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Research Article

In-Silico Study of Zingiber Officinale for Its Anti-Diabetic Activity

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ABSTRACT

Today, diabetes is recognized as a prevalent and major disease condition worldwide. According to recent WHO studies, approximately 422 million people globally suffer from diabetes, with the majority residing in low- and middle-income countries, and diabetes directly causes 1.5 million deaths annually. The use of computational techniques in the drug discovery and development process is increasingly being implemented and appreciated. Terms such as computer-aided drug design (CADD) are commonly applied in this area. In-silico virtual screening, or high-throughput virtual screening (HTVS), has emerged as an excellent complement to the time-consuming and expensive experimental techniques of high-throughput screening. Diabetes mellitus is a group of diseases that influence how the body utilizes blood sugar. This type of diabetes predominantly affects middle-aged or older individuals and was previously referred to as adult-onset diabetes or diabetes mellitus. However, type 2 diabetes also affects children and teenagers, primarily due to childhood obesity. Zingiber officinale, commonly known as flowering plant, contains numerous chemical constituents with anti-diabetic properties. A study was conducted to employ in-silico methods for the discovery of potential anti-diabetic constituents derived from the hypoglycemic properties of Z. officinale. Tools such as Chemsketch, Avogadro, Discovery Studio Biovia, and Pyrx were utilized. Furthermore, molecular docking, drug likeness testing, ADME, and toxicity studies were conducted using SwissADME, Protox2, and Autodockvina programs. Visualization of 2D interactions was also carried out using Discovery Studio Biovia software.

INTRODUCTION

In today's date Diabetes is the major and most common disease condition prevailing in the world. According to the recent studies of who About 422 million people worldwide have diabetes, the majority living in low-and middle-income countries, and 1.5 million deaths are

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directly attributed to diabetes each year. Both the number of cases and the prevalence of diabetes have been steadily increasing over the past few decades. The coexistent drugs have most side effect and many of them have become less responsive to this multifactorial disorder.[1] Use of computational techniques in drug discovery and development process is rapidly gaining in popularity, implementation and appreciation. Different terms are being applied to this area, including computer-aided drug design (CADD), computer-aided molecular modelling (CAMM), rational drug design, *In-silico* drug design, computer-aided rational drug design.[2] Term Computer-Aided Drug Discovery and Development (CADDD) will be employed in this overview of the area to cover the entire process.[3]

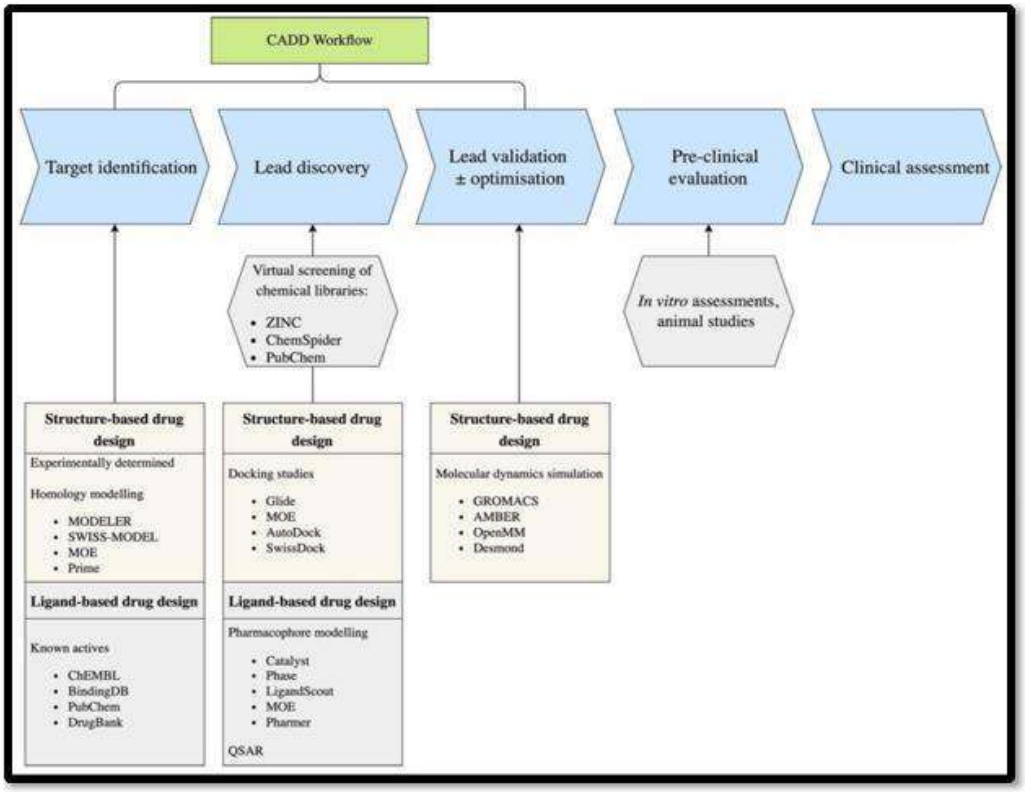


Fig No.1. Stages of Computer Aided Drug Design

Computer-Aided Drug Design (CADD) is an interdisciplinary field essential to modern drug discovery, integrating computational techniques with biological knowledge to identify and optimize drug candidates efficiently[4]. Its versatility stems from a range of methodologies, including molecular modelling and predicting drug metabolism. Adherence to Lipinski's rule within CADD ensures optimal oral drug likeliness by minimizing violations of criteria such as molecular weight and lipophilicity. This strategic integration accelerates and refines the drug discovery process, demonstrating CADD's impactful role across various fields and the pharmaceutical industry.[5] CADD can be classified into two general categories: structure-based and ligand-based. Structure-based CADD relies on the knowledge of the target protein structure to calculate interaction energies for all compounds tested, whereas ligand-based CADD exploits the knowledge of known active and inactive molecules through chemical similarity searches or construction of predictive, quantitative structure-activity relation (QSAR) models.[6] CADD is

capable of increasing the hit rate of novel drug compounds because it uses a much more targeted search than traditional HTS and combinatorial chemistry. It not only aims to explain the molecular basis of therapeutic activity but also to predict possible derivatives that would improve activity.[7]

In a drug discovery campaign, CADD is usually used for three major purposes:

1. Filter large compound libraries into smaller sets of predicted active compounds that can be tested experimentally;
2. Guide the optimization of lead compounds, whether to increase its affinity or optimize drug metabolism and pharmacokinetics (DMPK) properties including absorption, distribution, metabolism, excretion, and the potential for toxicity (ADMET);
3. Design novel compounds, either by "growing" starting molecules one functional group at a time or by piecing together fragments into novel chemotypes.[8]

The National Institute of Health, defines the term 'In-silico' as a modern word usually used to mean experimentation performed by computer and is related to the more commonly known biological terms in vivo and in vitro.9 In a more recent book, Danchin (2002) provides a quotation that offers a concise and cogent depiction of the potential of computational tools in chemistry, biology and pharmacology: 'Informatics is a real aid to discovery when analysing biological functions.10 In-silico virtual screening, or high-throughput virtual screening (HTVS), has yielded an excellent complement to the time-consuming and expensive experimental techniques of high-throughput screening.11 The ability to virtually screen compound libraries to improve enrichment of ligands progressed to experimental validation has provided countless lead compounds.12

