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Aphrodisiac Performance of Bioactive Compounds from *Trigonella foenum-graecum* Seed.: *In -Silico* Molecular Docking and Dynamics Simulation Approach

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Abstract

Background*:** Male sexual dysfunction is a group of disorders that affect sexual function, most notably erectile dysfunction (ED), Peyronie's disease (PD), and premature ejaculation (PE). More than 50% of men between the ages of 40 and 70 report having some form of erectile dysfunction, which is a rather high prevalence that rises with age. Age, diabetes mellitus (DM), cancer, stroke, hypertension, penile trauma, depression, anxiety, and disturbances in central serotonin neurotransmission and 5-HT postsynaptic receptor function are risk factors for male sexual dysfunction. The International Index of Erectile Dysfunction, the Sexual Health Inventory for Men, and the Premature Ejaculation Diagnostic Tool are three sexual questionnaires that can be used to screen for these illnesses. Fenugreek, or Trigonella foenum-graecum L., also known as methi (in Hindi), has been used as a culinary spice, flavouring ingredient, and medicinal plant for a long time. Despite being more well-known for its seeds, fenugreek leaves and stems have also been claimed to offer medical benefits, including laxative, lactation stimulant, and labour assist properties. ***Method*:** The purpose of the current study was to assess the efficiency of flavonoids and steroid saponin found in Trigonella foenum-graecum seed for their inhibitory influence on PDE-5 enzyme to elicit the aphrodisiac potency. The Auto Dock software used a grid-based docking algorithm to determine the bond. ***Result*:** Fenugreek found to be effective aphrodisiac agent and effectively binds to be target protein PDE-5 with binding energy -9.8, -10.6, -7.88 & -6.61 kcalmol^{-1}for Diosgenin, gitogenin, Naringenin and Vitexin respectively. ***Conclusion*:** The outcome of findings revealed that steroidal saponin(diosgenin) and flavonoid(vitexin) showed potent inhibitory effect on *PDE-5* enzyme which reflects the efficacy of fenugreek seed as potent aphrodisiac agent ***via synergetic effect of steroidal saponin and flavonoid.

Keywords: *Trigonella foenum-graecum* seed, Molecular docking**,** Diosgenin, gitogenin, Naringenin and Vitexin.

INTRODUCTION

Both men and women can experience sexual dysfunction, and it gets more common as people get older. 1 In addition to the desire, arousal, and orgasmic phases of the normal sexual response cycle, pain can also cause dysfunction [1]. Human sexual function is highly complex and includes both physiologic and psychological elements [2]. Since the diagnosis is based on clinical symptoms, it is essential to have a thorough sexual history and targeted physical examination. Men's sexual dysfunction can have a variety of causes, each with unique risk factors and therapies. Lack of interest in thinking about or engaging in sexual activity, whether alone or with a partner, is a sign of low sexual desire [2]. The persistent or recurrent inability to achieve or sustain a penile erection strong enough to provide sexual satisfaction is known as erectile dysfunction (ED). A rather frequent condition is erectile dysfunction. Since many men do not self-report their erectile dysfunction symptoms, doctors must inquire about sexual function and health in order to make a diagnosis [3].

Pathophysiology and Risk Factors

Low levels of sexual interest or desire and various sexual dysfunctions, like ED and PE, are likely to be correlated with poor health. Age has been identified as a major risk factor for male sexual dysfunction, however, independent of health [4, 5]. Regardless of health state or prior erectile function, one conclusion of this study indicated a 10-fold difference in relative risk

for ED linked with older age. The risk of ED was lowest in men who had healthy lives and did not have any chronic illnesses. 5 Conversely, physical activity, leanness, moderate alcohol intake, and a lack of a smoking history were related with decreased risk. Comorbid diseases, such as diabetes mellitus (DM), cancer, stroke, and hypertension, were associated with higher risk for ED. Both vascular and neuronal mechanisms are necessary for ED [6]. *Plant Trigonella foenum-graecum*, sometimes referred to as fenugreek, is a member of the Fabacea family [7]. Since the beginning of time, people have used it as food and flavouring. It is indigenous to the region that stretches from the Eastern Mediterranean to Ethiopia and Central Asia, and it is widely cultivated in both countries [8]. Plant seeds and leaves are utilised in traditional remedies as well as being consumed [9]. In many parts of the world, trigonella foenum-graecum is used to treat a variety of diseases, including urotoxicity, immunomodulatory, antiradical and antioxidant, chemopreventive, anticancer, antidiabetic, gastro protective, anti-inflammatory and antipyretic, regulation of hyperthyroidism, anthelmintic, antigenotoxic, anti-plasmodial, hepatotoxicity and nephrotoxicity & wound healing.

Experimental Work

Molecular Docking Studies

Ligand Preparation:

2D Structure of ligands like diosgenin, gitogenin, naringenin, and vitexin were drawn using ChemSketch [11], the two-dimensional structures of the prepared ligands were converted into their 3-D structures optimized with 3D geometry. The optimized structures were saved in PDB format for AutoDock compatibility. The basic structures of the prepared ligands were given below:

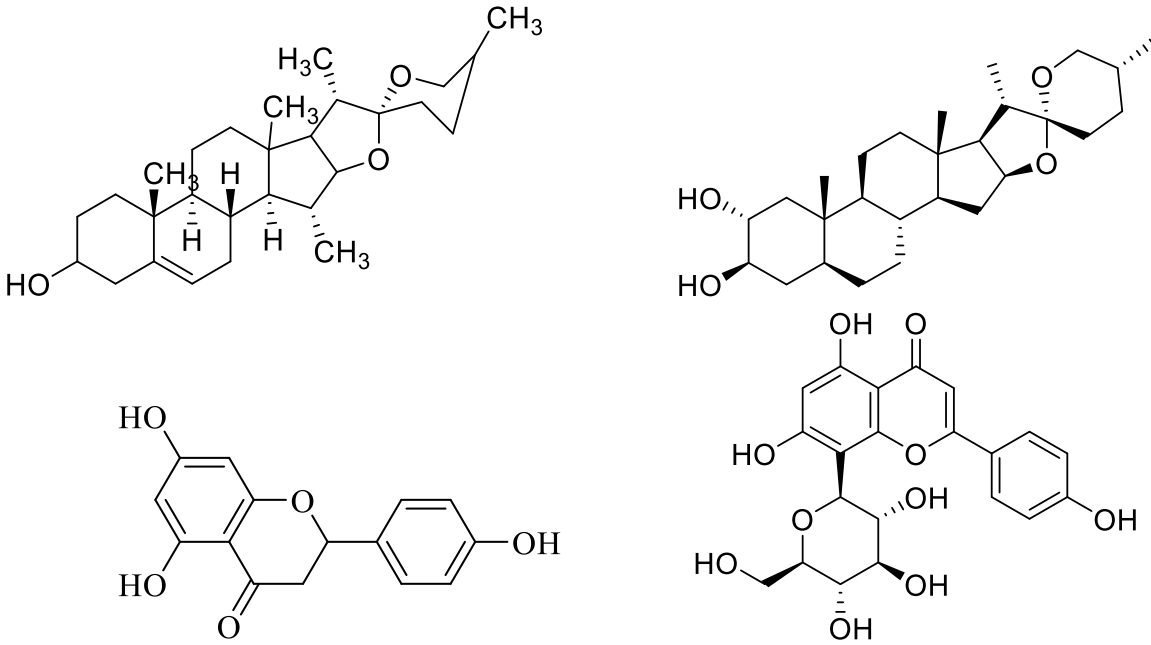


Figure 1: 2D structure of diosgenin, gitogenin, naringenin, and vitexin

Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing between grid points can be adjusted with another thumbwheel, the value in the study taken is 0.408 Å and No. of points considered are 40, 40 and 40 points in the x, y, and z dimensions and 30.79, 119.342 and 11.038 as x, y, z centers [12, 13].

