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Allium cepa Bulb Bioactive against Glycogen Synthase Kinase 3 β -protein: An *in-silico* Study on the Mechanism of Wound Healing Validation

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Abstract: ***Background*:** Hemostasis, inflammation, proliferation, and remodelling are all stages in the multiphase process of wound healing. Poor blood flow to the wound bed and microbial infection are two additional variables that might hinder the wound healing process. Enhancing the patient's systemic and local health as well as creating the perfect environment for wound healing are essential components of successful wound care. Numerous products have been developed to influence the wound environment in order to produce a pathogen-free, safe, and moist environment for healing. In the wound healing cascade, newer materials are being used to supplement or replace older substrates. ***Method*:** In the current work, glycogen synthase kinase-3 β (GSK-3 β) protein inhibitors were sought after using a molecular docking approach. The binding was determined by the Auto Dock software utilising a grid-based docking method. Compounds' 2D structures were constructed using the Merck Molecular Force Field, converted to 3D, and then energetically reduced up to an arms gradient of 0.01. (MMFF). ***Results*:** The molecular docking result revealed that β- sitosterol & kaempferol showed encouraging docking score. The docking score found to be with binding energy -9.26 & -7.41 kcalmol^{-1} for β- sitosterol & kaempferol respectively. ***Conclusion*:** The investigation's results showed that the presence of β -sitosterol and kaempferol in the ethanolic bulbs extract resulted in considerable wound healing efficacy.

Keywords: Wound healing, *In-silico* molecular docking, glycogen synthase kinase-3β (GSK-3β) protein β- sitosterol & kaempferol.

Research Paper

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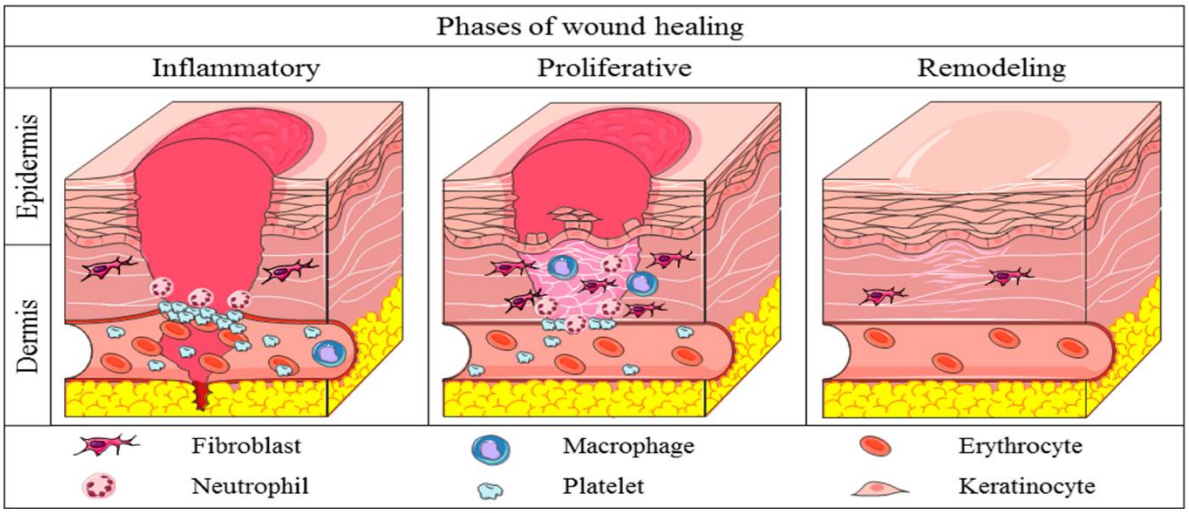
INTRODUCTION