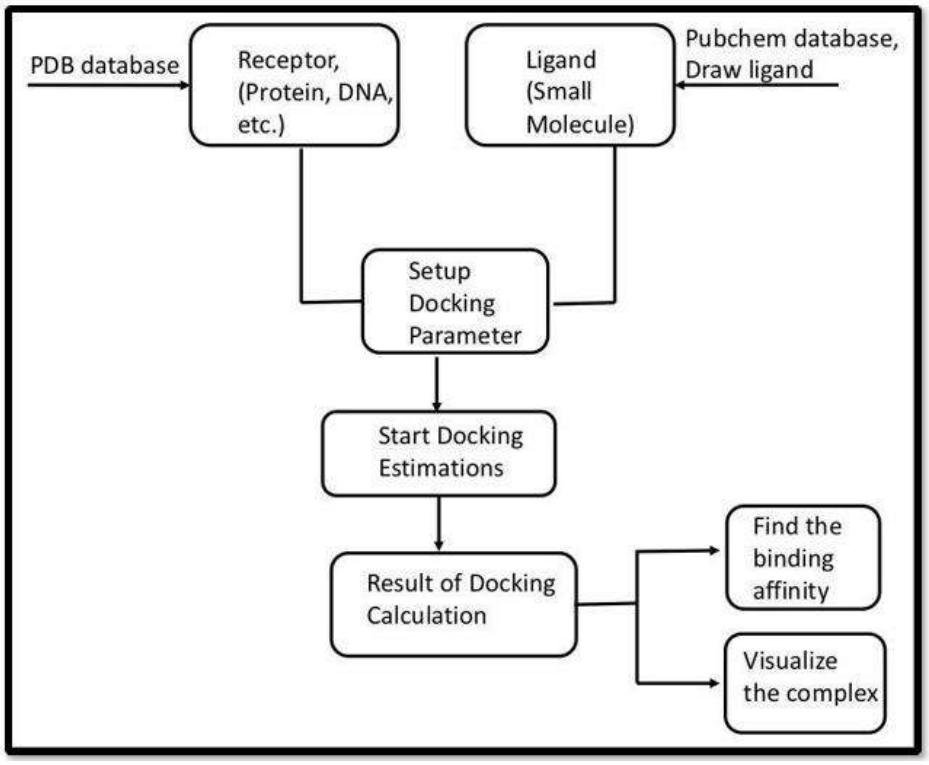


Fig No.2. Stages Of Molecular Docking

Molecular docking is a computational method developed to model these interactions at the molecular level by predicting the 3D structures of complexes.[13] Predicting the binding site through docking can help to unveil protein structure-function relationship and aid drug design in numerous ways.[14] focus on the fundamentals of protein docking by describing docking methods including search algorithm, scoring, and assessment steps as well as illustrating recent successful applications in drug discovery.[15] Especially address protein– small-molecule docking by comparatively analysing available tools implementing different approaches such as ab initio, structure-based, ligand-based (pharmacophore-/shape-based), information-driven, and machine learning approaches.[16] *Zingiber Ofiicinale* commonly known as passion fruit have many chemical constituents having anti-diabetic property.[17] This study was carried out to use *In-silico* method to discover best anti-diabetic constituents developed from hypoglycaemic properties of p edulis using tools like Chemsketch, Avogadro, Discovery studio Biovia, *Pyrx*. Along with molecular docking drug likeness test, ADME and toxicity study were performed using *Swiss ADME*, and Protox2 program respectively. Visualization of 2d-interaction is also carried out in this study using software 'discovery studio *biovia'.*

Diabetes

a. Definition

Diabetes mellitus refers to a group of diseases that affect how the body uses blood sugar (glucose). Glucose is an important source of energy for the cells that make up the muscles and tissues. It's also the brain's main source of fuel.[18] In the islets of Langerhans in the pancreas, there are two main subclasses of endocrine cells: insulin-producing beta cells and glucagon secreting alpha cells. Beta and alpha cells are continually changing their levels of hormone secretions based on the glucose environment. Without the balance between insulin and glucagon, the glucose levels become inappropriately skewed. In the case of DM, insulin is either absent and/or has impaired action (insulin resistance), and thus leads to hyperglycemia.[19]

b. Types of diabetes mellitus

There are a few different types of diabetes:

- Type 1 Diabetes
- Type 2 Diabetes

Type 1: This type is an auto immune disease in which your immune system attacks and destroys insulin-producing cells in your pancreas for unknown reasons. Up to 10% of people who have diabetes have Type 1. It's usually diagnosed in children and young adults, but it can develop at any age.20 Type 1 diabetes mellitus (T1D) is an autoimmune disease that leads to the destruction of insulin-producing pancreatic beta cells. Individuals with T1D require life-long insulin replacement with multiple daily insulin injections daily, insulin pump therapy, or the use of an automated insulin delivery system.21

Type 2: Type 2 diabetes occurs when your body becomes resistant to insulin, and sugar builds up in your blood. It's the most common type—about of people living with diabetes have type 2.22 People who are middle-aged or older are most likely to get this kind of diabetes. It used to be called adult-onset diabetes or diabetes mellitus. But type 2 diabetes also affects kids and teens, mainly because of childhood obesity.23

c. Symptoms24

Symptoms of both type one and type two are common and given below:

- Urinate a lot, often at night
- Are very thirsty
- Lose weight without trying
- Are very hungry
- Have blurry vision
- Feel very tired
- Have sores that heal slowly
- Have more infections than usual
- Have numb hands or feet

D. Risk Factors[25]

Risk factors responsible for the occurrence of diabetes are given below:

Type 1 Diabetes:

- Weak Immunity
- Family History Type 2 Diabetes:
- Lifestyle Factor
- Genes
- Family History
- Overweight (Obesity)
- Are Physically Less Active
- Insulin Resistance
- Impaired Glucose Tolerance

Literature Review:

A) Al-Snafi, A. E, (2024) : Zingiber officinale (Ginger) a genus classified within the family "Zingiberaceae". This family is known because of its high content of dietary and biochemical components, especially the impacts against diabetes mellitus and inflammation. Ginger, perform the antihyperglycemic activity by causing restoration for β-cells in pancreas, providing protection for the morphology of β-cells, amelioration of the pathological injuries of kidneys, control of the concentration of pro-inflammatory cytokines, regulation of hepatic gene expression of enzymes associated with glucose metabolism, inhibition oxygen free radicles caused during glucose metabolism, translocation of GLUT4, inhibition of liver glucose production, stimulation of pancreatic insulin release, inhibition of carbohydrate metabolizing enzymes, high production of hepatic glycogen through the improvement of glycogen synthesis in the liver, and decreasing insulin resistance, and peripheral consumption of blood sugar as well as its antioxidant activity.

B) Zhijun Yang, (2023): Ginger (Zingiber officinale) belongs to the Zingiberaceae family of flowering plants, which originated in South-East Asia and has long been a popular culinary ingredient. It has been demonstrated that ginger contains a variety of nutrients, such as carbohydrates, proteins, lipids, minerals and vitamins, as well as healthpromoting phytochemicals, mainly phenolic compounds and ginger essential oil. In addition, ginger has excellent antioxidant, antibacterial, and many other biological activities. Therefore, ginger has potential applications in functional food development, aquatic products and meat preservation, and food packaging. However, the full utilization of ginger's benefits and its incorporation into food products remain largely unexplored. Therefore, this review provides a comprehensive summary of the nutritional components, phytochemical compositions, biological properties, and potential applications of ginger in the food industry.28

C) Dos Santos FAR, et al (2022) : This author said that, the antidiabetic, antiglycation, and antioxidant potentials of ethanolic extract of seeds of Brazilian Zingiber Ofiicinale fruits (PESE), a major by-product of the juice industry, and piceatannol (PIC), one of the main phytochemicals of PESE. several biological properties of passion fruits have been demonstrated using in vitro and in vivo studies, such as antioxidant, analgesic,

antidepressant, sedative and anxiolytic-like, anti-inflammatory, antimicrobial, antihypertensive, hepatoprotective, and antidiabetic (peel, juice, and seed) activities. The in vitro evidence of the inhibition of alpha-amylase, alpha-glucosidase, and DPP-4 enzymes as well as antioxidant and antiglycation activities, warrants further investigation of the antidiabetic potential of Z.officinale seeds and PIC.17

D) Antoine Daina A, et al (2017) : To be effective as a drug, a potent molecule must reach its target in the body in sufficient concentration, and stay there in a bioactive form long enough for the expected biologic events to occur. Drug development involves assessment of absorption, distribution, metabolism and excretion (ADME) increasingly earlier in the discovery process, at a stage when considered compounds are numerous but access to the physical samples is limited. In that context, computer models constitute valid alternatives to experiments. Here, we present the new SwissADME web tool that gives free access to a pool of fast yet robust predictive models for physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry friendliness, among which inhouse proficient methods such as the, iLOGP and Bioavailability Radar. Easy efficient input and interpretation are ensured thanks to a user-friendly interface through the loginfree website http://www.swissadme.ch. Specialists, but also nonexpert in cheminformatics or computational chemistry can predict rapidly key parameters for a collection of molecules to support their drug discovery endeavours.

