ISOLATION AND IDENTIFICATION OF PIPERINE FROM FRUIT EXTRACT AND INSIGHT TO MOLECULAR DOCKING FOR ANTI-DIABETIC POTENTIAL

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Abstract:

Piperine is a natural alkaloid found in black pepper and other Piper species. It has been shown to have a variety of biological activities, including anti-diabetic potential. In this study, piperine was isolated from black pepper fruit extract and its anti-diabetic potential was investigated using molecular docking. Piperine was isolated from black pepper fruit extract using a combination of solvent extraction and thin layer chromatography. To investigate the anti-diabetic potential of piperine, molecular docking studies were performed with PPAR-γ. PPAR-γ enhances glucose metabolism. The molecular docking results showed that piperine binds to PPAR-γ with high affinity. The binding energy of piperine to PPAR-γ was -88.29 kcal/mol. These results suggest that piperine may be a potential inhibitor of PPAR-γ which could lead to anti-diabetic activity. Overall, this study demonstrates that piperine can be easily isolated from black pepper fruit extract. The molecular docking results suggest that piperine may have anti-diabetic potential by inhibiting PPAR-γ. Further studies are needed to confirm the anti-diabetic activity of piperine *in vivo*.

Keywords:

Piperine**,** *Piper nigrum* , iGEMDOCK , Antidiabetic , Docking .

Introduction:

Piper nigrum belongs to the family Piperaceae, it is a perennial shrub native to southern India, and has been extensively cultivated there and in other tropical regions. As of 2013, Vietnam is the world's largest producer, as well as exporter, of pepper, producing 34% of the global P. nigrum crop. Due to its strong pungency, it is regarded as the „King of spices" and it has valuable medicinal potency. It is one of the world most common kitchen spices and well known for its pungent chemical constituent piperine (1-peperoyl piperidine,), discovered in 1819 by Hans Christian, which has diverse pharmacological activities [1, 2]. It is commonly known as Kali mirch in Urdu and Hindi, Marich in Nepali, Pippali in Sanskrit, Milagu in Tamil, and Black Pepper, Peppercorn, Green pepper, White pepper, Madagascar pepper in English. It is widely accepted and most used in different traditional systems of medicine, like the Unani and Ayurvedic systems [3-5]. It has long been used to treat many diseases, such as antihypertensive, antioxidant, antiplatelets, antitumor, anticonvulsant, antithyroid, analgesic, anti-inflammatory, antidiarrheal, antispasmodic, antidepressants, immunomodulatory, antibacterial, antifungal, hepatoprotective, etc.

Diabetes mellitus is a chronic disorder of carbohydrates, fats and protein metabolism. A defective or deficient insulin secretary response, which translates into impaired carbohydrates (glucose) use, is a characteristic feature of diabetes mellitus, as is the resulting hyperglycemias Diabetes mellitus (DM) is commonly referred to as a "sugar" and it is the most common endocrine disorder and usually occurs when there is deficiency or absence of insulin or rarely, impairment of insulin activity (insulin resistance). The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025 [6-10].

Insulin and glucagon hormones both are secreted by the pancreas. Insulin is secreted by the beta (ß) cells and glucagon is secreted by the alpha (α) cells both are located in the islets of Langerhan"s. Insulin decreases the blood glucose level by the glycogenesis and transport glucose into the muscles, liver and adipose tissue. Neural tissue and erythrocytes do not required insulin for glucose utilization whereas alpha (α) cells plays an important role in controlling blood glucose by producing the glucagon and it increases the blood glucose level by accelerating the glycogenolysis [11-14].

In addition to increased risk of obesity, metabolic and cardiovascular disorders, and malignancy in future life of fetus after delivery. Type II diabetes mellitus comprises 80% to 90% of all cases of diabetes mellitus. Geographical variation can contribute in the magnitude of the problems and to overall morbidity and mortality. Moreover, people with diabetes who undertake moderate amounts of physical activity are at inappreciably lower risk of death than inactive persons. It is now well established that a specific genetic constitution is required for such an event to cause The growing burden of diabetes and other noncommunicable diseases is one of the major health challenges to economic developments bedeviling WHO African Region states.

In diabetes, there is an aberration either in the synthesis or secretion of insulin as seen in Type 1 diabetes mellitus (T1DM) and stenosis in the pancreatic duct, or the development of resistance to insulin or its subnormal production as in the case of Type 2 diabetes (T2DM) and certain secondary diabetes [15-18].