A wound is defined as the destruction of the cellular and anatomic discontinuities in a tissue [1]. The tissue may be injured by a chemical, physical, thermal, microbiological, or immunological attack. Infected wounds and other troublesome issues are more likely to occur in uncomfortable wounds [2]. Diabetes, immune system problems, ischaemia, malnutrition, ageing, local infections, and local tissue damage from burns or gunshots are just a few of the diseases that can delay wound healing. One of the most common outcomes of burn injuries is infection, which accounts for 50–75% of hospital mortality [3]. Wound healing is a coordinated set of procedures that repair the integrity of the damaged tissue. Researchers are looking for alternatives because herbal-based medicines used to heal wounds are typically not just less expensive but also rife with problems including drug resistance and allergy [4]. More than 80% of people still treat their ailments with traditional drugs worldwide [5]. They are particularly important for wound management [6] because they encourage the formation of the optimal environment by supplying a moist environment. The traditional system of medicine asserts that numerous medicinal plants can help with wound healing even if their mechanisms of action and efficacy have not been adequately investigated. The first phase of this process is an acute inflammatory phase, which is followed by the production of collagen and other extracellular macromolecules, which are ultimately altered to generate a scar [7]. Essentially, this mechanism is a connective tissue reaction. When the skin is torn, sliced, burned, or punctured [8], it results in a wound. Numerous physiological systems, including white blood cells [9], fibroblasts, keratinocytes, etc., normally participate in wound healing at the wound site. Protein, lipid, and glucose metabolism all increase as resting energy consumption increases (RES). Antibiotics and anti-cancer drugs may also have an influence. Other disorders, such as diabetes and others, have an effect on wound healing. Wound infection is one of the most common diseases in less developed countries because of the poor hygienic conditions [10–13]. The restoration of broken anatomical continuity and impaired functional status of the skin depend on an effective wound healing technique. Physical injuries that cause the skin to split or

open are called wounds [14]. For the treatment of burns and wounds, many traditional healers around the world, particularly in countries like India and China, have vital knowledge of a variety of lesser-known, previously uncovered wild herbs. One of the newer areas of study in the biomedical sciences today is the study of drugs that treat wounds [15–18]. Traditional medical practises that have been utilised for many years in Asia and Africa to treat wound-related illnesses are currently the subject of scientific study. According to numerous traditional medical practises around the world, wounds are often treated topically with different medicinal plants or with their extracts alone or in combination with some other plant parts [19].



Phases of wound healing

The perennial herb *Allium cepa*, often known as the onion, has its stem in the subterranean bulb. In the family Liliaceae are onions. Antimicrobial, antioxidant, analgesic, anti-inflammatory, anti-diabetic, hypolipidemic, anti-hypertensive, and immunoprotective actions are just a few of the many pharmacological qualities [20].

It has been found that the Wnt/b-catenin pathway, which promotes wound healing, inhibits the glycogen synthase kinase-3 (GSK-3) protein, an essential regulatory enzyme. The possibility of using a variety of medicinal plants as potential sources of drugs to treat wounds has been looked at. In the present study, we in-silico screened the phytoconstituents β- sitosterol & kaempferol on GSK-3 β protein and described the proposed mechanism of action of flavonoids for their wound-healing properties.

S. No	Bioactive	Description
1.	β- sitosterol	Numerous cell signalling pathways, including those involved in the cell cycle, apoptosis, proliferation, survival, invasion, angiogenesis, metastasis, anti-inflammatory, anticancer, hepatoprotective, antioxidant, cardioprotective, and antidiabetic effects have been shown to be affected by beta-sitosterol in studies, with little to no toxic side effects [21, 22].
2.	kaempferol	Kaempferol has been shown to modify a variety of essential components in cellular signal transduction pathways connected to apoptosis, angiogenesis, inflammation, and metastasis on a molecular level. Significantly, kaempferol suppresses cancer cell proliferation, angiogenesis, and death while also appearing to maintain normal cell viability and occasionally exhibiting a protective impact [23, 24].

Experimental work

The *A. cepa* bulb contains -sitosterol, kaempferol, myritic acid, and ferulic acid, according to a literature review (Arka Jyoti Chakraborty *et al*., 2022) [26]. Additionally, according to the current experiment, chromatographic analysis proved that the ethanolic bulb extract contained kaempferol and β -sitosterol. So, for the *in-silico* validation investigation, β-sitosterol and kaempferol were chosen as the lead molecules.