E) Akram T Kharroubi, et al. (2015): This author said that, the prevalence of diabetes mellitus in diverse regions is examined, highlighting its epidemic nature. The Middle East and North Africa stand out with the highest diabetes prevalence in adults while the Western Pacific region leads in the sheer number of diagnosed adults and hosts countries with the highest diabetes prevalence. This comprehensive review delves into various diabetes classes—type 1, type 2, gestational diabetes, and other types—comparing them based on diagnostic criteria, aetiology, and genetics. Recent years have witnessed a surge in molecular genetics exploration, with a focus on mutations and single nucleotide polymorphisms in genes associated with glucose metabolism, pancreatic cell development, control, and function.27

F) V.K. Vyas, et al. (2012) : This author said that, in order to understand the binding specificity of the ligand with protein the knowledge of 3D structure is very important. With increase in the known protein structure, and the modelling software, homology modelling is rapidly becoming the method of choice for obtaining 3D coordinates of protein. Homology modelling is a representation of the similarity of environmental residues at topologically corresponding positions in the reference proteins. In the absence of experimental data, model building on the basis of a known 3D structure of a homologous protein is at present the only reliable method to obtain the structural information. Knowledge of the 3D structures of proteins provides invaluable insights into the molecular basis of their functions.29

G) Xuan-Yu, et al. (June 2011) : This author said that, molecular docking plays a growing role in drug discovery. the overview investigates into various molecular docking methods, examining their evolution and usage in drug discovery. It outlines essential theories, about sampling processes and scoring functions. The discourse extends to the distinctions and effectiveness of existing docking software. Notably, accommodating flexibility in receptor structures, particularly incorporating backbone flexibility,

poses a hurdle for current docking methods. A promising resolve surfaces in the form of the recently introduced Local Move Monte Carlo (LMMC) approach, tailored to address challenges in flexible receptor docking.26

H) I. M. Kapetanovic (30 January 2008) : The author highlighted the increasing use of computational power in drug discovery to expedite hit identification, streamline development processes, optimize drug properties, and address safety concerns. Term Computer-Aided Drug Discovery and Development (CADDD) will be employed in this overview of the area to cover the entire process. Computational strategies include ligandbased and structure-based drug design, along with quantitative structure-activity relationships. Regulatory agencies and the pharmaceutical industry are collaborating to advance computational tools, aiming to improve efficiency, reduce animal testing, and enhance predictability in drug development. The trajectory suggests ongoing growth in Computer-Aided Drug Discovery and Development (CADDD) as technology progresses.2

Aim and objective

The Aim of this research work is to perform *In-silico* study on phytoconstituent of *Zingiber officinale* for antidiabetic activity and compare its results with standard drug (Metformin).

Objective of this study:

Evaluation of phytoconstituent on basis of ADMET study for selection of best suited constituent

- To Perform Homology modelling to modify protein
- To Perform Molecular docking on selected constituents
- To Study 2D- interaction between ligand and modified protein
- To Compare the constituent with the standard drug

Plan of Work

- Evaluation of Phytoconstituents on Basis of Drug likeness, ADME and Toxicity
- Preparation of Ligand
- Preparation of Receptor
- Molecular Docking
- Visualization Study
- Results and Discussion
- Conclusion

MATERIALS AND METHODS

Ginger (Zingiber officinale Roscoe), which belongs to the Zingiberaceae family and the Zingiber genus, has been commonly consumed as a spice and an herbal medicine for a long tim. Ginger root is used to attenuate and treat several common diseases, such as headaches, colds, nausea, and emesis. Many bioactive compounds in ginger have been identified, such as phenolic and terpene compounds. The phenolic compounds are mainly gingerols, shogaols, and paradols, which account for the various bioactivities of ginger. In recent years, ginger has been found to possess biological activities, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activities.30 In addition, accumulating studies have demonstrated that ginger possesses the potential to prevent and manage several diseases, such as neurodegenerative diseases, cardiovascular diseases, obesity, diabetes mellitus, chemotherapy-induced nausea and emesis, and respiratory disorders. In this review, we focus on the bioactive compounds and bioactivities of ginger, and we pay special attention to its mechanisms of action.31

Taxonomical Name*

Kingdom: Plantae
Family: Zingiberaceae
Division: Magnoliophyta
Class: Liliopsida
Order: Ingiberales
Genus: *Zingiber*
Common Name: Ginger[32]

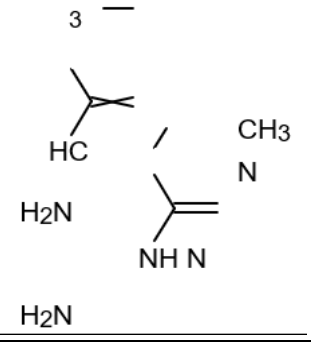
Fig No.3. *Zingiber Ofiicinale* Plant with Flower and Fruit

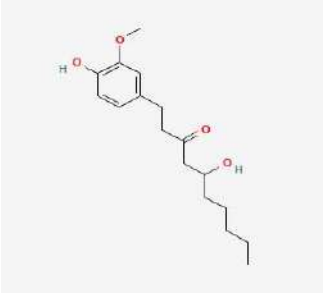
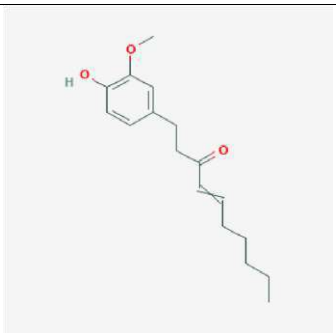
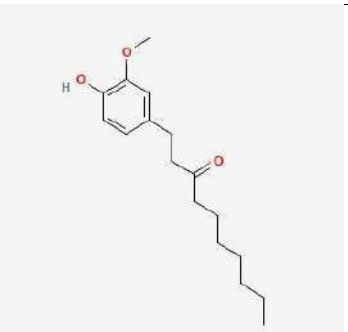
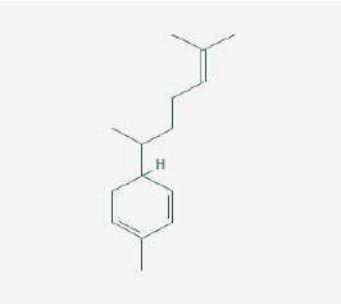
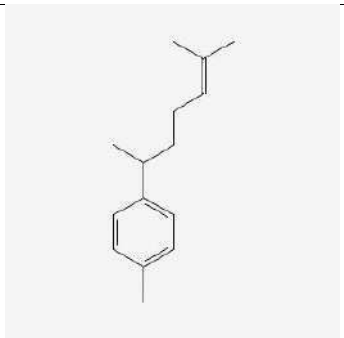
Diabetes mellitus is known as a severe metabolic disorder caused by insulin deficiency and/or insulin resistance, resulting in an abnormal increase in blood glucose. Prolonged hyperglycemia could accelerate protein glycation and the formation of advanced glycation end products (AGEs). Many research works have evaluated the antidiabetic effect of ginger and its major active constituents.[33] Additionally, 6-gingerol reduced the levels of plasma glucose and insulin in mice with high-fat diet-induced obesity. Nε-carboxymethyl-lysine (CML), a marker of AGEs, was decreased by 6-gingerol through Nrf2 activation.[34] In 3T3-L1 adipocytes and C2C12 myotubes, 6-paradol and 6-shogaol promoted glucose utilization by increasing AMPK phosphorylation. In addition, in a mouse model fed a high-fat diet, 6-paradol significantly reduced the level of blood glucose. In another study, 6-gingerol facilitated glucose-stimulated insulin secretion and ameliorated glucose tolerance in type 2 diabetic mice by increasing glucagon-like peptide 1 (GLP-1). Besides, 6-gingerol treatment activated glycogen synthase 1 and increased cell membrane presentation of glucose transporter type 4 (GLUT4), which increased glycogen storage in skeletal muscle. Furthermore, the consumption of ginger could reduce the levels of fasting plasma glucose, glycated hemoglobin A (HbA1C), insulin, TG, and TC in patients with type 2 diabetes mellitus (DM2). Moreover, ginger extract treatment improved insulin sensitivity in rats with metabolic syndrome, which might have been relevant to the energy metabolism improvement induced by 6-gingerol.[35]