Table 1: Grid parameters used in current docking analysis of PDE5 enzyme.

S. No.	Receptor	x-axis	y-axis	z-axis	Spacing	x center	y center	z center
1	PDE5	40	40	40	0.408	30.79	119.342	11.038

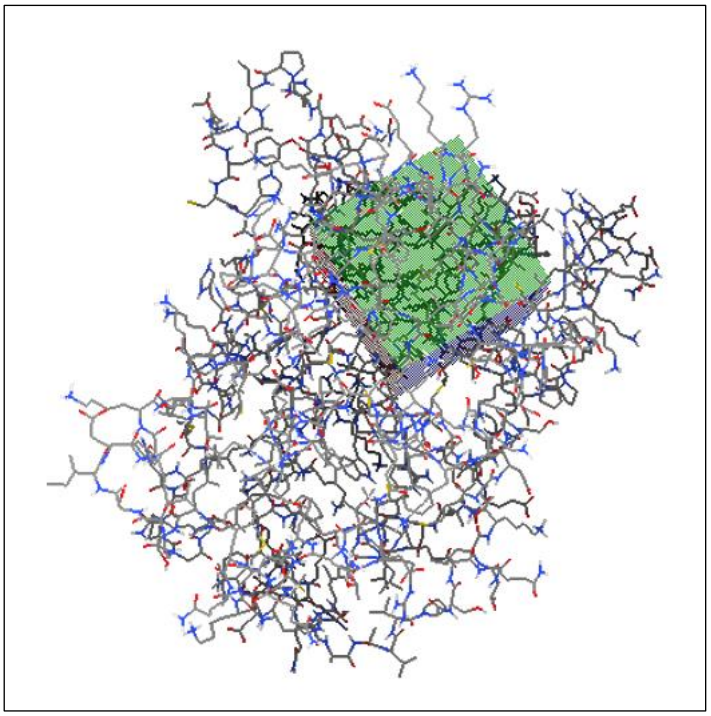


Figure 2: Grid box covering all active sites in Human PDE5 enzyme

Preparation of the docking file

All the calculations were carried out by using Autodock 4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [14, 15].

Docking of beta-tubulin with Quercetin

Crystal structure

The crystal structure of the protein consisting of Human PDE5 enzyme is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (2h42.pdb) registered in the Protein data bank was used [16-18].

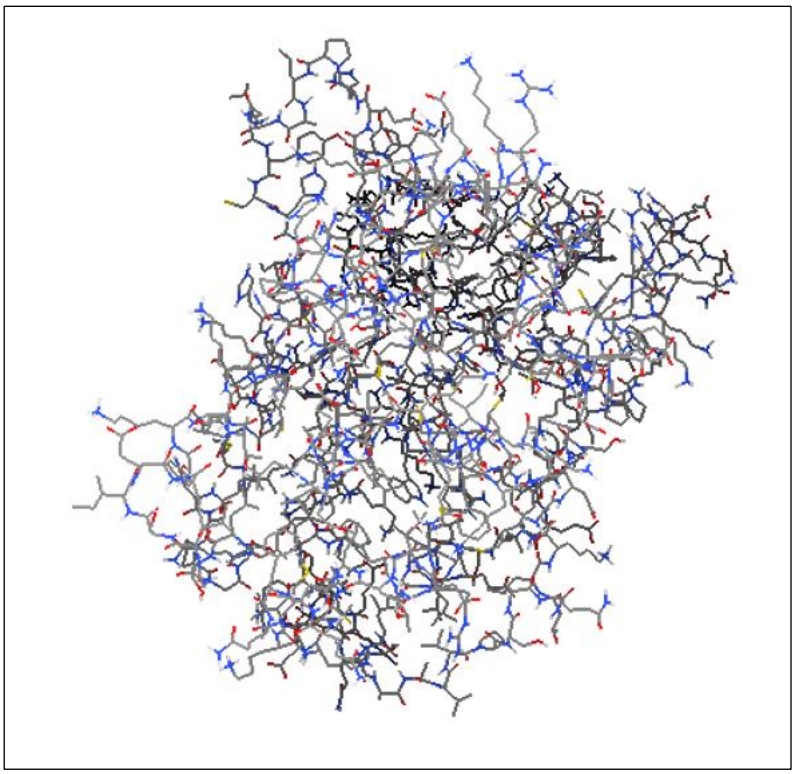


Figure 3: Crystal structure of Human PDE5enzyme (PDB ID-2h42)

Processing of Protein

The downloaded receptor protein is having two chains, i.e. chain A, and B. Out of these two chains, chain B was selected for experimental purpose and other chains were removed from it. The bound ions were separated from the macromolecular complex by using software Chimera [19, 20].

Molecular Docking Simulation Studies

Docking of ligands like diosgenin, gitogenin, naringenin, and vitexin against viral Human PDE5enzyme was performed by Autodock. All the bonds of each ligand were kept flexible, while no residues in receptor were made flexible [21, 22].

Toxicity & ADME-T Studies

The ligand molecules viz. diosgenin, gitogenin, naringenin, and vitexin were studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties [23 & 26].

RESULT & DISCUSSION

Aphrodisiacs were sought after to assure both male and female potency since reproduction was seen as a significant moral and religious matter. Erectile dysfunction, arousal issues (reduced libido), compulsive sexual behaviour, orgasmic disorder, and failure of detumescence are all examples of sexual dysfunction. Sexual dysfunction also includes premature ejaculation, retrograded, delayed, or suppressed ejaculation, erectile dysfunction, arousal difficulties (reduced libido), and erectile dysfunction. When Viagra (sildenafil) was introduced as the first pharmaceutically authorised treatment for erectile dysfunction in the 1990s, it generated a great deal of media interest, thanks in large part to aggressive advertising. Such compounds have long been sought after. A substance (food or drug) that stimulates sexual desire is known as an aphrodisiac. The search for natural supplements made from medicinal plants is becoming more intense, mostly due to the lack of negative side effects.

Men with ED often turn to nutraceuticals and nutritional supplements as an affordable solution to treat their sex problems. The market for dietary supplements to address sexual health concerns is projected to rise as public knowledge of these problems rises. The Trigonella foenum-graecum plant, from which fenugreek is derived, has long been a staple of Ayurvedic, Chinese, and Unani medicine. It has been suggested that fenugreek seed extract can help with circadian rhythm, glycemic regulation, cholesterol levels, and libido. The extract contains a variety of lipids, vitamins, and amino acids, including arginine.

High levels of flavonoids, a class of bioactive dietary components, are present in the diet, which might account for some of the positive benefits that have been noticed. An increase in dietary flavonoid intake has been linked to improvements in endothelial function and blood pressure which raises the possibility that flavonoids are more likely than other dietary components to enhance erectile function. Flavonoids have anti-inflammatory properties, inhibit LDL oxidation and endothelial NADPH oxidase, modify endothelial nitric oxide (NO) synthase activity, and increase NO status. They are found in many plant-based foods and drinks, such as fruit, vegetables, tea, herbs, and wine.