Ligand Preparation

2D Structure of ligands like beta-sitosterol and kaempferol were drawn using ChemSketch, the two-dimensional structures of the prepared ligands were converted into their 3-D structures optimized with 3D geometry [25]. The optimized structures were saved in PDB format for AutoDock compatibility. The basic structures of the prepared ligands were given below:

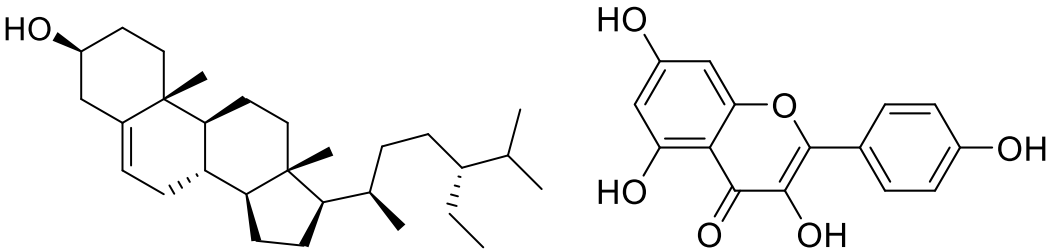


Fig. 1: 2D structure of beta-sitosterol and kaempferol

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 and grid points is given in table 1 [26, 27].

Table 1: Grid parameters used in current docking analysis of GSK-3-beta

S. No	Receptor	x-axis	y-axis	z-axis	Spacing	x center	y center	z center
1	GSK-3-beta	40	40	40	0.392	23.936	-17.104	9.189

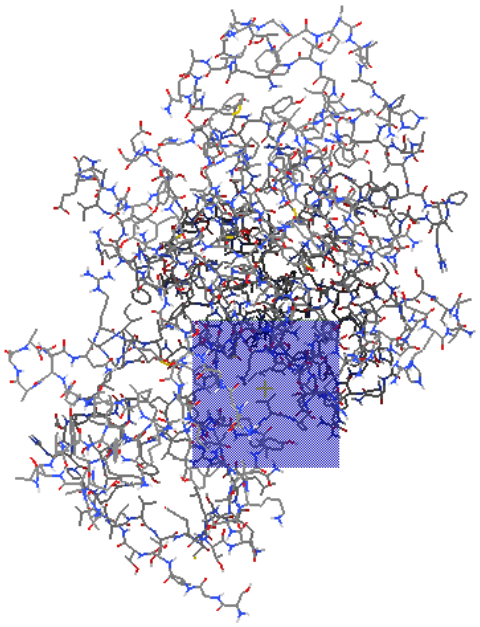


Fig. 2: Grid box covering all active sites in GSK-3-beta receptor

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 [28-30].

Docking Study

Crystal structure

The crystal structure of the protein consisting of GSK-3-beta receptor is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (7oy5.pdb) registered in the Protein data bank was used [31-33]. The complex ligand was separated by using Chimera software.

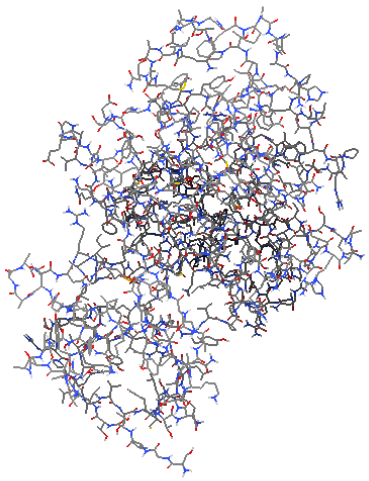


Fig. 3: Crystal structure of GSK-3-beta receptor (PDB ID-7oy5)

Processing of Protein

The downloaded receptor protein is having only one chains, i.e. chain A, which has been selected for experimental purpose and complex ligand was removed from it. The bound ligand was separated from the macromolecular complex by using software Chimera [34, 35].

Molecular Docking Simulation Studies

Docking of ligands like beta-sitosterol and kaempferol against GSK-3-beta receptor was performed by Autodock. All the bonds of each ligand were kept flexible, while no residues in receptor were made flexible [36].

Toxicity & ADME-T Studies

The ligand molecules viz. beta-sitosterol and kaempferol 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 [37].

RESULT AND DISCUSSION

Hemostasis, inflammation, proliferation, and remodelling are all stages in the multiphase process of wound healing. Poor blood flow to the wound bed and microbial infection are two additional variables that might hinder the wound healing process.

