545, TRP A:629, TYR A:547, VAL A: 711, TYR A:662, and HIS A:740.

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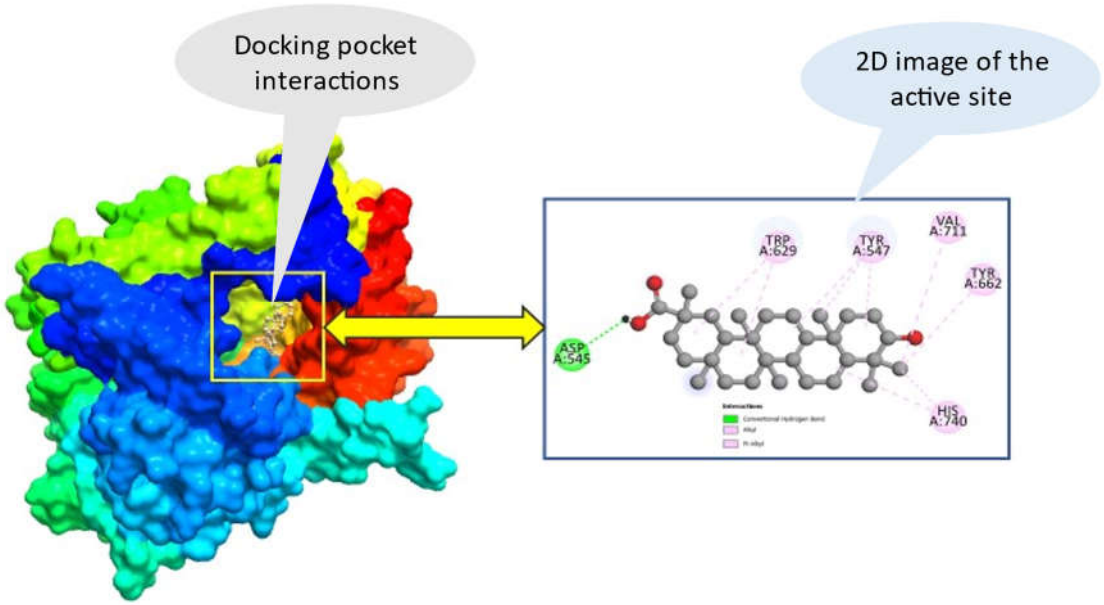

Figure 1. Human dipeptidyl peptidase-IV (PDB ID: 4A5S) with Bryonolic acid.

2.5. ADMET studies

The ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties are calculated using the web server pkCSM and presented in Table 4. Except for ligand 2, the results of the water solubility test showed that the remaining ligands are highly soluble in aqueous media, with the maximum Caco-2 permeability being 1.21 for ligands 06, 08, and 10. The G.I. absorption refers to how quickly the medications break down after entering the digestive system. Most of the compounds in this study have an excellent bioavailability rate, ranging from 92.03% to 99.55%, but only a small number of them dissociate badly in the gastrointestinal tract, with ranges of 39.15% and 31.48%. In order to produce a prospective drug candidate, the overall absorption rate is, therefore, fulfilled. In addition, none of them can cross the BBB. The VDss level varies from -1.009 to +0.338, with a maximum total clearance rate of 0.631 mL/min/kg. No ligand produces positive result for CYP450 1A2 Inhibitor, CYP 450 2C9 Substrate, or CYP450 3A4 Inhibitor; however, for CYP450 3A4 Substrate, ligand 01, 03, 04, 06, 08, 09, and 10 exerted a positive result.

Table 4. ADME studies of selected compounds reported from *L. siceraria*.

Sl. No	Absorption			Distribution		Metabolism				Excretion	
	Water solubility LogS	Caco-2 permeability	Human Intestinal	VDss (human)	BBB Permeability	CYP450 1A2 Inhibitor	CYP 450 2C9 Substrate	CYP450 3A4 Substrate	CYP450 3A4 Inhibitor	Total Clearance (mL/min/kg)	Renal OCT2 substrate
01	-3.45	1.14	98.11	-0.853	No	No	No	Yes	No	-0.036	No
02	-1.55	-0.25	39.15	0.218	No	No	No	No	No	0.631	No