Molecular docking is a virtual methodology for screening of lead optimization from the huge library of compounds using the binding energy and scoring function as a parameter. Molecular docking is a part of computer-aided drug design (CADD) that is a combination of computational techniques, bioscience and chemical sciences that ease the drug discovery process and scale-up the process. It is important to have the knowledge of position and orientation of ligand (binding site) for effective docking studies. This information can be obtained by the comparison with the other family members of same protein and/or by the online tool like GRID, POCKET, SurfNet, PASS and MMC [19-22].

Materials &Methods:

Plant Material:

The dried fruit of *Piper nigrum* were purchased from the local market, washed and then dry to remove any extraneous material from the crude drugs. The crude drugs were approved from the department of Pharmacognosy, Faculty of Pharmacy, Raja Balant Singh Engineering Technical Campus Bichpuri Agra, Uttar Pradesh (Reg. No. 202012). The crude drugs were subjected to pulverization.

Chemicals:

Various chemicals like ethanol (95%) (changshuyangyuan chemical), Potassium hydroxide(CDH), calcium chloride (CDH), sodium oxalate (Qualigen fine chemicals thermoelectronLLS), sulphuric acid (Thermo fisher scientific), ammonia solution (CDH), hydrochloric acid (Thermo fisher scientific)hexane (Thermo fisher scientific) and Cystone (Himalaya herbal healthcare) were procured by the institute and used in the research work.

Extraction & Isolation of Piperine:

Piperine was extracted and isolated from the black pepper by the soxhlet apparatus. The powdered drug (40g) was mixed with 500mL of ethanol (95%) in a Soxhlet apparatus for three hours.Filter the solution and concentrate under vacuum on a water bath at 60^{o}C. Add 20 ml of 10% alcoholic KOH with constant stirring to concentrated extract and filter. Allow alcoholic solution to stand overnight whereupon needles of piperine separate out. The yield was found to be 2.5% w/w [23].

Chemical Identification:

After extraction and isolation the piperine was chemically identified by Mayer’s and Wagner’s test and analytically by the thin layer chromatography methods. The melting point of isolated piperine was found to be 124^{0}C (M.P. 123^{0}C).

Mayer's test

Alkaloids are precipitated from neutral or slightly acidic solution by Mayer‟s reagent to give white to a ceramic colored precipitate. Mayer‟s reagent was freshly prepared by dissolving a mixture of mercuric chloride (1.36 g) and of potassium iodide (5.00 g) in water (100.0 mL). Few crystals of piperine alkaloid were dissolved in few 2mL of ethanol then add 2 drops of HCl and two drops of Mayer's reagent, a yellowish white precipitate formed.

Wagner's test

Wagner‟s reagent was freshly prepared by dissolve 2 g of Iodine and 6 g of potassium iodide in 100 mL of distilled water. Take few crystals of piperine and dissolved in few 2 mL of ethanol then add two drops of HCl and 2 drops of Wagner‟s reagent a brown precipitate formed.

Thin Layer Chromatography

Isolated Piperine was subjected to thin-layer chromatography (TLC), by using Silica gel G. as a stationary phase and Ethanol:hexane (7:3) as a mobile phase18. The piperine spot was identified by visualising reagent i.e. dragendorff‟s reagent.The standard R_f value of Piperine was 0.25 (literature). The R_f value of isolated Piperine was found to be 0.26 [24].

MOLECULAR DOCKING:

Docking studies were performed between the bioactive molecules (ligand) from the plant of *P.nigrum* and the protein (PPAR-γ) responsible for the pathogenesis of diabetes by using the iGEMDOCK suit v 2.1.

Preparation of Protein structure:

The three dimensional structure of required proteins i.e. PPAR-γ (PDB ID: 2P4Y) has been fetched from the Protein Data bank (https://www.rcsb.org) at 2.25Å resolution for the in-silico molecular docking studies. PPAR-γ protein represents the Crystal structure of human PPAR-gamma-ligand binding domain complexed with an indole-based modulator (Fig1 &2).