A. cepa's different medicinal and pharmacological properties were evaluated because it is one of the most significant condiment plants farmed and consumed globally. The perennial herb known as the onion (Allium cepa) is high in dietary fibre and is a member of the Amaryllidaceae family. In addition to vitamins and minerals, it has a high concentration of folic acid, vitamin B6, magnesium, calcium, potassium, and phosphorus. It is extensively used as an antibacterial drug, but it also has actions against cancer, diabetes, hypertension, platelets, and depression. It also has neuroprotective, anti-inflammatory, and antiparasitic properties. The immune system, as well as the digestive, circulatory, and respiratory systems, are reported to benefit from its use.

The FGF/TGF- and Wnt/-catenin pathways are two crucial cell signalling pathways that promote wound healing and regeneration. The function of fibroblast cells and the speed of wound healing may be impacted by a disruption in the release of these substances. The Wnt/-catenin pathway is one of them and is essential for cell proliferation during wound healing. The GSK3 β - enzyme phosphorylates and degrades the -catenin protein, making it the most significant signalling step in this pathway. When GSK3 β - is inhibited, activated -catenin travels to the nucleus to control gene expression. Small compounds like glycogen synthase kinase 3- β (GSK3- β) inhibitors may be beneficial agents for enhancing wound healing, according to previous studies utilising a variety of animal models.

It was discovered through a computationally based docking analysis that both lead compounds exhibit strong *GSK3*- β inhibiting effects. The results demonstrated a promising docking score and lead molecule's pattern of binding to the target protein's active region with strong covalent bonding.

It has been investigated whether a range of medicinal plants could serve as potential sources of medications that treat wounds. We performed an *in-silico* screening of the phytoconstituents β-sitosterol and kaempferol on ***GSk-3β*** enzyme. *A.cepa* found to be effective wound healing agent and their lead molecules effectively binds to be target protein ***GSk-3β*** enzyme

with binding energy -9.26 & -7.41 kcalmol^{-1} for β-sitosterol & kaempferol respectively. The result was tabulated in table 2. The binding mode of selected lead molecules showed in fig.4-5. The 2D and 3D interaction of selected compound displayed in Fig 6-11. The interaction of β- sitosterol & kaempferol with active site at ***GSk-3β*** enzyme showed as follows:

Compound	**Conventional Hydrogen bounding**	**Pi-sigma bounding**	**Covalent bounding**	**Week Vander's interaction**
β- sitosterol	ASP^{200} ASN^{186},	PHE ^{67}	LYS^{85}, VAL^{110} LEU^{132}, CYS^{197} ALA^{83}, LEU^{188} TYR^{134}, ILE^{62} VAL^{70}	VAL^{135}, Pro^{136}
Kaempferol	VAL^{135} ASP^{200} ASP^{133}	LEU^{132} ALA^{83} LYS^{85} VAL^{110}	LEU^{188}	Met^{101}, ILE^{62}, PRO^{136}, THR^{138}, GLU^{137}, TYR^{134}

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 β- sitosterol & kaempferol were shown in Figure 12 & 13. Theoretically, all the ligand molecules have shown encouraging docking score.

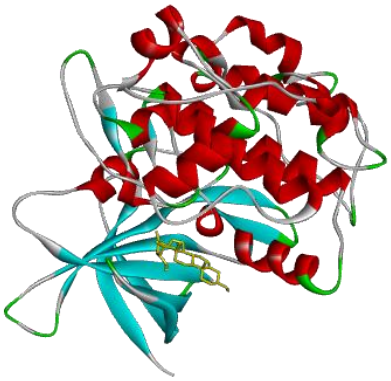


Fig. 4: Binding mode of beta-sitosterol within the active site of GSK-3-beta receptor

