

IMMUNOSUPPRESSANTS IN SJOGREN'S SYNDROME

Submitted in partial fulfillment of the
requirements for the award of Bachelor
of Science Degree in Bioinformatics and
Data Science

By

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ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **SAMHITHA S (Reg.No: 40738016)** who carried out the project entitled "**IMMUNOSUPPRESSANTS IN SJOGREN'S SYNDROME**" under my supervision in October 2023.

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DECLARATION

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ABSTRACT

Sjogren's syndrome is a lymphoproliferative disease with autoimmune features characterized by mononuclear cell infiltration of exocrine glands, notably the lacrimal and salivary glands. There is no particular drug for the treatment of this syndrome. To overcome and control this condition, medicinal plants are the versatile solution with less side effects when compared to synthetic drugs. The medicinal plants are the natural reservoir of medicinal compounds to cure many diseases since the compounds have no adverse side effects. The current study is focused on their in-silico activity by adopting molecular drug designing methods. The main research of this work is to investigate the naturally available phyto-compounds by In-silico molecular drug designing approach against Nf-Kb inducing kinase. Molecular docking analysis of phyto-compounds - Catechin, Secoisolariciresinol, Epicatechin, Hesperetin, Maslinic Acid, Hydroxytyrosol, Lariciresinol, Ferule acid, Sinapic acid, Caffeic Acid, Quercetin, Apigenin and Octanoic acid showed best result with Nf- Kb inducing kinase. Their pharmacotherapy and adverse effects have also been summarized. The molecular drug designing software BioDiscovery studio and 3D molecular visualization CDOCKER-BIOVIA software were used. Moreover, this study suggested performing further in-silico, in-vitro and in-vivo experiments in order to understand the therapeutic potential of these phytocompounds on this prototypical anti-inflammatory pathway that is involved in both active and adaptive immunity. This could lead to novel insights in the therapeutic treatment of other autoimmune diseases and inflammatory conditions.

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CHAPTER 1

INTRODUCTION

Sjogren's syndrome (SS) is an immune-mediated inflammatory condition characterized by sicca syndrome, musculoskeletal pain, and fatigue. Extra glandular manifestations are common and there is a markedly increased risk of lymphoma development. SS is associated with a high health-economic burden driven largely by the symptom burden on patients. Currently, there is no approved disease-modifying treatment and management is based on empirical evidence. Progress in the understanding of SS pathogenesis has led to an expanding portfolio of more targeted therapies under development. It is histologically characterized by the lymphocytic infiltration of exocrine glands. It is estimated that 30–40% of patients develop extra- glandular features including pulmonary, articular, cutaneous, muscular, renal and neurological manifestations (Båve et al ., 2005).

In addition, there is an estimated lifetime risk of lymphoma between 5–10% (Begovich, et al ., 2005).

Patients with SS carry significant symptom burden, accompanied by reduced health-related quality of life and high health economic burden to the patients and society (Blomhoff et al ., 2005).

Despite considerable clinically unmet needs, effective evidence-based treatments have not been developed.

1.1 COMMON SYMPTOMS AND OCCURENCE

It is characterized by dryness of the mouth, eyes, and other mucous membranes (sicca syndrome) due to lymphocytic infiltration of exocrine glands and secondary gland dysfunction. Sjogren syndrome can affect various exocrine glands or other organs.

Sjogren syndrome occurs most frequently among middle-aged women. The disease is classified as primary when there is no other associated disease. In about 30% of patients with autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus,

systemic sclerosis, mixed connective tissue disease, Hashimoto thyroiditis, primary biliary cirrhosis, or chronic autoimmune hepatitis, Sjogren syndrome develops and, in such cases, is classified as secondary.

Genetic associations have been found (e.g., HLA-DR3 antigens in White people with primary Sjögren syndrome) but are not necessary for diagnosis or clinical management. Diagnosis is by specific criteria relating to eye, mouth, and salivary gland involvement, autoantibodies, and (occasionally) histopathology (Leverenz, St Clair.,2019).

Sjögren's syndrome may have different effects on the body, and the symptoms vary from person to person, individuals affected with this autoimmune condition have flare ups of symptoms that can cycle between mild and severe.

Typically,the symptoms are;

Dry eyes that may burn or itch, the dryness causes blurry vision or sensitivity to bright light. It can also cause the eyelids to get irritated, itchy due to inflammation and mainly,reduced or no tear production.

Dry mouth that can cause trouble swallowing, speaking, and tasting. Due to the lack the protective effects of saliva, the condition could lead to development of more dental decay (cavities) and mouth infections, such as candidiasis (also called thrush). The affected individuals may have either one or both dry eyes and mouth is dry mouth problems. In some cases, Sjögren's syndrome affects other tissues and organs and has more widespread effects on the body.

These other effects can be:

- Fatigue.
- Joint pain.
- Dry skin.
- Dry nasal passages and throat, and a dry cough.

- Skin rashes.
- Muscle aches.
- Acid reflux.
- Vaginal dryness.
- Swelling of the glands around the face and neck.
- Trouble sleeping.
- Poor concentration and memory problems.
- Numbness, tingling, and weakness, especially in the extremities.
- Shortness of breath or trouble breathing.
- Muscle weakness.

The symptoms can be severe, with some people reporting debilitating pain and fatigue. About 5% of individuals affected with Sjögren's syndrome are at a risk of getting cancer. They have a higher chance of developing Lymphoma, due to severely swollen and frequent inflammation of the lymph nodes.