Previous investigation proven the efficacy and protection profile of diallyl thiosulfinate associated with nuciferine and diosgenin in the remedy of primary and secondary erectile dysfunction. These compounds are accomplished to improve the control of ejaculation in candidates suffering from premature and erectile dysfunction devoid of any side effects.

In the current study, an effort was made to evaluate the effectiveness of flavonoids and steroid saponin discovered in *Trigonella foenum-graecum* seed for their inhibitory impact on PDE-5 enzyme to elicit the aphrodisiac potency. The precise mechanism by which flavonoid and steroid saponin inhibit the PDE-5 enzyme is still unknown. The docking-based computational study against the PDE-5 enzyme has been carried out with the intention of recommending the most likely mechanism of action of the chosen lead phytoconstituent.

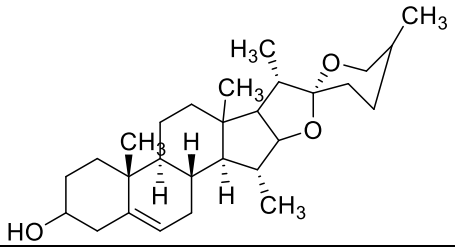
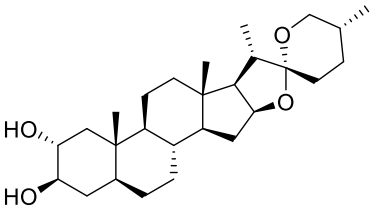
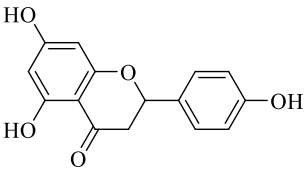
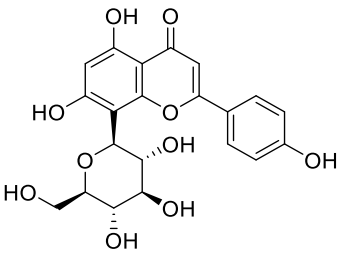
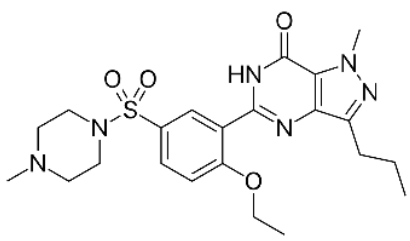
PDE5 is an enzyme found primarily in the smooth muscle of the corpus cavernosum that selectively cleaves and degrades cGMP to 5′-GMP. *PDE5* inhibitors are similar in structure to cGMP; they competitively bind to PDE5 and inhibit cGMP hydrolysis, thus enhancing the effects of NO. This increase in cGMP in the smooth muscle cells is responsible for prolonging an erection. *PDE-5* inhibitors lack a direct effect on corpus cavernosum smooth-muscle relaxation. Therefore, after administration, adequate sexual stimulation is necessary for an erection to occur [24].

The grid parameter used in docking analysis of *PDE-5* tabulated in table 1. The finding revealed that selected flavonoids *i.e.* Diosgenin, gitogenin, Naringenin and Vitexin are potent inhibitor of PDE-5 enzyme in following manner: Diosgenin > gitogenin > Naringenin >Vitexin. Although Vitexin showed more or less similar with Sildenafil as per binding energy.

Fenugreek found to be effective aphrodisiac agent and effectively binds to be target protein PDE-5 with binding energy -9.8, -10.6, -7.88 & -6.61 kcalmol-1for Diosgenin, gitogenin, Naringenin and Vitexin respectively. The result was tabulated in table 2. The binding mode showed in fig.4-7. The 2D and 3D interaction of selected compound displayed in fig.8-18. The interaction of diosgenin showed that ligand binds specifically at THR A:723 (conventional hydrogen binding), LEU A:804,PHE A:820,ILE A:824,MET

A:816,LEU A:725, HIS A:613 on PDE-5 protein through Pi-sigma binding along with week Vander walls interaction at SER A: 663,PHE A:786, ASN A:662,ASN, A:661, ASP A 764, IEU A: 665 whereas vitexin showed binding interaction at PHE A:820,TYR A:612, PHE A:786,VAL A:782 covalently on *PDE-5* protein. Conventional hydrogen binding with ASN A:661 & GLN A:817 along with pi-Sigma binding at ALA A:767, ALA A:779, LEU A:765 & week Vander wall interaction at GLU A:775, ILE A:788,ASP A:764,HIS A:617,HIS A:613, LEU A:725,LEU A:804,MET A:816,ILE A:813,ALA A:783.Both lead compound (diosgenin & vitexin) showed good interaction with *PDE-5* protein and had potent inhibitory effect. The pharmacokinetic profile reveals that it is having good pharmacokinetic profile but with the presence of any major toxic effects including mutagenicity, tumorogenicity and reproductive effects. The pharmacokinetic and toxicity profiling results of ligands like Diosgenin, gitogenin, Naringenin, Vitexin and Sildenafil were shown in figure 19-23. Theoretically, all the ligand molecules have shown encouraging docking score. The docking result of diosgenin revealed that their docking scores was -10.6 kcal mol^{-1}, and it can be predicted as good inhibitor of *PDE-5* enzyme whereas vitexin showed resemble docking score with sildenafil (standard).

Table 2: Results of docking of ligands like diosgenin, gitogenin, naringenin, and vitexin against Human PDE5 enzyme

Sl.	Compound	Structure	Binding Energy
1	Diosgenin		-9.8
2	Gitogenin		-10.6
3	Naringenin		-7.88
4	Vitexin		-6.61
5	Sildenafil		-6.58