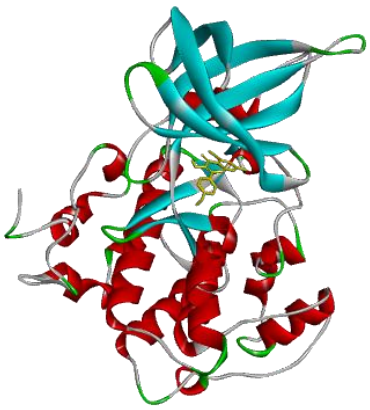
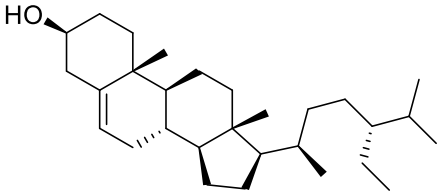
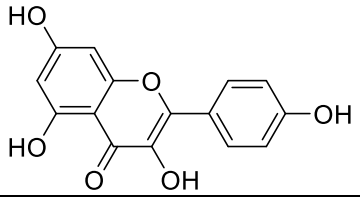


Fig. 5: Binding mode of kaempferol within the active site of GSK-3-beta receptor

Table 2: Results of docking of ligands like beta-sitosterol and kaempferol against GSK-3-beta receptor

Sl. No	Compound Name	Structure	Binding Energy (Kcal/mole)
1	Beta-sitosterol		-9.26
2	Kaempferol		-7.41

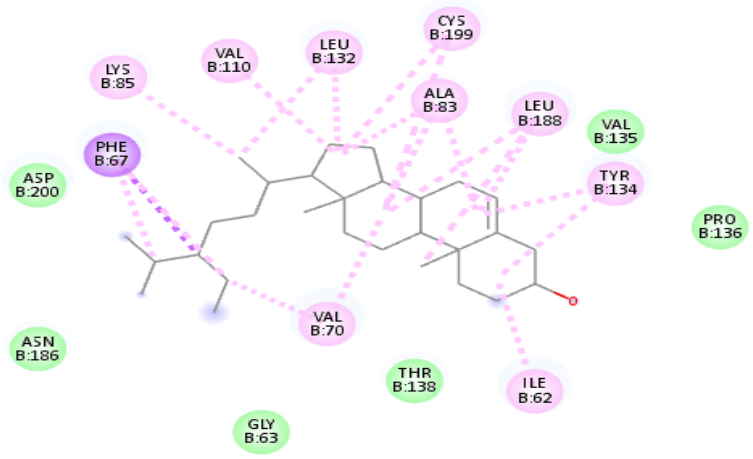


Fig. 6: Two-dimensional binding mode of beta-sitosterol within the active site of GSK-3-beta receptor

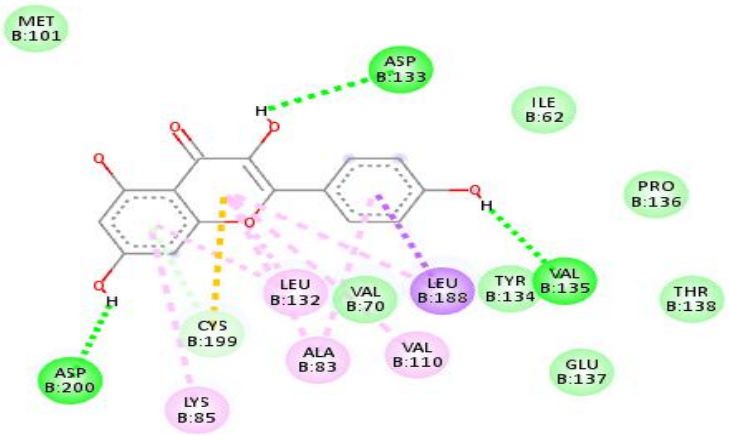


Fig. 7: Two-dimensional binding mode of kaempferol within the active site of GSK-3-beta receptor

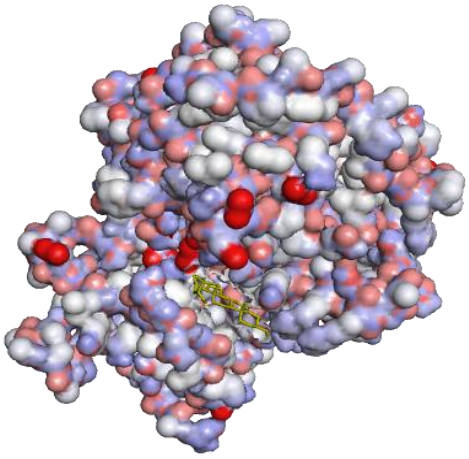


Fig. 8: Three-dimensional binding conformation of beta-sitosterol within the active site of GSK-3-beta receptor