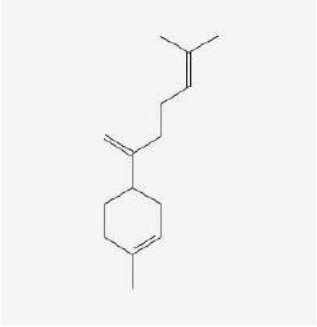
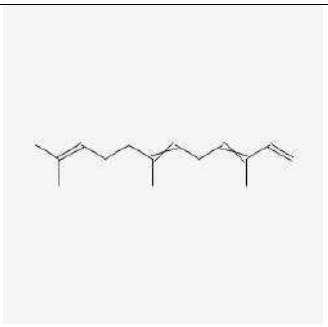
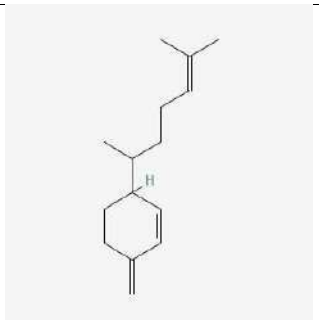
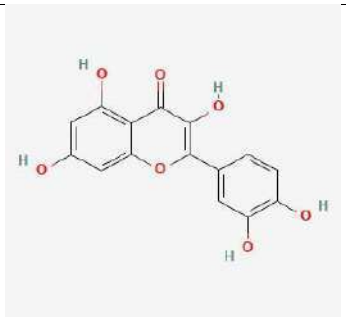
a. Chemical Constituents

9 constituents were selected from the list of 110 chemical constituent present in *Zingiber officinale.*

Table.1. Name Of Chemical Constituents Along with Their Canonical Smiles and Structures

Sr. No.	Compound	Canonical Smiles	Structures
A.	**Metformin (Standard)**	**Cn(C)C(=N) N=C(N)N**	

B.	6-Gingerol	Cccccc(Cc(=O)Ccc1=CC(=C(C=C1)O)Oc)O	
C.	6-Shogaol	Cccccc=Cc(=O)Ccc1=Cc(=C(C=C1)O)Oc	
D.	6-Paradol	Ccccccccc(=O)Ccc1=CC(=C(C=C1)O)Oc	
E.	Zingiberene	Cc1=Ccc(C=C1)C(C)Ccc=C(C)C	
F.	Acurcumene	Cc1=Cc=C(C=C1)C(C)CcC=C(C)C	

G.	Beta Bisabolene	Cc1=Ccc(Cc1)C(=C)Ccc=C(C)C	
H.	A-Farnesene	Cc(=Cccc(=Ccc=C(C)C=C)C)C	
I.	B-Sesquiphella Ndrene	Cc(Ccc=C(C)C)C1ccc(=C)C=C1	
J.	Quercetin	C1=Cc(=C(C=C1c2=C(C(=O)C3=C(C=C(C=C3o2)O)O)O)O)O	

Smiles of all the constituent were recovered using PubChem website. and the structure were drawn using Chemsketch

Software and Websites

List of software and websites that are used in study is given below in Table.2:

Table.2. List And Information of Software Used

Sr No.	Software	Websites	Application
1	*Chemsketch*	ACD labs	To create and modify images of chemical structures

2	*Avogadro*	Soft tonic	A molecule editor and visualizer designed for cross platform use and converting files into mol file
3	*Pyrx*	Source fordge	Virtual Screening software for Computational Drug Discovery and molecular docking
4	*Biovia*	Dassault system	Visualization, simulation and pharmacophore mapping, etc.
5	*Pubchem*	National institute of health	Retrieve data related to any compound
6	*Swiss ADME*	Molecular modelling group of the SIB (Swiss Institute of Bioinformatics).	Drug likeness and ADME study
7	*Protox 2*	U.S. Environmental Protection Agency	Study the Toxicity data
8	*PDB*	RCSB	Retrieval of 3D-structure of protein
9	*SWISS model*	Swiss institute of bioinformatics	Homology modelling of the required protein
10	*NCBI national library of medicine (gene)*	National institute of health	Retrieval of gene sequence

Experimental Work

a. Evaluation of Ligand on basis of Drug Likeness, ADME and Toxicity study i. Drug Likeness study

Drug-likeness test of phytochemicals based on the Lipinski rule using *SwissADME*[36] software.[37]

The Lipinski rule of five state that:

- Molecular weight less than equal to 500
- Log p should be less than 5
- H bond donor and acceptor should be less than equal to 5
- TPSA should be less than 140 angstroms
- No of rotatable bonds should be less than 10

To perform *In-silico* studies, of the 20 phytoconstituents retrieved from *Zingiber Ofiicinale* should satisfy drug likeness test (determines whether particular compound could be used as drug).

ADME Study

ADME Study is carried out using the software *Swissadme*. It is the quick, accurate and easy to use software used for absorption, distribution, metabolism, and excretion prediction. It predicts pharmaceutically applicable properties of organic molecules individually, or in groups.[38] Where GI Absorption states the absorption of drug, BBB Permeation states the distribution of drug, CYP1A2 inhibitor states the protein binding, and bioavailability states the excretion of drug. All other descriptors are also stated on the software.

Toxicity study

Toxicity study is performed to predict the possible toxicities present in the compounds based on their similarity with other compounds. For the assessment of toxicity *Protox 2*[39] software is used this software screens the compound on various toxicities such as organ toxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity etc. and give the results with accuracy of 99% individually. It also provides predicted toxicity score and predicted lethal dose (LD50).[40]

Preparation of ligand:

After the ADMET study, 10 constituents were selected from a large number of phytoconstituents present in passion fruit. Smiles of the phytoconstituents were retrieved from *PubChem*.[41] The 2D structure of the constituents were sketched on *Chemsketch*[42] with the help of smiles. The structures were saved in the working folder as a mol file Using control all ligands were loaded into the *Avogadro*[43] software. And, finally converting them into the PDB format and saving them in the working folder.[44]

Preparation of receptor:

Firstly, gene sequence of the required protein was retrieved from the website [NCBI national library of medicine (gene)][45] which was further used as the template for modelling of the required protein. After that gene sequence was inputted in the required targeted sequence and then validate the provided gene sequence after that search for template and select the best suited template. Swiss Model[46] is used for homology modelling. The template selected is the enzyme with PDB id 6B1U, 6B1U is the AMPK (AMswPactivated protein kinase) enzyme and build the model whose 3d structure is available on pdb[47]. The AMP-activated protein kinase (AMPK) αβγ heterotrimer regulates cellular energy homeostasis with tissue-specific isoform distribution. After the protein model is build that file of protein is opened in the discovery studio biovia[48] and the file is converted and saved in the working folder as mol file.[49]

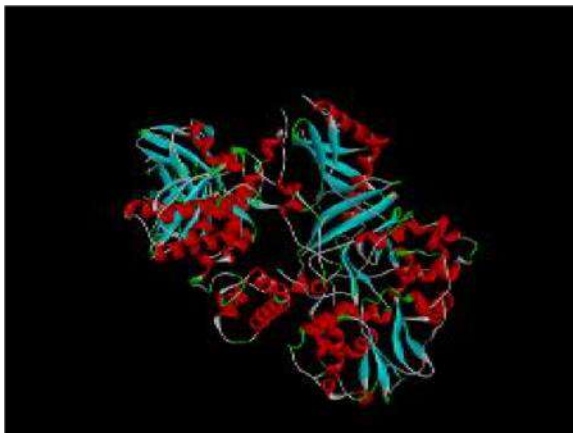


Fig No.5: 3D-structure of AMPK enzyme(6BIU)