Binding Mode

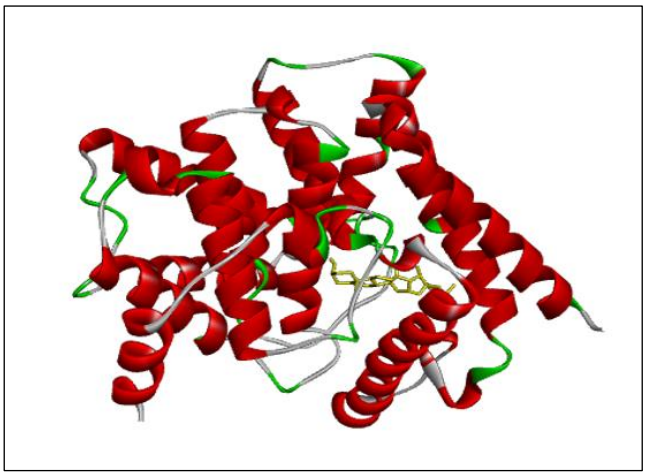


Figure 4: Binding mode of diosgenin within the active site of Human PDE5 enzyme

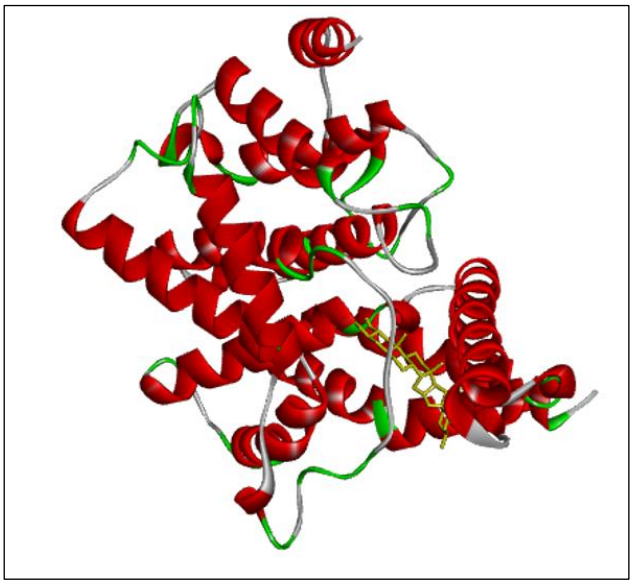


Figure 5: Binding mode of gitogenin within the active site of Human PDE5 enzyme

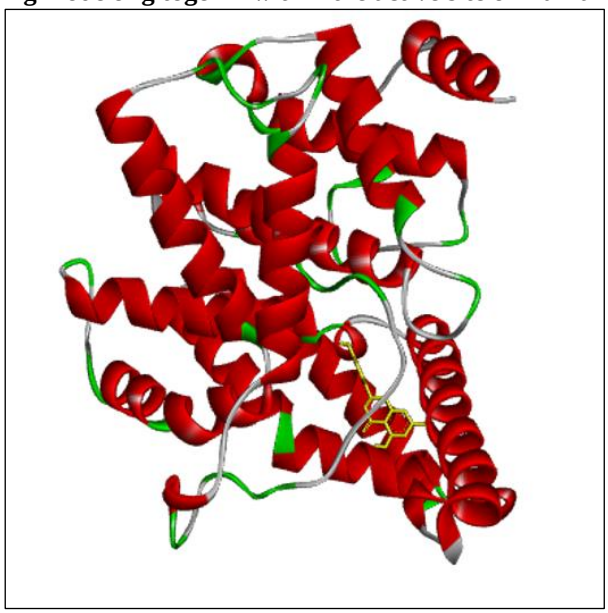


Figure 6: Binding mode of naringenin within the active site of Human PDE5 enzyme

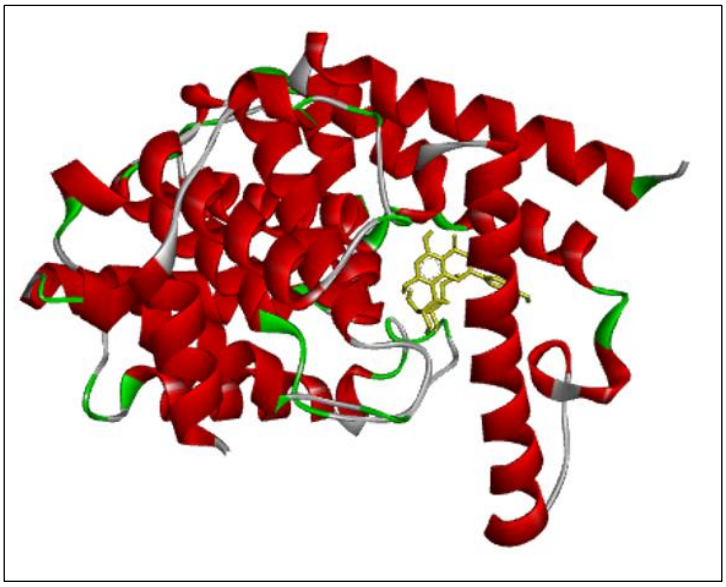


Figure 7: Binding mode of vitexin within the active site of Human PDE5 enzyme

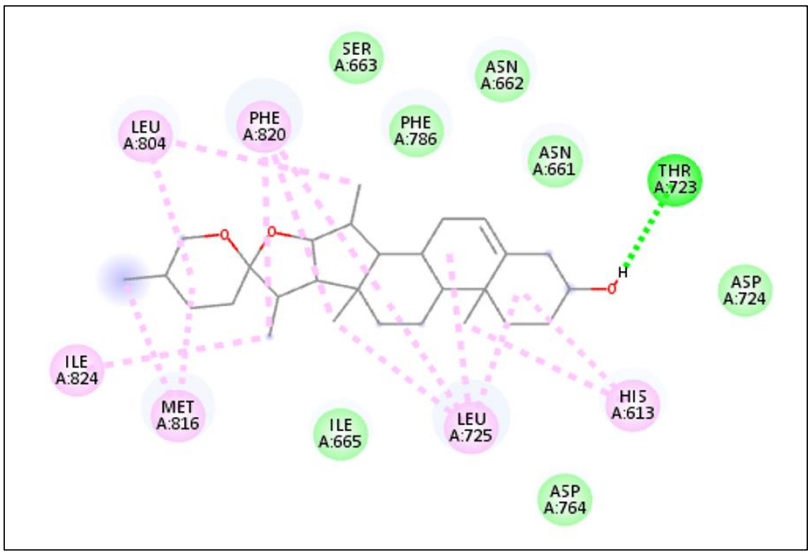


Figure 8: Two-dimensional binding mode of diosgenin within the active site of Human PDE5 enzyme

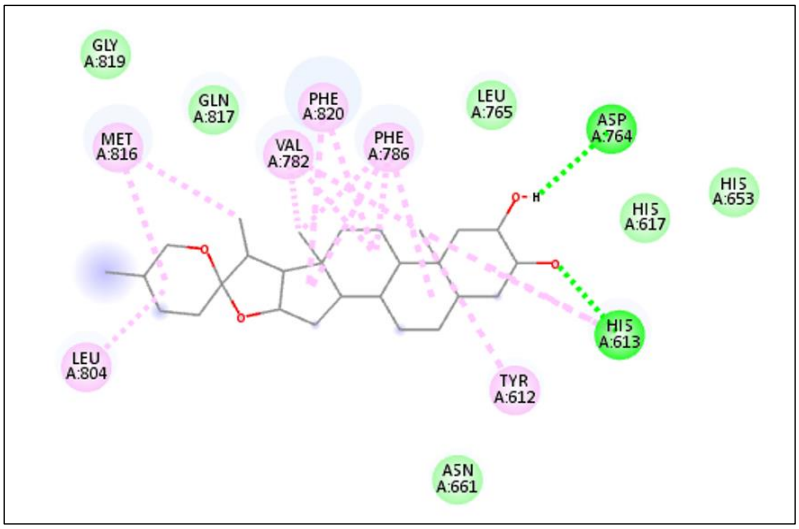


Figure 8: Two-dimensional binding mode of gitogenin within the active site of Human PDE5 enzyme