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03	-6.33	1.1	92.03	0.338	No	No	No	Yes	No	0.067	No
04	-3.46	1.15	98.42	-0.799	No	No	No	Yes	No	-0.036	No
05	-4.42	0.43	69.47	-0.398	No	No	No	No	No	0.322	No
06	-6.75	1.21	94.64	0.179	No	No	No	Yes	No	0.619	No
07	-3.01	0.50	31.48	0.996	No	No	No	No	No	0.211	No
08	-6.71	1.21	94.64	0.179	No	No	No	Yes	No	0.619	No
09	-3.26	1.16	99.55	-1.009	No	No	No	Yes	No	-0.081	No
10	-6.68	1.21	94.97	0.178	No	No	No	Yes	No	0.611	No

Maximum compounds are free from AMES toxicity, hepatotoxicity and skin sensitization, but bryonolic acid, bryononic acid, and oleanolic acid may possess hepatic toxicity, and cucurbitacin G may show AMES toxicity as shown in Table 5**.** The maximum tolerated dose is 1.598 mg/kg/day, the maximum oral rat acute toxicity level is 2.553mol/kg and oral rat chronic toxicity 3.186 mg/kg/day.

Table 5. Toxicity prediction of selected compounds reported from *L. siceraria*.

Sl. No.	Compound name	AMES toxicity	Max. tolerated dose (human) mg/kg/day	Oral Rat Acute Toxicity (LD50) (mol/kg)	Oral Rat Chronic Toxicity (mg/kg/day)	Hepatotoxicity	Skin Sensitization
01	Bryonolic acid	No	0.098	2.294	2.065	Yes	No
02	L-ascorbic acid	No	1.598	1.063	3.186	No	No
03	Bryonolol	No	-0.863	2.213	2.031	No	No
04	Bryononic acid	No	0.098	2.294	2.065	Yes	No
05	Cucurbitacin G	Yes	-0.461	2.502	2.001	No	No

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06	Fucosterol	No	-0.653	2.553	0.89	No	No
07	Hesperidin	No	0.525	2.506	3.167	No	No
08	Isofucosterol	No	-0.653	2.553	0.89	No	No
09	Oleanolic acid	No	0.094	2.196	2.109	Yes	No
10	Spinasterol	No	-0.664	2.54	0.872	No	No
Metformin hydrochloride		Yes	0.874	2.465	2.122	No	Yes

3. Discussion

Ten bioactive compounds of various types reported from *Lagenaria siceraria* have been selected for assessing anti-diabetic activity. Pharmacological drug-likeness is a revolutionary step of the bioavailability assessment of a specific compound as an oral treatment [30]. It is predicted that nine out of ten targeted pharmaceuticals are not changed transparently owing to their adverse effects, leading to significant drug expenses, time, and human resources being squandered. This issue arises from an inability to determine the precise pharmacological properties [31,32]. Yet, by applying Lipinski's five-rule (Ro5), it is feasible to easily assess the characteristics of lead compounds, including their bioavailability and G.I. absorption [33]. Orally active medicines are required to adhere to this guideline, which states that there cannot be more than one violation of the predetermined criterion. Hence, it was determined whether or not each docking chemical satisfied Lipinski's Ro5 requirements [34,35]. With the exception of L-ascorbic acid (2), all of the targeted compounds, including bryonolic acid (1), bryonolol (3), bryononic acid (4), cucurbitacin G (5), fucosterol (6), hesperidin (7), isofucosterol (8), oleanolic acid (9), and spinasterol (10) exhibited at least one violation towards Ro5. Hesperidin (7), on the other hand, exhibited a maximum of three violations, which implies that it cannot be employed for oral dosage form. According to this rule, it is also suggested that, if a drug has two or more Ro5 violations, such as hesperidin does, then it demonstrates poor solubility or permeability [36]. As a result, this rule serves as a foundation for making predictions regarding the high possibility of success or failure of the development of a medicine based on a single chemical exhibiting a certain pharmacological or biological function [37,38].

Molecular docking is becoming increasingly popular among researchers, drug developers, and scientists as a result of its low cost and what appears to be its ease of usage [39]. This approach makes it feasible to make accurate predictions regarding the interaction that takes place in the coordination sphere between a ligand and a receptor [40,41]. In order to successfully conduct out molecular docking, there are three primary requirements that must be met: bond intensity, molecular interactions, and bond characterization. Lead compounds exhibited relatively low binding energies, hydrogen bonding, and van der Waals interactions as well as a favorable ADME profile [42–44].