Molecular Docking

Pyrx[50] was used to identify binding energy between ligand and macromolecule. *Pyrx* is the software that uses autodock vina as a default software for molecular docking. As per the inverse relation of energy and stability, the conformation with greater binding energy is less stable. The Default parameters of the software program have been applied similar to the protocol followed elsewhere. The grid box can generally be minimised or maximised as per need and presence of active pocket but in this research the grid box set on default. As the active pocket was screened Briefly, Lamarckian Genetic Algorithm (LGA) with default atomic salvation parameters 126 Å (x, y, and z) grid box in ratio of (60:60:60) for scoring energy was set at coordinates as X =33.442; Y = 5.8935 and Z = -48.4118 with 0.375 angstroms grid points spacing as of centre.[51]

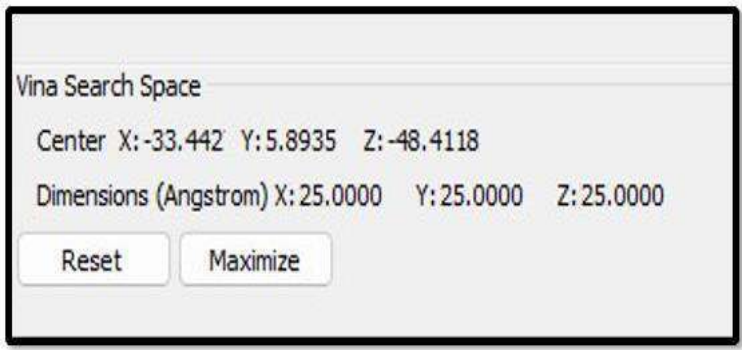


Fig No.6. Dimensions of Grid Box for Molecular Docking

e. Visualization Study

2d-interaction was studied using *Discovery Studio Biovia* software. 2d-interaction states the number of amino acids present, types of bond present between macromolecule and ligand, etc. The out files of the ligands were retrieved from the file extensions of the software. After the out files are retrieved the .PDB file of the macromolecule is opened in the *biovia* software along with all the out files. Then the macromolecule is copied and pasted on the out file of ligand and 2d-interaction was studied.[52]

7. RESULTS AND DISCUSSION

a. Drug Likeness and ADME Study:

Drug Likeness Study: The results of Drug Likeness study given in table.3.

Table.3. Drug Likeness Study Results

Sr No	Constituent Name	Molecular Weight (G/Mol)	Rotatable Bonds	H Bond Acceptor	H Bond Donor	Log P	Follows Lipinski	No Of Violations
1	6-gingerol	294.39 g/mol	10	4	2	3.48	Yes	0
2	6-shogaol	276.37 g/mol	9	3	1	3.28	Yes	0
3	Zingiberene	204.35 g/mol	4	0	0	3.65	Yes	0
4	6-paradol	278.39 g/mol	10	3	1	3.65	Yes	0
5	α-curcumene	202.34 g/mol	4	0	0	3.50	Yes	1
6	Beta bisabolene	204.35 g/mol	4	0	0	3.67	Yes	1
7	α-farnesene	204.36 g/mol	6	0	0	3.89	Yes	1
8	β-sesquiphellandrene	204.35 g/mol	4	0	0	3.58	Yes	1
9	Quercetin	302.24 g/mol	1	7	5	1.63	Yes	0
10	Metformin	129.16 g/mol	2	2	3	0.34	Yes	0

Discussion Based on Drug Likeness Study:

Based on the drug likeness study it is observed that most of the constituents of *Zingiber Officinale* follows Lipinski rule. As it is important for all the constituents to follow Lipinski rule, for its formulation or synthesis. All the other constituents follow Lipinski rule except for certain violations. The constituents having one violation are namely, alpha Curcumene, beta Bisabolene, alpha sisquiphellandrene, farnesene.

II. ADME Study: Results of ADME study are given below in Table.4:

Table.4. ADME Study Result

Sr. No	Constituents Name	GI Absorption	BBB Permeant	CYP1A2 Inhibitor	Bioavailability Score
1.	6-gingerol	High	Yes	Yes	0.55
2.	6-shogaol	High	Yes	Yes	0.55
3.	6-paradol	High	Yes	Yes	0.55
4.	Zingiberene	Low	No	No	0.55
5.	α-curcumene	Low	No	No	0.55
6.	Beta bisabolene	Low	No	No	0.55
7.	α-farnesene	Low	No	Yes	0.55
8.	β-sesquiphellandrene	Low	No	Yes	0.55
9.	Quercetin	High	No	Yes	0.55
10.	Metformin	High	No	No	0.55

Discussion based on ADME Study:

Based on the results of ADME study it is observed that most of the constituents of *Zingiber Ofiicinale* have significant with average bioavailability of 0.55 and as most of the constituents have higher absorption but some of them have lower absorption. Where as certain constituents also cross BBB permeability.

b. Toxicity study: The Results of toxicity study is given below in Table.5:

Table.5. Results of Toxicity Study

Sr. No.	Constituents name	Predicted Toxicity Score	Predicted LD50 (mg/kg)
1	6- gingerol	3	250
2	6-shogaol	4	687
3	6-paradol	5	2580
4	Zingiberene	4	1680
5	α-curcumene	4	2000
6	Quercetin	3	159

7	Beta bisabolene	4	4400
8	Metformin	4	680
9	β-sesquiphellandrene	5	5000
10	α-farnesene	5	3650

Discussion based on toxicity study:

Based on the results of toxicity study most of the drugs shows class, 4 and 5 toxicity which are safe classes but some of the constituents show toxicity of class 3 toxicity which is not suitable for administration as a formulation or as drug, because instead of giving expected results they are producing their own toxicity and are harmful for human body. As per the combined results of drug likeness, ADME and toxicity study out of all the 9 constituents selected of *Zingiber Ofiicinale* all constituent follow Lipinski rule and some constituents have higher amount of toxicity present in them. After the drug likeness, ADME and toxicity study the phytoconstituents competent are given in table 6:

Table.6. List of Chemical Constituents

Sr No.	Name of constituents	Molecular formula
1	6-gingerol	C17H26O4
2	6-shogaol	C17H24O3
3	6-paradol	C17H26O3
4	zingiberene	C15H24
5	α-curcumene	C15H22
6	Beta bisabolene	C15H24
7	α-farnesene	C15H24
8	β-sesquiphellandrene	C15H24
9	quercetin	C15H10O7
10	metformin	C4H11N5

Molecular Docking: Results of molecular docking are stated below:

Table.7. Results of Molecular Docking

Sr No	Chemical Constituent	Binding affinity	rmsd/ub	rmsd/lb
1	6-gingerol	-4.8	0	0
2	6-shogaol	-4.4	0	0
3	6-paradol	-4.1	0	0
4	Zingiberene	-4.6	0	0
5	α-curcumene	-4.6	0	0
6	Beta bisabolene	-4.5	0	0
7	α-farnesene	-4.4	0	0
8	βsesquiphellandrene	-4.6	0	0
9	Quercetin	-5.7	0	0
10	Metformin	-4.3	0	0

Discussion Based on Molecular Docking:

The docking of the macromolecule modelled AMPK enzyme with chemical constituents of *Zingiber Ofiicinale* has been done. The table 7 shows the Binding Energy of 10 compounds including the standard. *In-silico* studies revealed that all the synthesized molecules show good binding affinity toward the target protein ranging from -5.7- -4.1.

d. Visualization Study

The active pocket was considered to be the site where modelled 6B1U complexes with active chemical constituents of *Zingiber Ofiicinale*. The 2d-interaction

is generally based on the binding between ligand and macromolecule. and the bonds between ligand and macromolecule.