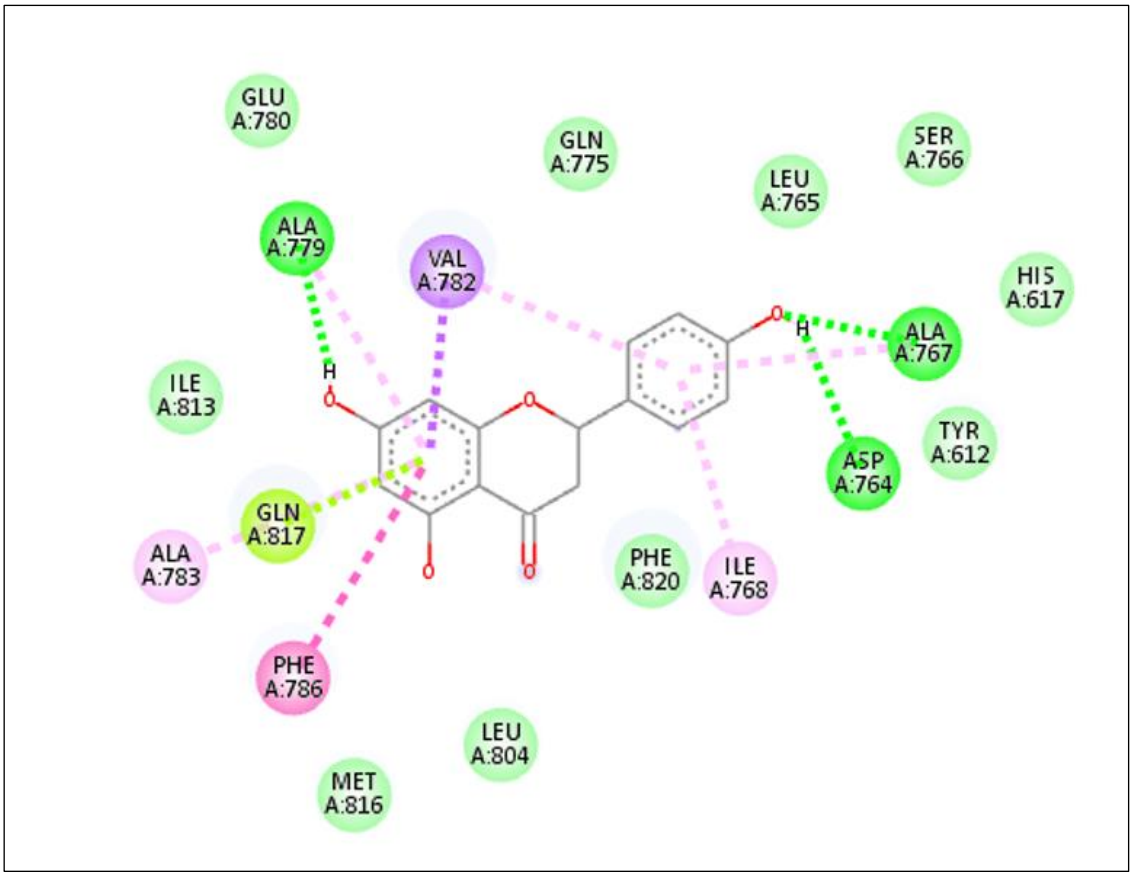


Figure 9: Two-dimensional binding mode of naringenin within the active site of Human PDE5 enzyme

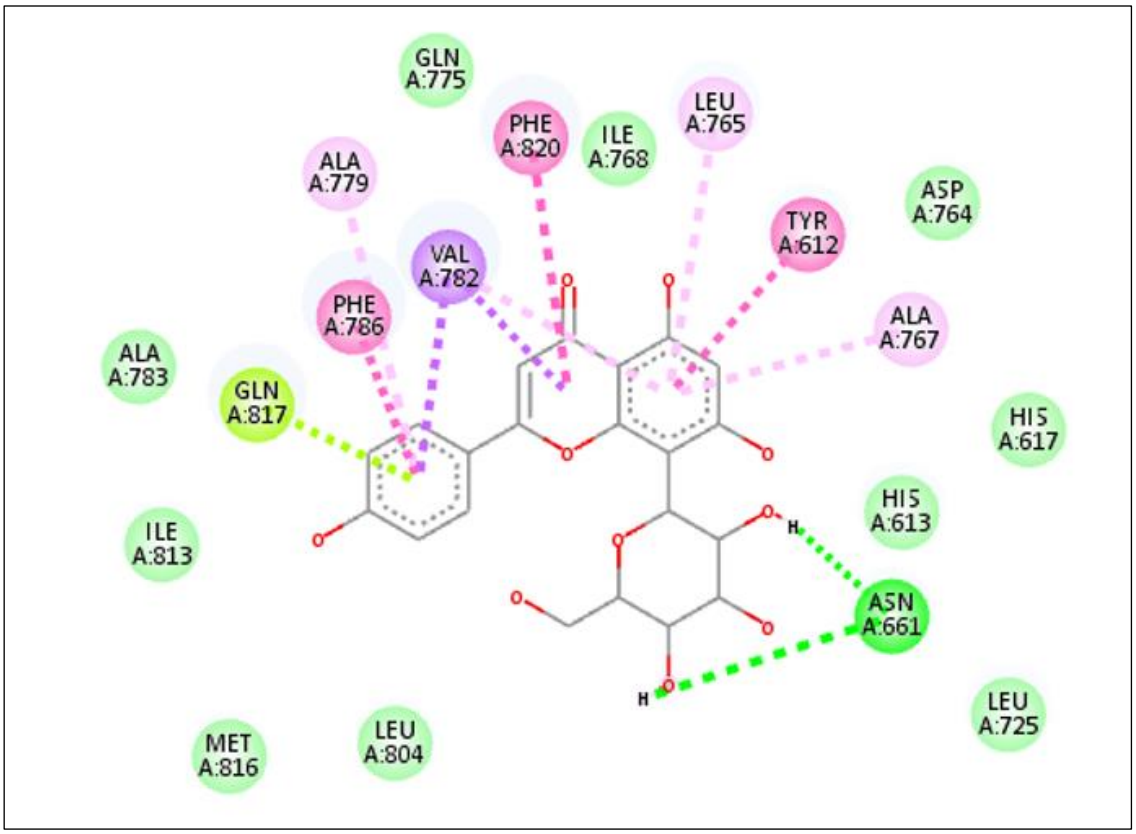


Figure 10: Two-dimensional binding mode of vitexin within the active site of Human PDE5 enzyme

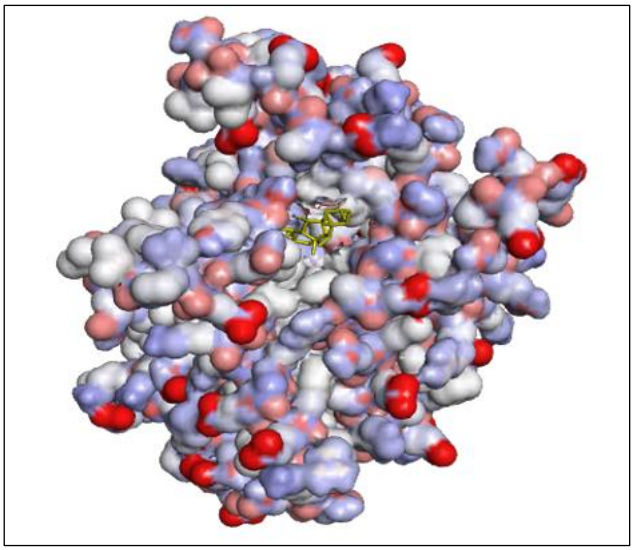


Figure 11: Three-dimensional binding conformation of diosgenin within the active site of Human PDE5 enzyme

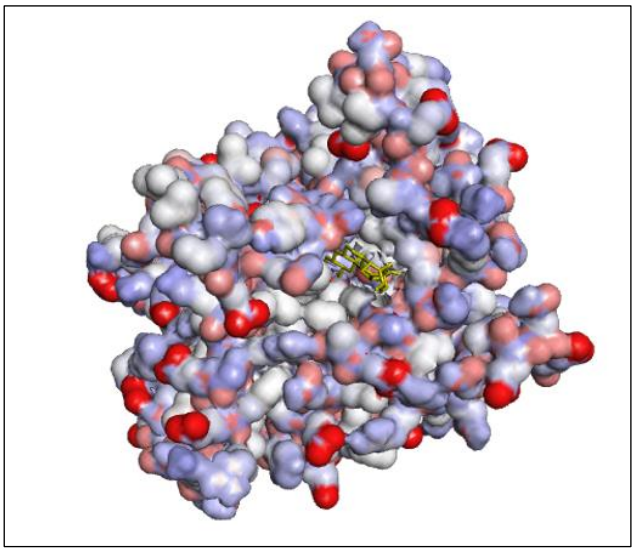


Figure 12: Three-dimensional binding conformation of gitogenin withiAn the active site of Human PDE5 enzyme