Any biologically active compounds are regarded to have the potential to have pharmacological effects if they have a minimum binding energy of -6.0 Kcal/mole [45,46]. Therefore, all ten ligands showed greater affinity than standard Metformin hydrochloride towards two receptor-binding proteins. But, the highest affinity (-10.7 Kcal/mol) was shown by Hesperidin for Human CYP3A4 bound to metformin. It follows that the targeted molecules exerted better affinity than the original Metformin hydrochloride.

Non-covalent interactions, including hydrogen bond and hydrophobic interactions, play an important role in maintaining energetically favorable ligands at the active site of a specific

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macromolecular structure, hence boosting improved binding affinity and drug efficacy [47]. In an effort to realize that the protein-ligand complex system remained in a stable state throughout the molecular docking simulation, it was determined that the system maintained a steady state [48]. This experiment has been conducted to analyze and compare the stability of these conceptual connection models. In the binding pocket of protein ligands, it is evident how they interacted during the synthesis of complex molecules. Thus, amino acid residues that function throughout the interaction may be the most important determinant of the binding affinity of protein-ligand complexes [49,50]. Lastly, a 2D representation of active amino acid residues revealed the presence of distinct kinds of amino acids with varying bond angles. ASP A:545, TRP A:629, TYR A:547, VAL A: 711, TYR A:662, and HIS A:740 are among the amino acid residues formed during the formation of the protein-bryonolic acid complex.

In modern years, the *in silico* ADMET studies of potential bioactive compounds have been exploited in drug development as a main screening technique for the early discovery phase [51,52]. Early ADMET research screening may lower the risk of failure during the clinical study [53]. The pkCSM is one of the greatest predictors of these necessary ADMET data [54]. Except for ligand 2, the majority of the chemicals in this study were very soluble in water. Maximum substances were discovered to have an absorption rate greater than 90%, but there were also compounds with poor solubility; these data showed that these compounds have varying levels of absorption ability in the gastrointestinal system. Caco-2 permeability values varied between -0.25 and 1.21. The ligands cannot cross the blood-brain barrier (BBB), whereas their volume of distribution (VDss) ranged from -1.009 to 0.996 log L/kg. Only ligands 01, 03, 04, 06, 08, 09, and 10 may block the CYP450 3A4 substrate during metabolism. The total clearance rate was determined as -0.081mL/min/kg to 0.63mL/min/kg. The majority of them were found to be devoid of AMES mutagenicity, skin sensitization, and hepatotoxicity, as predicted by toxicological modelling. The toxicological data implied that these compounds have good physiochemical and pharmacokinetics properties.

The molecular docking of selected compounds with Human CYP3A4 and Human dipeptidyl peptidase-IV demonstrated that all of the examined ligand molecules exhibited excellent binding energy, and bryonolic acid displayed hydrogen and hydrophobic bonding with several amino acids in the active pockets. Thus, bryonolic acid had the highest affinity for human dipeptidyl peptidase-IV and possessed substantial antihyperglycemic effects.

4. Materials and Methods

4.1. Optimization and Ligand preparation

All chemical structures were constructed using the ChemDraw 2012 program [55]. The structures were then optimized using the Avogadro Application in order to rectify the geometry arrangement [56]. All chemical structures were then stored as protein data bank (PDB) files for molecular docking study.

4.2. Protein preparation and Molecular Docking study

Two proteins (Human CYP3A4 linked to metformin with PDB ID: 5G5 and Human dipeptidyl peptidase-IV with PDB ID: 4A5S) were selected from the RSCB Protein Data Bank and were retrieved as PDB files. It was then analyzed and cleaned by Pymol (version 2) of all the excess molecules such as water and ligands in order to obtain a fresh protein. The nonbonding interaction of drug-protein was then done using the AutoDock Vina docking software tool. Docking studies were carried out utilizing the configurable ligand and receptor grid boxes in PyRx and AutoDock Vina Wizard, respectively. Finally, the visual depiction of the 2D interaction and active amino acid residues specific for the ligand in protein structure was created using Discovery Studio as shown in Table 6.