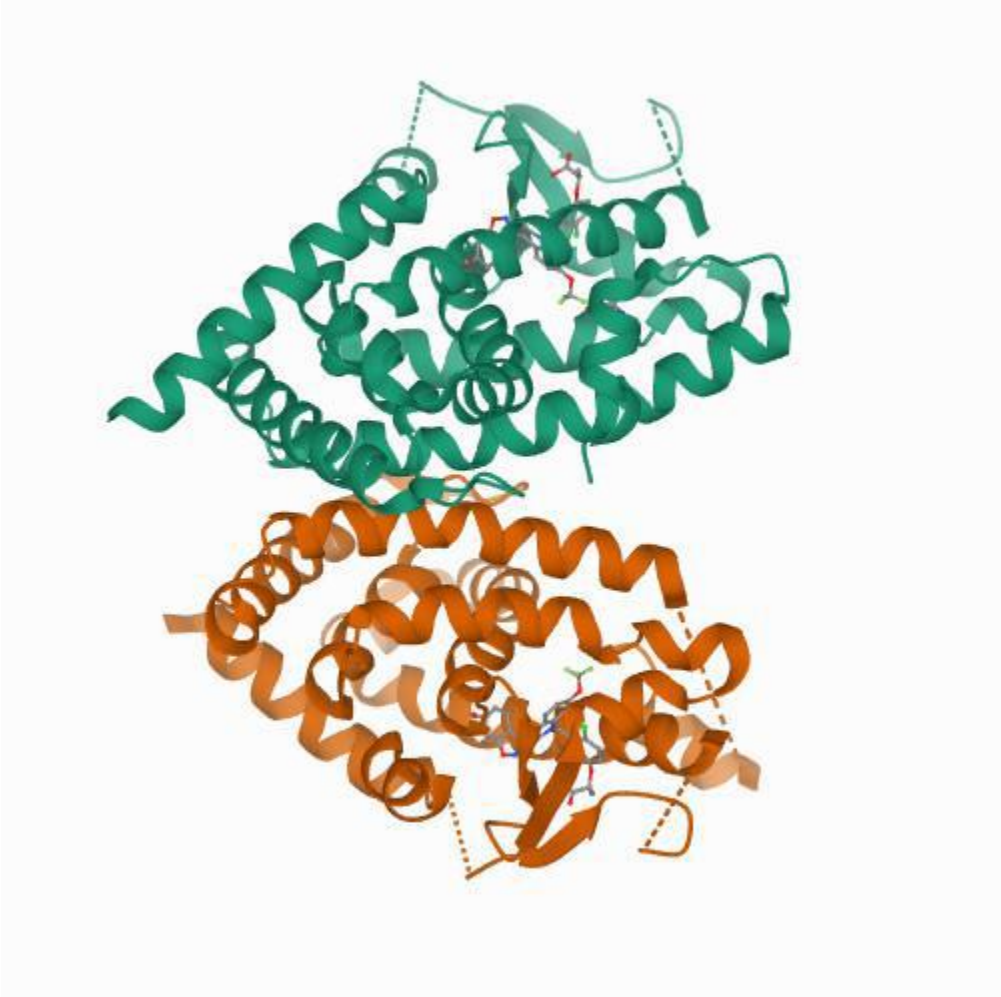

Fig.1: Crystal structure of PPAR-γ

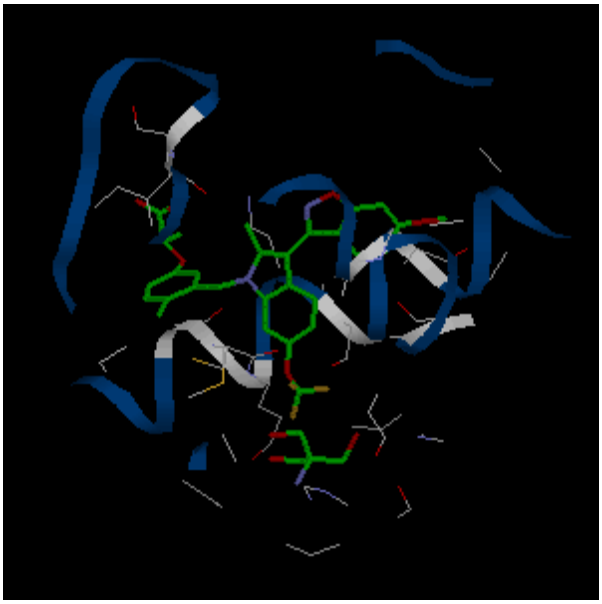

Fig.2: Binding site of PPAR-γ (C03)

Assembling of Ligand:

The bioactive ligands were developed from the molecules that are built in the P.nigrum for the molecular docking profiling. The bioactive molecule (Piperine) was taken from the database of PubChem. The three dimensional conformer of the molecules were utilized in Mol file (.mol) format for the protein ligand interaction by using the conversion software openbabel (https://openbabel.org).