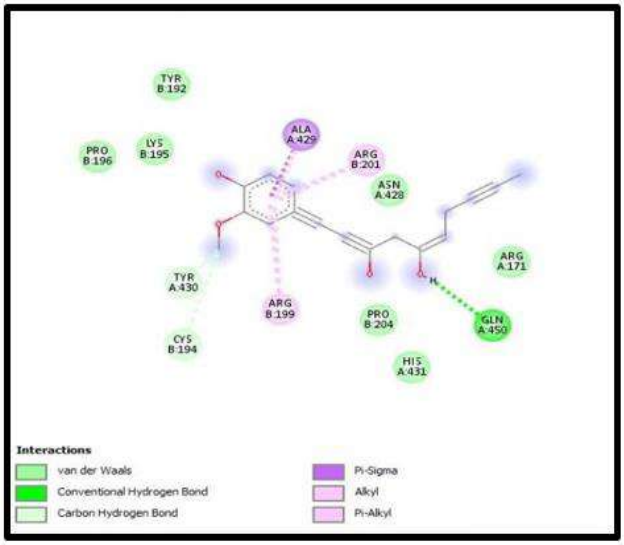


Fig No.7. 2D-interaction between Gingerol and 6B1U

The active pocket I (gingerol and 6B1U) consisted of 13 amino acids. Whereas the amino acids are attached to ligand molecules using Vander Waals force, conventional hydrogen bond, Pi-sigma, Pi-Alkyl, Alkyl, carbon hydrogen bond. As shown in fig No.7.

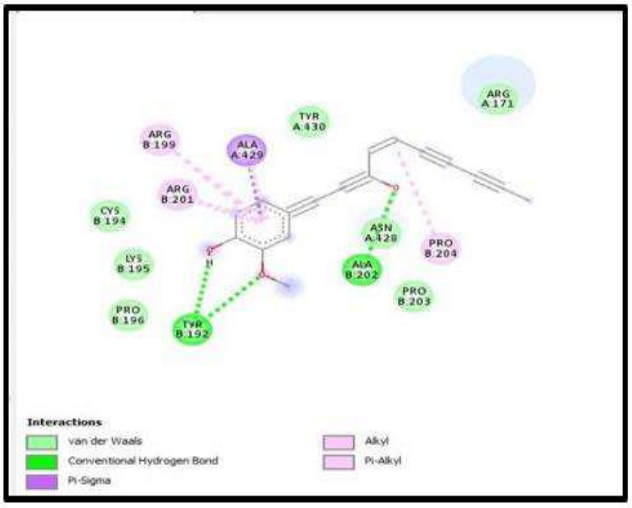


Fig No.8. 2D-interaction between Shagaol and 6B1U

As shown in fig No.8. The active pocket II (Shagaol and 6B1U) consisted of 13 amino acids. Whereas the amino acids are attached to ligand molecules using Vander Waals force, conventional hydrogen bond, pi-sigma, pi-alkyl and Alkyl bonds.

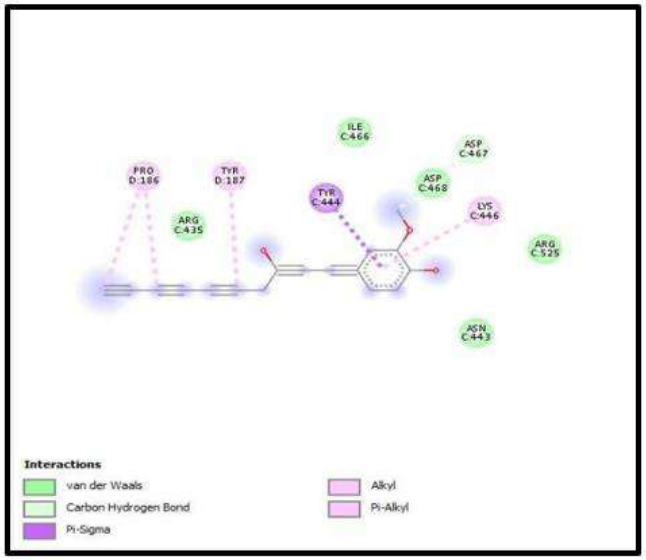


Fig No.9. 2D-interaction between Paradol and 6B1U

The active pocket III (Paradol and 6B1U) consisted of 10 amino acids. Whereas the amino acids are attached to ligand molecules using Vander Waals force, Pi-Alkyl bonds, PiSigma, conventional hydrogen bond, alkyl bonds. As shown in fig No.9.

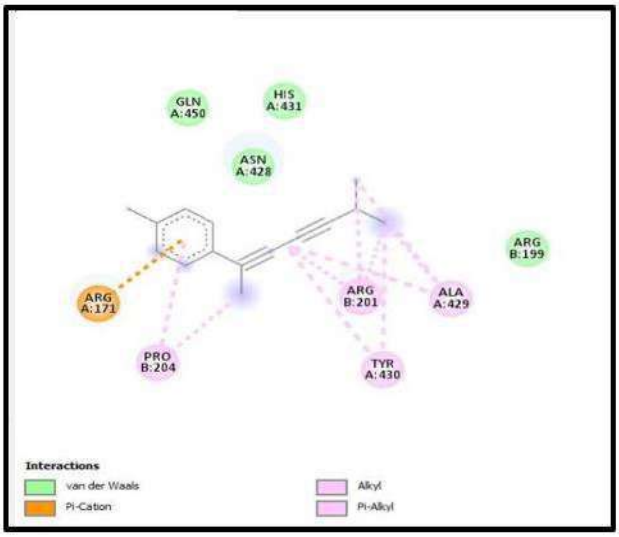


Fig No.10. 2D-interaction between α-curcumene and 6B1U

The active pocket IV (curcumene and 6B1U) consisted of 9 amino acids. Whereas the amino acids are attached to ligand molecules using Vander Waals force, Pi-cation, Pi-alkyl, alkyl bond. As shown in fig No.10.

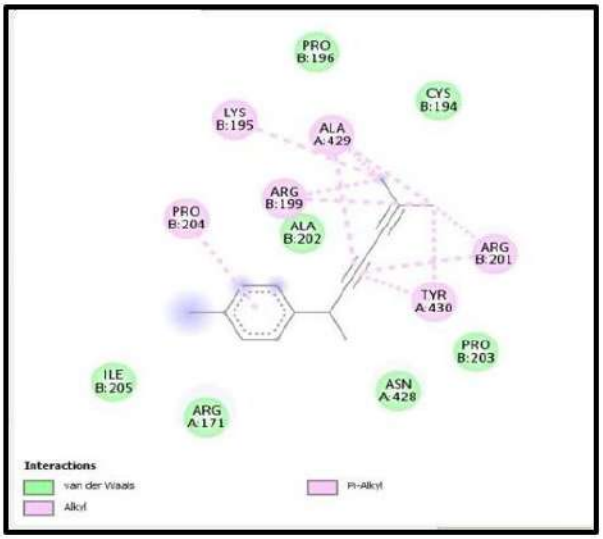


Fig No.11. 2D-interaction between Zingiberene and 6B1U

The active pocket V (zingiberene and 6B1U) consisted of 13 amino acids. Whereas the amino acids are attached to ligand molecules using Vander Waals force, Pi-alkyl, alkyl bonds. As shown in fig No. 11.

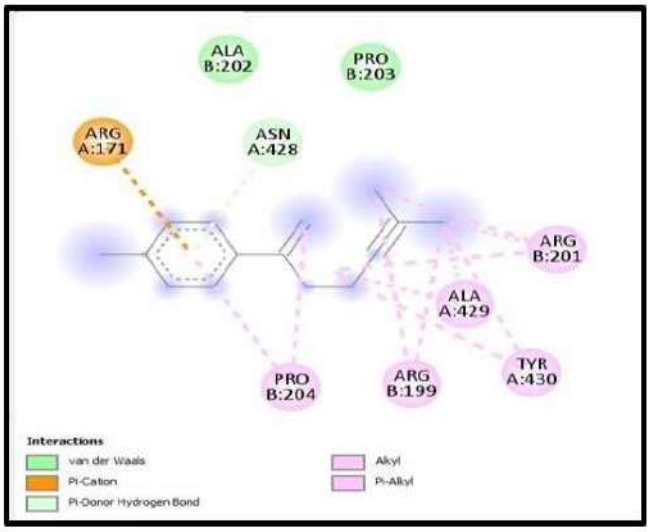


Fig No.12. 2D-interaction between Beta bisabolene and 6B1U

The active pocket VI (beta bisabolene and 6B1U) consisted of 9 amino acids. Whereas the amino acids are attached to ligand molecules using Vander Waals force, Pi-cation, alkyl, pialkyl, pi-donor hydrogen bond. As shown in fig No.12.