4.3. Lipinski rule, Pharmacokinetics and Drug likeness

Christopher A. Lipinski was the first person to present a description of the Lipinski rule in 1997 [34]. This rule describes the pharmacokinetic properties of a molecule by emphasizing the

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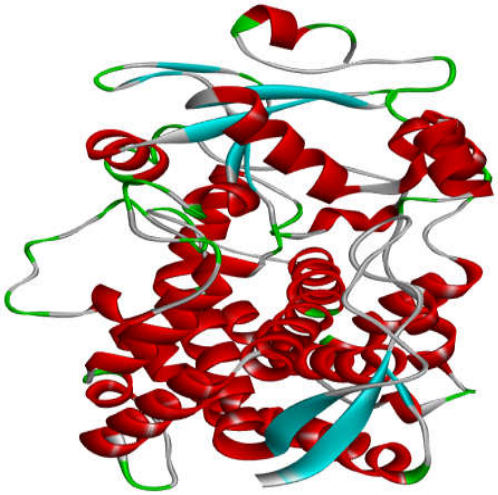
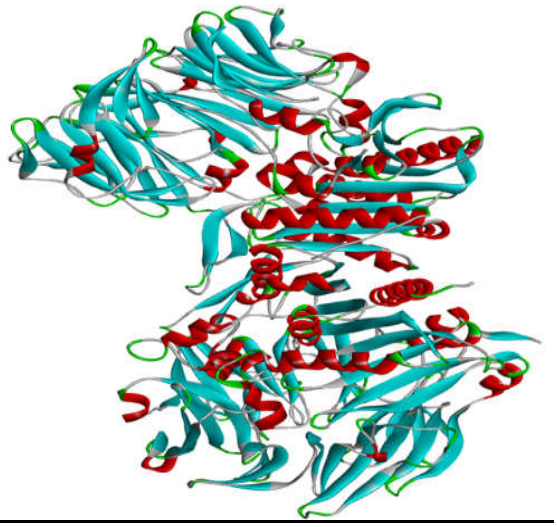
significance of molecular characteristics. This rule applies specially to oral drugs. Then, these drug similarity attributes have been evaluated using the Swiss ADME online open access database. Lipinski rule includes - Molar mass of the compound, Number of hydrogen bond acceptors, Number of hydrogen bond donors, Number of rotatable bonds, Molar refractivity, Topological Polar Surface Area (TPSA), Solubility, Lipinski's rule of five, and Bioavailability score [57].

Table 6. Optimization of target for in silico assessment.

Human CYP3A4 bound to metformin (PDB ID: 5G5J)	Human dipeptidyl peptidase-IV (PDB ID: 4A5S)
Organism: Homo sapiens	Organism: Homo sapiens
Resolution: Å	Resolution: Å
Method: X-RAY DIFFRACTION [58]	Method: X-RAY DIFFRACTION [59]
	

4.4. ADMET Properties

When developing a novel drug, it is essential to perform a comprehensive analysis of the pharmacokinetic properties of the compound [60]. The pkCSM program package (http://biosig.unimelb.edu.au/pkcsm/) is an online application that aids in the evaluation of physicochemical descriptors, ADME parameter prediction, pharmacokinetic characteristics, and toxicity aspects of any chemical compound [54]. In this study, the Water solubility Log S, Caco-2 Permeability, volume of distribution (VDss) of human, CYP450 1A2 Inhibitor, CYP450 3A4 Substrate, CYP450 3A4 Inhibitor, CYP450 2C9 Substrate, and Total Clearance (mL/min/kg) have been enumerated. Another important parameter, toxicity was assessed. This parameter included AMES (Salmonella/microsome mutagenicity assay) toxicity, maximum tolerable dose for humans (mg/kg/day), oral rat acute toxicity (LD_{50}), oral rat chronic toxicity (LOAEL), hepatotoxicity, and skin sensitization.

5. Conclusions

In this study, the mechanism underlying the antidiabetic effects of some natural compounds obtained from Lagenaria siceraria is confirmed by computational approaches such as molecular docking, ADMET and drug likeness. The findings revealed that some of the reported molecules have antidiabetics activity, which suggested that these compounds could be potential to manage diabetes. The overall study has been conducted based on the concept of in silico method. Overall, analysis has documented that these natural ligands are capable of establishing various H-bonds as well as hydrophobic and van der walls interactions and are strongly bonded with the target receptors. Finally, these compounds should, therefore, undergo extensive in vitro and in vivo tests in wet lab to establish them as potential drug candidates.

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