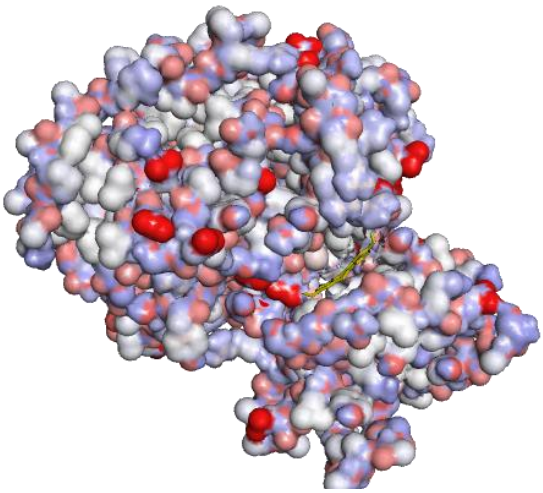


Fig. 9: Three-dimensional binding conformation of kaempferol within the active site of GSK-3-beta receptor

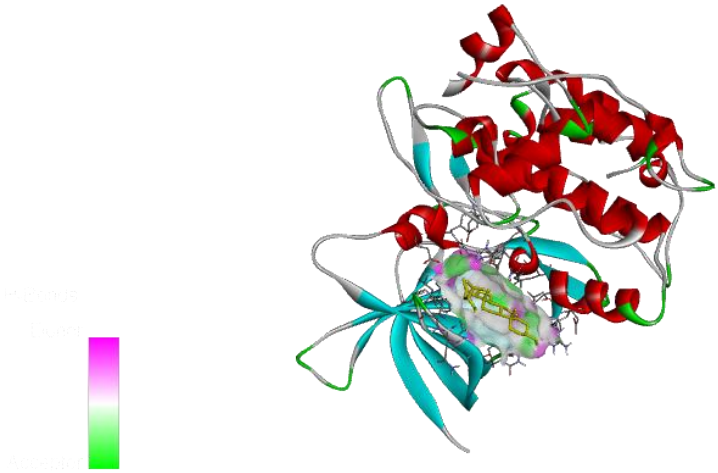


Fig. 10: Three-dimensional binding mode of beta-sitosterol within the active site of GSK-3-beta receptor

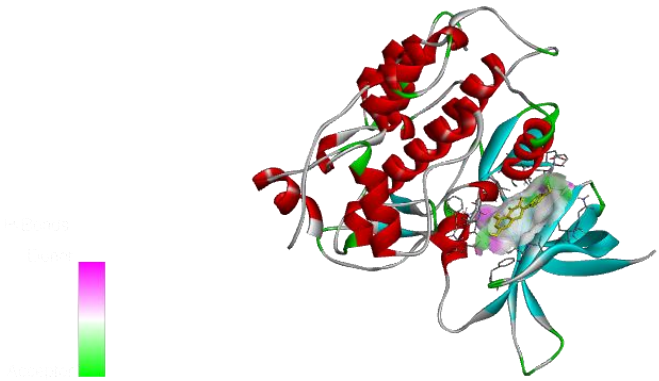


Fig. 11: Three-dimensional binding mode of kaemferol within the active site of GSK-3-beta receptor