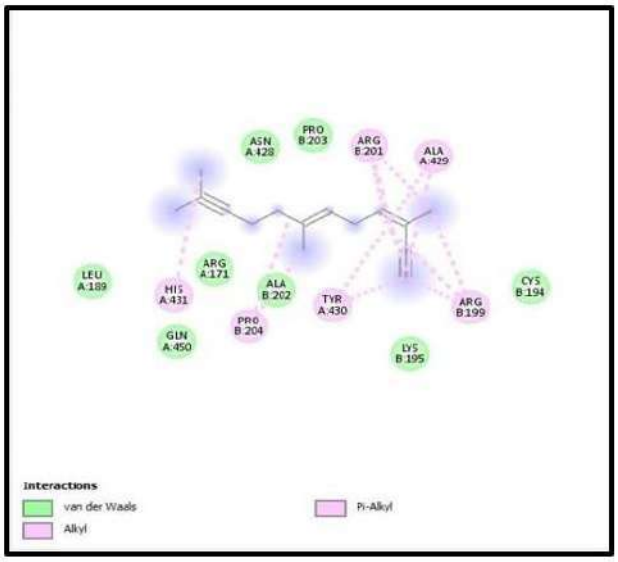


Fig No.13. 2D-Interaction Between A-Farnesene And 6B1U

The active pocket VII (Farnesene and 6B1U) consisted of 14 amino acids. Whereas the amino acids are attached to ligand molecules using Vander Waals force, Pi-Alkyl, Alkyl. As shown in fig No. 13.

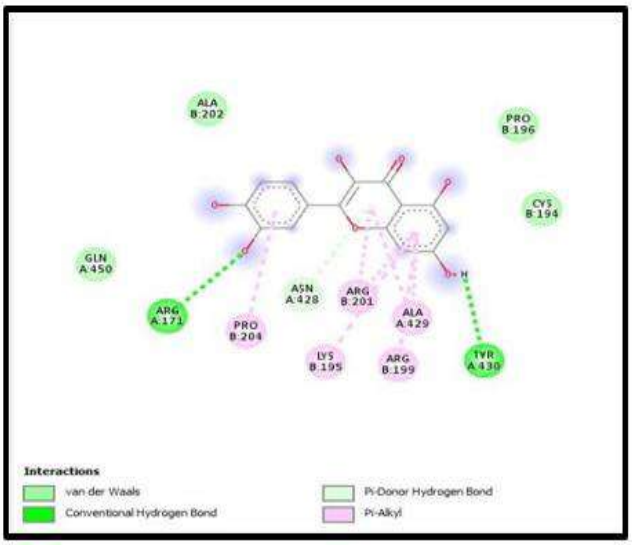


Fig No.14. 2D-Interaction Between Quercetin And 6B1U

The active pocket VII (quercetin and 6B1U) consisted of 12 amino acids. Whereas the amino acids are attached to ligand molecules using Vander Waals force, Pi-Alkyl, pi-donor hydrogen bond, conventional hydrogen bond. As shown in fig No.14.

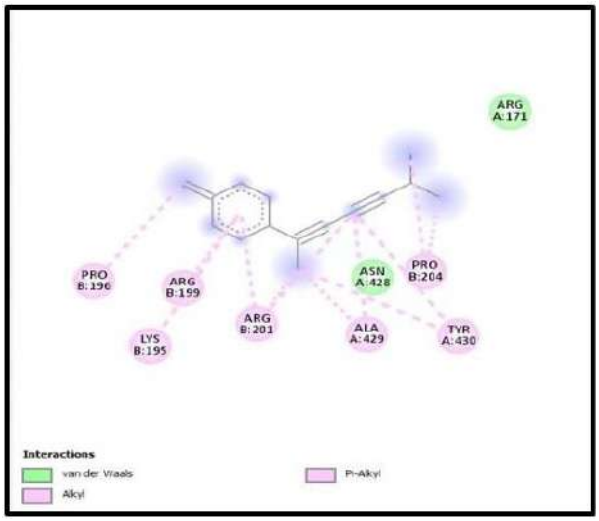


Fig No.15. 2D-Interaction Between B-Sesquiphellandrene And 6B1U

The active pocket VII (sesquiphellandrenel and 6B1U) consisted of 9 amino acids. Whereas the amino acids are attached to ligand molecules using Vander Waals force, Pi-Alkyl, alkyl. As shown in fig No. 15.

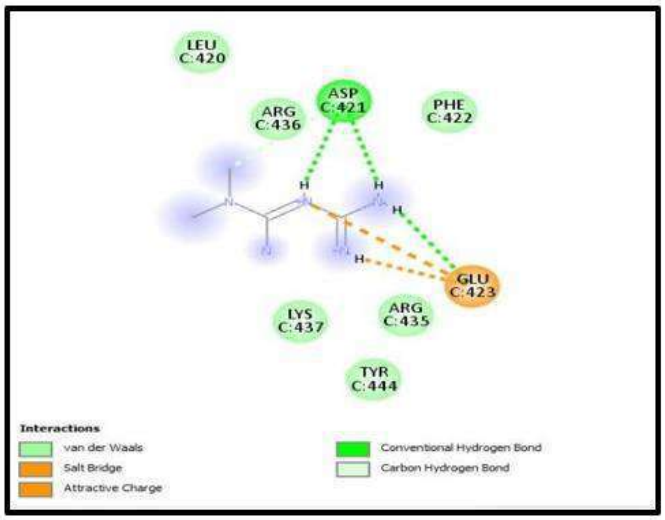


Fig No.16. 2D-Interaction Between Metformin And 6B1U

The active pocket VII (metformin and 6B1U) consisted of 8 amino acids. Whereas the amino acids are attached to ligand molecules using Vander Waals force, conventional hydrogen bond, salt bridge, carbon hydrogen bond, attractive charge. As shown in fig No. 16.

Discussion based on 2d-interaction

The docking of modelled receptor with all the constituents exhibits well established bonds with one or more amino acids in the receptor active pocket. Fig No 7-16 – shows the docked 2D images of all the ligands (Constituents of *Zingiber Ofiicinale*) including the standard drug i.e., Metformin.

CONCLUSION:

The molecular docking studies were conducted for the chemical constituents of *Zingiber Ofiicinale* on the receptor (AMPK enzyme; PDB ID: 6BIU).

with 6BIU serving as the template macromolecule. The *In-silico* study revealed that the constituent exhibit good binding affinity toward the target protein, Quercetin demonstrated the highest binding affinity, while gingerol and shagaol, has anti-diabetic activity according to the literature review. Whereas the constituents named above have higher binding affinity towards the protein compared to the standard drug Metformin. The ligands underwent pre-screening for their Drug Likeness and ADMET properties, and the most suitable ligands were selected for further study. 2D interactions were analysed for all 10 ligands along with standard drug (Metformin). The study revealed that the interaction of gingerol is similar to Metformin, binding with amino acids such as ARG A:263, GLU A:194, ALA A:191, and others. Quercetin, with higher binding affinity, interacts with amino acids like PRO A:193, HIS A:131, and others. It is evident from these interactions that Quercetin has greater binding with the AMPK enzyme, potentially enhancing its activity as an antidiabetic agent. Quercetin also shows potential as an anti-diabetic compound due to its high receptor affinity. Thus, this study expands the potential application of gingerol as a promising drug for diabetes, while further research could uncover the anti-diabetic properties of quercetin.