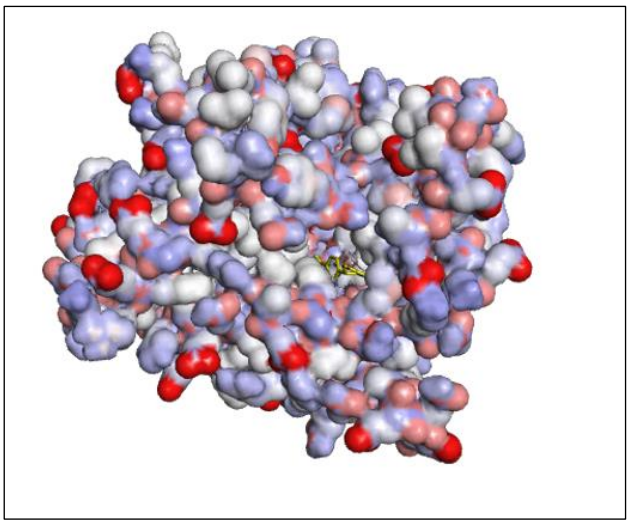


Figure 13: Three-dimensional binding conformation of naringenin within the active site of viral Human PDE5 enzyme

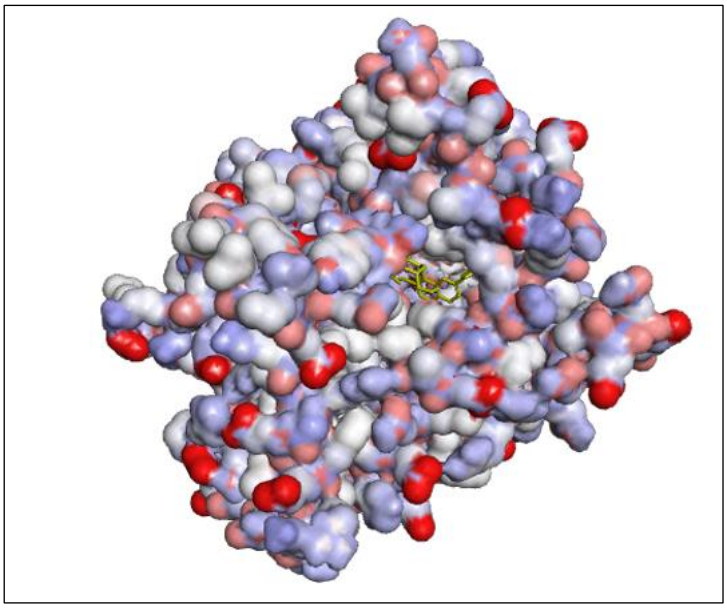


Figure 14: Three-dimensional binding conformation of vitexin within the active site of Human PDE5 enzyme

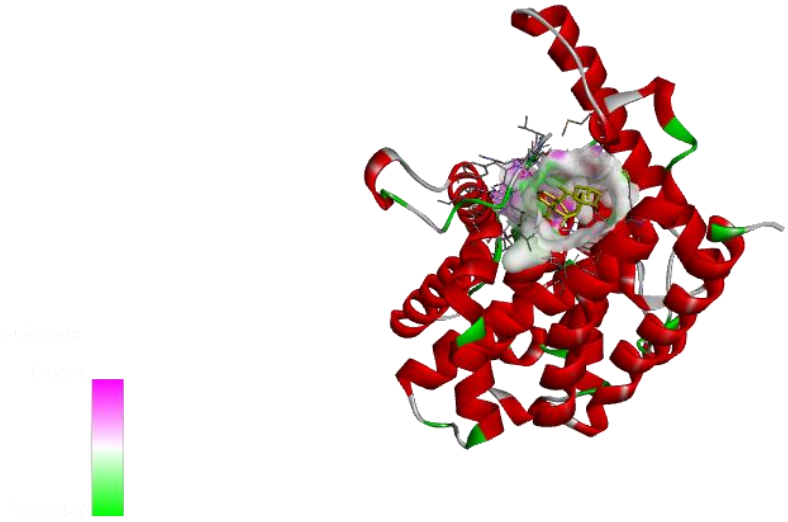


Figure 15: Three-dimensional binding mode of diosgenin within the active site of Human PDE5 enzyme

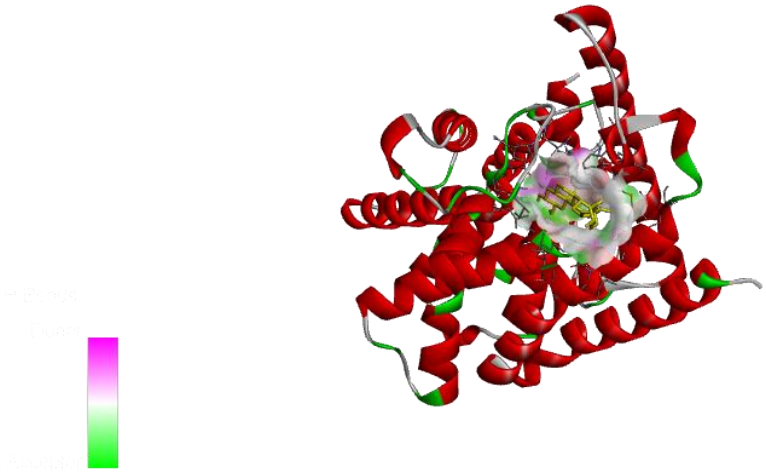


Figure 16: Three-dimensional binding mode of gitogenin within the active site of Human PDE5 enzyme

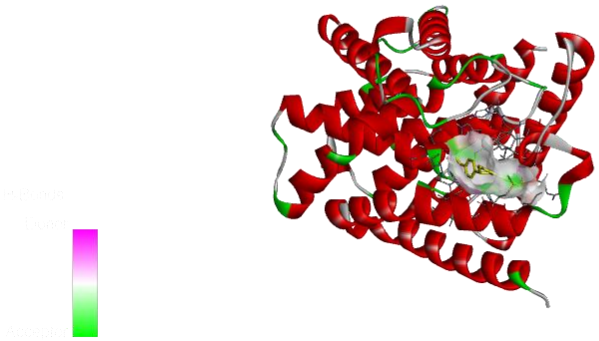


Figure 17: Three-dimensional binding mode of naringenin within the active site of Human PDE5 enzyme

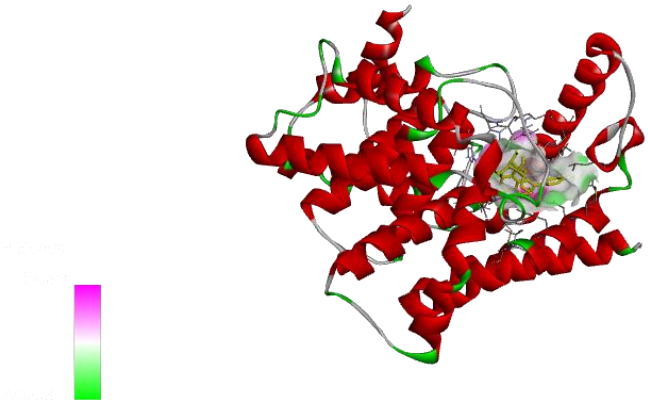


Figure 18: Three-dimensional binding mode of vitexin within the active site of Human PDE5 enzyme