Docking Procedure:

From preparations to post-screening analyses including pharmaceutical interactions, iGEMDOCK provides a comprehensive VS environment. To begin, iGEMDOCK includes interactive interfaces for preparing the target protein's binding region as well as the screening chemical library. Docking was performed by the docking software iGEMDOCK for proteins (PPAR-γ) and ligand (Piperine and Rosiglitazone) binding interaction for diabetic treatment approach. IGEMDOCK provides the integrated virtual screening (VS) environment from preparations through post screening analysis with pharmacological interactions. iGEMDOCK gives biological insight by deriving the pharmacological interactions from screening compounds without relying on the experimental data of active compounds.

IGEMDOCK next generates electrostatic (E), hydrogen-bonding (H), and van der Waals (V) interaction profiles between proteins and compounds. IGEMDOCK infers pharmacological interactions and clusters the screening compounds for post-screening analysis based on these profiles and compound structures.

In addition iGEMDOCKgive raise to protein-ligand interaction profile of electrostatic, hydrogen-bonding and Van der Waal‟s binding energies. Depending on these data and bioactive molecule structures, iGEMDOCK provides the interactions based on pharmacology and collect the ligands for the post screening analysis. Finally the iGEMDOCK ranks and visualizes the screening molecules by combining the pharmacological interactions and energy based scoring function of GEMDOCK.

Dock Profiling:

During the docking by iGEMDOCK software the protein and ligands molecules were assigned for bonds, bond orders, explicit hydrogen charges and flexible torsions. In docking accuracy setting the standard docking with population size of 200, generations of 70 and number of solutions of 2 was kept for genetic algorithm(GA) parameters. In docking scoring functions the ligand intra energy of hydrophobic and electrostatic preferences were kept on 1.00. Docking was conducted between protein and inhibitors which result in binding affinities in kcal/mol and docking run time. The molecule with lowest energy is taken as the best inhibitor [25].

Results:

Table I: Result of Chemical Identification of Piperine

SN	Chemical Identification	Inference
1	Mayer's Test	Yellowish white precipitate
2	Wagner's Test	Brown precipitate
3	TLC	Rf = 0.26
4	Melting Point	124^0C

Table II: Molecular docking:

SN	Molecule	Energy (kcal/mol)	VDW	HBond	Elec
1	Piperine	-88.29	-88.29	0	0
2	Rosiglitazone	-97.76	-83.32	-14.44	0

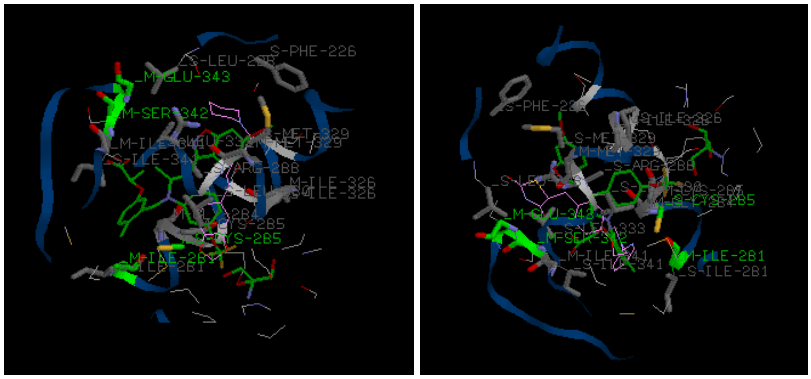

Fig.3: Docked poses of Piperine (A) and Rosiglitazone (B) showing the binding (Pink color) with PPAR-γ (C03) green color.

Discussion:

Black pepper is a rich source of biologically active compounds, including piperine, which is responsible for its pungent flavor. Piperine has been shown to have a variety of pharmacological activities, including antidiabetic, anti-cancer, and anti-inflammatory effects.

In the study, the researchers identified piperine as the major active compound in black pepper using chemical and analytical methods. They then used molecular docking to study the interaction of piperine with the PPAR-γ receptor, which is a target for antidiabetic drugs. The results showed that piperine has the potential to inhibit the PPAR-γ receptor, suggesting that it may be a useful antidiabetic agent.

The researchers also discuss the potential of black pepper and its constituents for the treatment of other diseases, such as cancer, obesity, hypertension, and diarrhea. They note that further research is needed to confirm the efficacy and safety of black pepper for these conditions.

Conclusion:

Black pepper is a versatile spice with a wide range of potential medicinal applications. Piperine, the major active compound in black pepper, has been shown to have antidiabetic, anti-cancer, and anti-inflammatory effects. Further research is needed to confirm the efficacy and safety of black pepper for the treatment of various diseases.

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