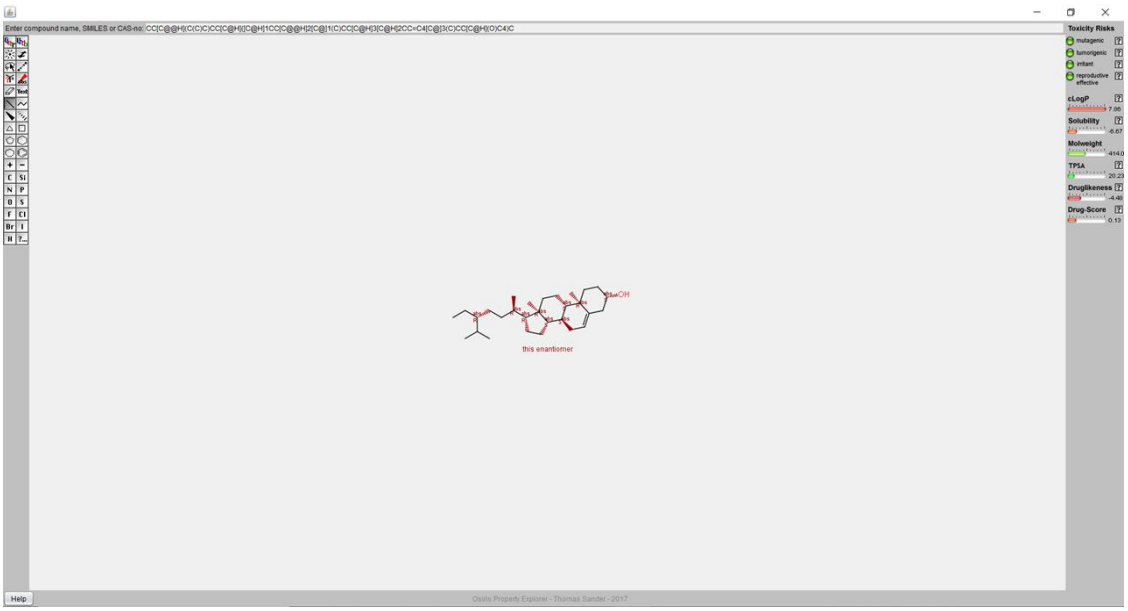


Fig. 12: Pharmacokinetic and toxicity profiling of beta-sitosterol

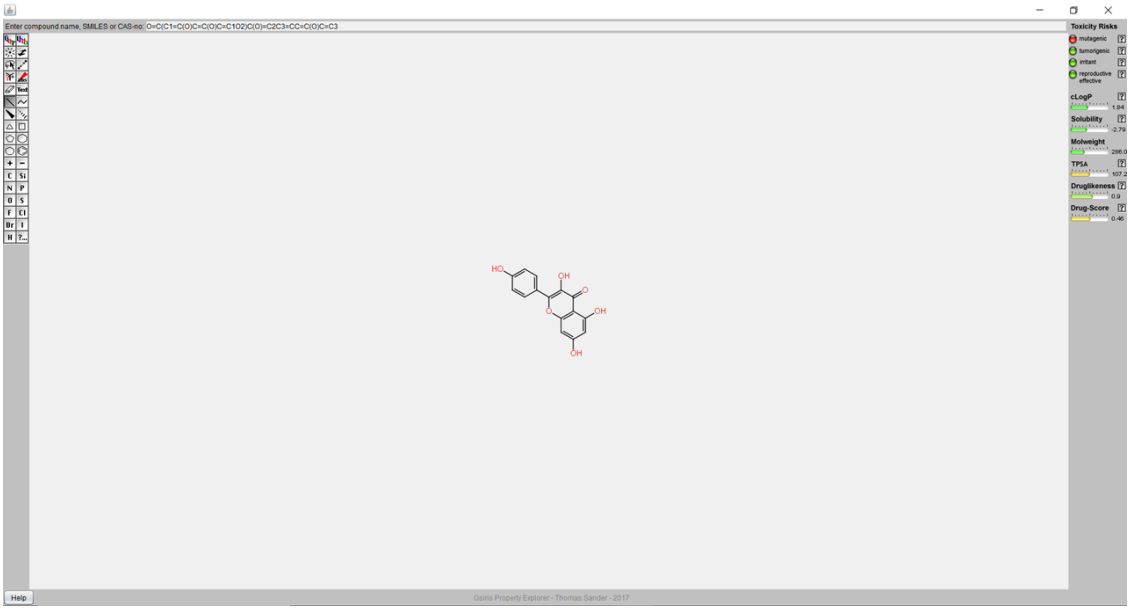


Fig. 13: Pharmacokinetic and toxicity profiling of kaempferol

CONCLUSION

It was discovered through a computationally based docking analysis that both lead compounds exhibit strong ***GSK3***- β inhibiting effects. The results demonstrated a promising docking score and lead molecule's pattern of binding to the target protein's active region with strong covalent bonding. The synergistic impact of kaempferol and β -sitosterol is what gives ethanolic bulb extract from *A. cepa* its ability to heal wounds.

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