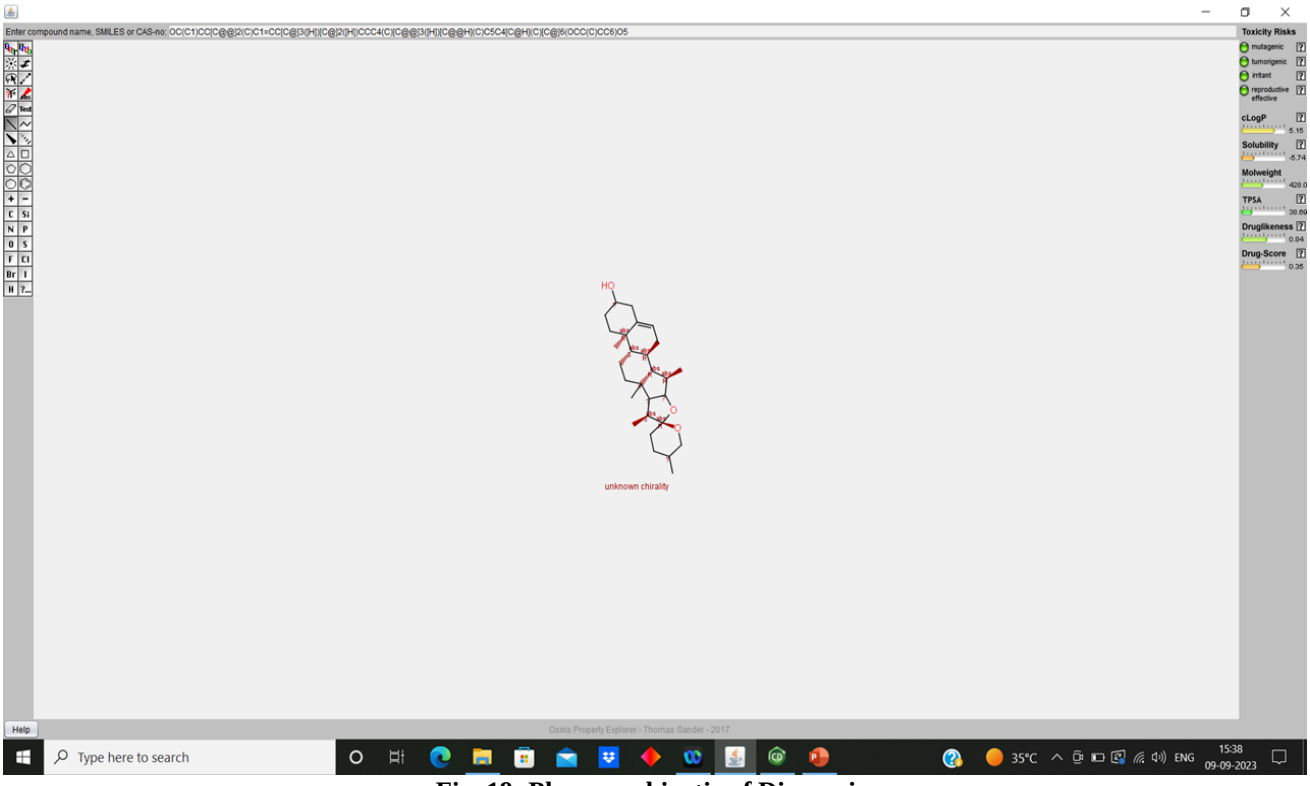


Fig. 19: Pharmacokinetic of Diosgenin

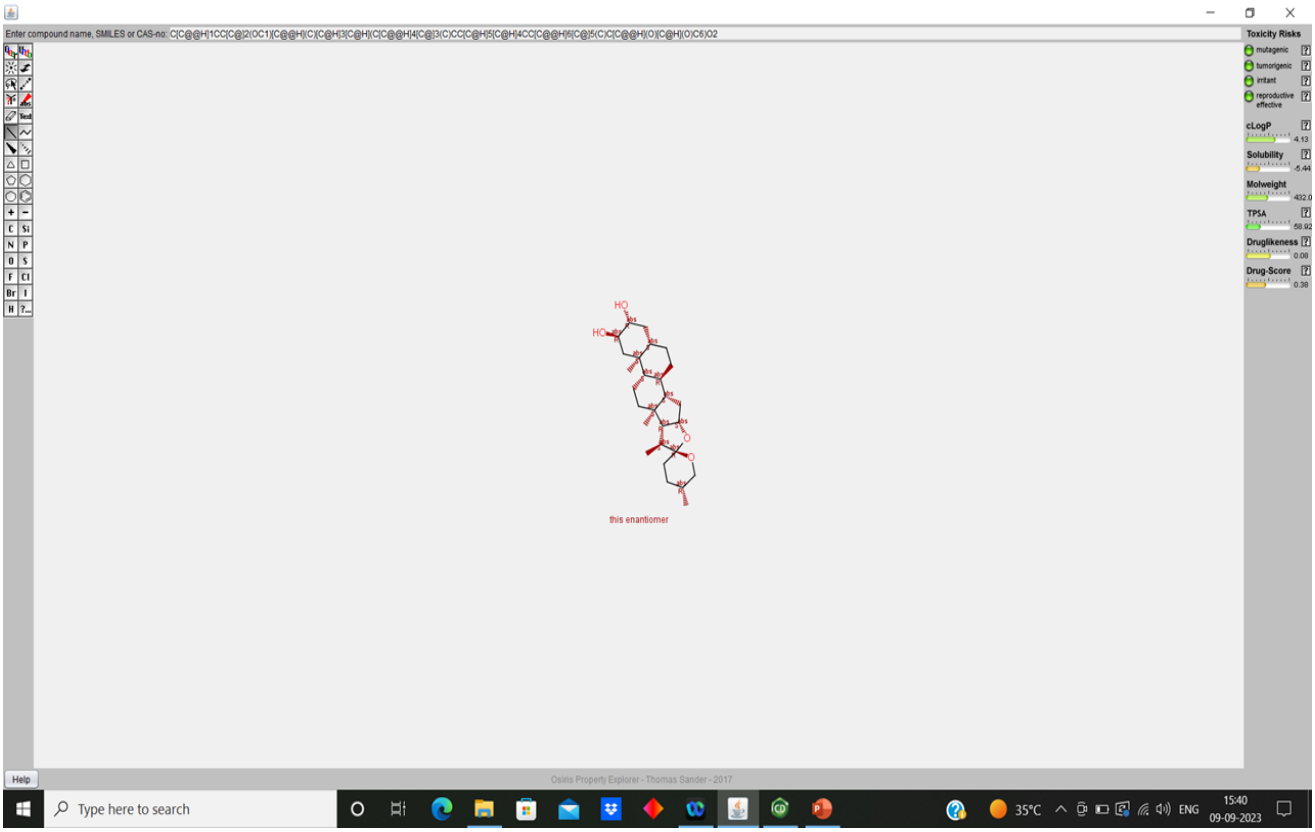


Fig. 20: Pharmacokinetic of Gitogenin

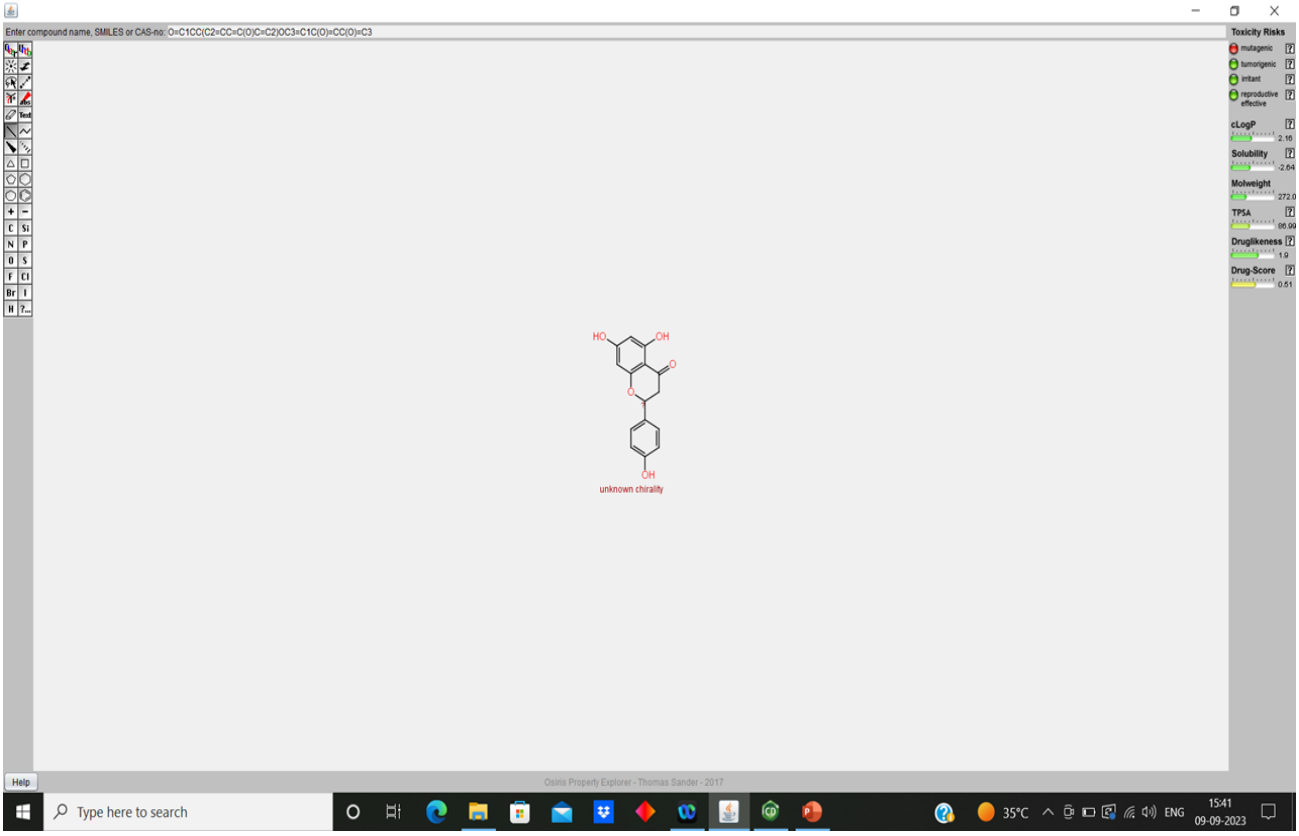


Fig. 21: Pharmacokinetic of Naringenin

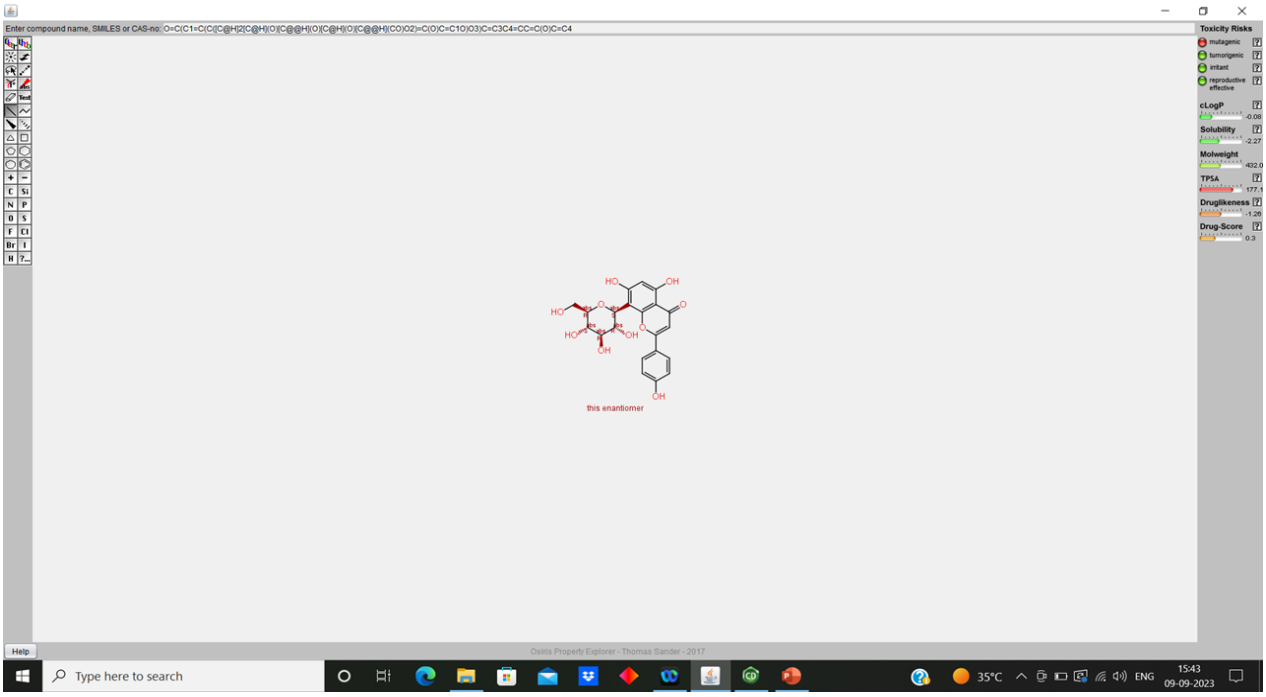


Fig. 22: Pharmacokinetic of vitexin

Fig. 23: Pharmacokinetic of Sildenafil

CONCLUSION

Herbal preparations in Ayurveda system of medicine always have a unique range of beneficial effect because of its wonderful therapeutic value without causing adverse effects. Here the plant fenugreek seed was scientifically validated for aphrodisiac potential. The active constituents found in the seed was taken as lead molecule for computational based molecular docking study against PDE-5 enzyme. In the present design computational-based experiment an attempt had been made to evoke aphrodisiac potential of fenugreek seed constituents against *PDE-5* enzyme. The outcome of findings revealed that steroidal saponin(diosgenin) and flavonoid(vitexin) showed potent inhibitory effect on *PDE-5* enzyme which reflects the efficacy of fenugreek seed as potent aphrodisiac agent *via* synergetic effect of steroidal saponin and flavonoid. So, the clinical trials have to be followed on the drug fenugreek seed. Thus, can treat the infertile males effectively and help them to achieve their dream of producing their own offspring.

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