REFERENCES

1. WHO, (https://www.who.int/news-room/fact-sheets/detail/diabetes), accessed on dated,15, Feb 2024
2. Kapetanovic IM. “Computer-aided drug discovery and development (CADDD): Insilico-chemico-biological approach.” Chem Biol Interact. 2008 Jan 30;171(2):165-76.
3. Niazi SK, Mariam Z. “Computer-Aided Drug Design and Drug Discovery: A Prospective Analysis.” Pharmaceuticals. 2024; 17(1):22.
4. Souza HCA, et al. “Molecular Docking and ADME-TOX Profiling of Moringa oleifera Constituents against SARS-CoV-2.” Adv Respir Med. 2023 Oct 27;91(6):464-485.
5. Walters WP. “Going further than Lipinski's rule in drug design.” Expert Opin Drug Discov. 2012 Feb;7(2):99-107.
6. Javed MR. “CADD and Molecular Dynamic Simulations: Potential Impacts to Conventional Medicines.” Comb Chem High Throughput Screen. 2022;25(4):658659.
7. Zhao L, et al. “Advancing computer-aided drug discovery (CADD) by big data and data-driven machine learning modeling.” Drug Discov Today. 2020 Sep;25(9):1624-1638.
8. Ece A. “Computer-aided drug design.” BMC Chem. 2023 Mar 24;17(1):26.
9. Ekins S, et al. “In-silico pharmacology for drug discovery: methods for virtual ligand screening and profiling.” Br J Pharmacol. 2007 Sep;152(1):9-20.
10. Sliwoski G, et al. “Computational methods in drug discovery.” Pharmacol Rev. 2013 Dec 31;66(1):334-95.
11. Radwa E. et al. “Using filters in virtual screening: A comprehensive guide to minimize errors and maximize efficiency.” AR in medchem, Volume 59. 2022; 99-136
12. Michael Berry, et al. “Practical Considerations in Virtual Screening and Molecular Docking.” ET in Comput Bio, 2015; 487-502.
13. Ferreira LG, et al. “Molecular docking and structure-based drug design strategies. Molecules.” Mol dock: r advan. 2015 Jul 22;20(7):13384-421.
14. Lohning AE, et al. “A Practical Guide to Molecular Docking and Homology Modelling for Medicinal Chemists.” Curr Top Med Chem. 2017;17(18):2023-2040.

15. Yu W, et al. "Computer-Aided Drug Design Methods." Methods Mol Biol. 2017; 1520:85-106.
16. Feyza Maden, et al. "Fundamentals of Molecular Docking and Comparative Analysis of Protein–Small-Molecule Docking Approaches." Biomed Engineering. Intech Open. 2023;1-40
17. Dos Santos FAR, et al. "Antidiabetic, Antiglycation, and Antioxidant Activities of Ethanolic Seed Extract of and Piceatannol In Vitro." Molecules. 2022 Jun 24;27(13):4064.
18. Tomic D, et al. "The burden and risks of emerging complications of diabetes mellitus." Nat Rev Endocrinol. 2022 Sep;18(9):525-539.
19. Mayoclinic, (https://www.mayoclinic.org/diseases-conditions/diabetes/symptomscauses/syc-20371444) accessed on dated,16, Feb 2024
20. Clevelandclinic, (https://my.clevelandclinic.org/) accessed on dated,16, Feb 2024
21. Lucier J, et al. "Type 1 Diabetes." [Updated 2023 Mar 3]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan; 190-202
22. Healthline (https://www.healthline.com/health/diabetes) accessed on dated,16, Feb 2024
23. National Institute of Diabetes And Digestive And Kidney Disesases (https://www.niddk.nih.gov/health-information/diabetes/overview/risk-factorstype-2-diabetes) accessed on dated,16, Feb 2024
24. WebMD, (https://www.webmd.com/diabetes/type-2-diabetes) accessed on dated,16, Feb 2024
25. CDC, centre of fisease control and prevention, (https://www.cdc.gov/index.htm), accessed on dated,15, Feb 2024
26. Xuan-Yu, et al. "Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery." Current Computer - Aided Drug Design, 7.2, 2011; 146-157(12)
27. Kharroubi AT, et al. "Diabetes mellitus: The epidemic of the century." World J Diabetes. 2015 Jun 25;6(6):850-67.
28. Zhijun Yang, et al. REVIEW "Zingiber Ofiicinale: An Insight into Current Researches on Phytochemistry and Pharmacology." Front. Pharmacol, 11. 20 May 2023;11:617
29. Vyas VK, et al. "Homology modeling a fast tool for drug discovery: current perspectives." Indian J Pharm Sci. 2012 Jan;74(1):1-17.
30. Wijeratnam SW, "Ginger Plant Fruit." Encyclopaedia of Food and Health, 2006;230-234
31. Taiwe S, et al,. "Zingiber Ofiicinale. Medicinal Spices and Vegetables from Africa" med plant. December 2017; 513-526
32. IMPAAT: Indian Medicinal Plants, Phytochemistry And Therapeutics. (https://cb.imsc.res.in/imppat/phytochemical/Passiflora%20edulis) accessed on dated,17, Feb 2024
33. Soumya RS, et al. "Zingiber Ofiicinale (var. Flavicarpa) Juice Supplementation Mitigates Isoproterenol-induced Myocardial Infarction in Rats." Plant Foods Hum Nutr. 2021 Jun;76(2):189-195.
34. Bhat R., et al, "Fruits of Tropical Climates: Dietary Importance and Health Benefits" Encyclopedia of Food and Health, 2016; 144-149

35. Zhang. Yan-shu, et al. "Identification of the passion fruit (Zingiber Ofiicinale Sims) MYB family in fruit development and abiotic stress, and functional analysis of PeMYB87 in abiotic stresses." Frontiers in Plant Science, 14: 2023; 1664-4672
36. Swiss adme, Molecular modelling group of the SIB (Swiss Institute of Bioinformatics), (http://www.swissadme.ch/), accessed on dated,17, Jan 2024
37. Ononamadu CJ, et al. "Molecular docking and prediction of ADME/drug-likeness properties of potentially active antidiabetic compounds isolated from aqueousmethanol extracts of Gymnema sylvestre and Combretum micranthum." BioTechnologia (Pozn). 2021 Mar 31;102(1):85-99.
38. Daina A, et al. "SwissADME: a free web tool to evaluate pharmacokinetics, druglikeness and medicinal chemistry friendliness of small molecules." Sci Rep. 2017 Mar 3;7:42717.
39. Protox 2, U.S. Environmental Protection Agency,(https://comptox.charite.de/protox3/index.php?site=home#) accessed on dated,17, Jan 2024
40. Banerjee P, et al. "ProTox-II: a webserver for the prediction of toxicity of chemicals." Nucleic Acids Res. 2018 Jul 2;46(W1):W257-W263.
41. Pubchem, National institute of health (https://pubchem.ncbi.nlm.nih.gov/) , accessed on dated,17, Jan 2024
42. Chemsketch, ACD LABS, (https://www.acdlabs.com/resources/free-chemistrysoftware-apps/chemsketch-freeware/) accessed on dated,17, Jan 2024
43. Avogadro, Soft tonic (https://avogadro.cc/) accessed on dated,17, Jan 2024
44. Yair T, et al. "Protein-Protein and Protein-Ligand Docking." Protein Engineering -
45. Tech and App. First edition may 2013;187 Ncbi nationl library of medicine (gene), National institute of health, (https://www.ncbi.nlm.nih.gov/gene) accessed on dated,20, Jan 2024
46. Swiss model, Swiss institute of bioinformatics,(https://swissmodel.expasy.org/) accessed on dated,20, Jan 2024
47. Protein data bank, RCSB, (https://www.rcsb.org/)accessed on dated,17, Jan 2024
48. Biovia, Dassault system, (https://www.3ds.com/products/biovia), accessed on dated,17, Jan 2024
49. Guedes IA, et al. "Receptor-ligand molecular docking." Biophys Rev. 2014 Mar;6(1):75-87.
50. Pyrx, Source fordge (https://pyrx.sourceforge.io/) , accessed on dated,17, Jan 2024
51. Kumar CJ, et al. "Computer Aided Molecular Docking Studies on a Series of Benzothiazepines as Potential Anti Convalescent Agents." Int. J. Pharm. Sci. Rev. Res., 47(2), 2017; 14-17
52. Baroroh U, et al. " Molecular interaction analysis and visualization of proteinligand docking using Biovia Discovery Studio Visualizer." Indonesian journal of comput biol.2; 2023;22-30.

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