1.2 TYPES AND STAGES OF SJOGREN'S SYNDROME

Sjögren's syndrome (SS) is a systemic as well as an organ-specific autoimmune disease, characterized by destructive lymphocytic infiltration of the salivary and lacrimal glands. SS patients are differentiated into three categories or stages:

Stage I is glandular SS

Stage II is extra-glandular SS

Stage III is extra-glandular SS with lymphoid malignancy

The lympho-aggressive nature of the disease appears to lead SS patients from stage I to

II and from stage II to III. However, stage III patients made up only 5% of SS patients. Many patients remain stable in stages I or II for as long as 10 or 20 years. Therefore, there are factors which trigger patients in stages I or II to progress to stages II or III and that only those patients who have such factors progress from stages I or II to stages II or III, respectively(Sugai et al .,1995).

1.3 DIAGNOSIS AND TESTS

To diagnose Sjögren's syndrome, doctors must detect specific antibodies (blood proteins) in the blood sample taken from the affected individual. A pattern of inflammation, found most often on the salivary glands of the lips, which is a very common characteristic of Sjögren's syndrome.

Observing these concerns the affected individuals are recommend to undergo some or all of the following tests:

- **Blood and urine tests**, to look for the presence of antibodies common in Sjögren's syndrome. The results of an ANA (antinuclear antibody) test will determine whether the patients have an autoimmune disorder.
- **Schirmer's test**, to check tear glands whether they are producing enough tears to keep the eyes moist.
- **Ocular surface staining** to look closely at the surfaces of the eyes for damage and dryness.
- **Salivary gland function scans**, which look at the glands on the sides of neck, below the ears and under the jaw.
- A **biopsy** of the lip to look for inflammation of the glands that produce saliva and tears. This test can determine the type of inflammation and the severity. A biopsy of the lip is performed because the salivary glands just under the lip's inner surface are the easiest glands to access.
- **Sialometry**, which is a test that measures the flow of saliva.

- **Ultrasonography** of the major salivary glands to reveal characteristic structural changes that can aid in diagnosis.

1.4 TREATMENT

Sjögren syndrome should be initially managed by topical therapy of dry eyes and dry mouth. Dry eyes should be treated with lubricating eye preparations (initially drops such as Hypro-mellose or methyl-cellulose and an over-the-counter ointment at bedtime). Other treatments include drainage (punctal) duct closure and topical cyclosporine. Skin and vaginal dryness can be treated with lubricants. Mouth dryness may be avoided by sipping fluids throughout the day, chewing sugarless gum, and using a saliva substitute containing carboxy-methylcellulose as a mouthwash. Drugs that decrease salivary secretion (e.g., antihistamines, antidepressants, other anti- cholinergics) should be avoided. Other systemic manifestations of the disease should be treated depending on the severity and the involved organ. Treatment is usually symptomatic, but visceral organ involvement is treated with cortico-steroids and immunosuppressants.

Overall health and life expectancy are mainly unaffected in patients whose only symptoms are dry eyes and mouth. Aggressive systemic treatment is occasionally indicated; it is usually reserved for patients with associated diseases (e.g., severe vasculitis, optic neuritis, or visceral involvement). Corticosteroids (e.g., oral prednisone 1 mg/kg once a day), or rituximab may be needed in severe disease with cryoglobulinemic vasculitis, peripheral neuropathy/multiple mononeuropathy, severe parotid swelling, severe lung involvement, or inflammatory arthritis that does not respond to non-biologic therapy. However, prognosis is less favorable in those with systemic organ involvement. In those with severe disease, death may occasionally result from pulmonary infection and, rarely, from renal failure or lymphoma. Associated systemic autoimmune disorders may dictate prognosis (Leverenz, St Clair.,2019).

1.5 DRUGS COMPOUNDS CURRENTLY USED IN THE TREATMENT OF SJOGREN'S SYNDROME

Table 1.1. Drug compounds currently used for SS

DRUG	TARGET	PHARMACOLOGICAL ACTION	THERAPEUTIC EFFECT
Pilocarpine	Muscarinic acetylcholine receptors M1,M2,M3,M4	Agonist Pharmacological active	Stimulates saliva production
Cevimeline	Muscarinic acetylcholine receptors M1,M3	Agonist Pharmacologically active	Stimulates saliva and tear production
Methotrexate	Thymidylate synthase	Inhibitor Pharmacologically active	Treatment of Sjögren's arthritis and fatigue
	Bifunctional purine biosynthesis protein PURH		
	Dihydrofolate reductase		
Hydroxy-chloroquine	Toll-like receptor 7	Antagonist Pharmacologically active	Treatment of Sjögren's arthritis, fatigue and rashes.
	Toll-like receptor 9		

1.5.1 Pilocarpine

Pilocarpine is a muscarinic cholinergic agonist used on the eye to treat elevated intraocular pressure, various types of glaucoma, and to induce miosis. Also available

orally to treat symptoms of dry mouth associated with Sjogren's syndrome and radiotherapy.

This compound belongs to the class of organic compounds known as alkaloids and derivatives. These are naturally occurring chemical compounds that contain mostly basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. Also some synthetic compounds of similar structure are attributed to alkaloids. In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulfur and more rarely other elements such as chlorine, bromine, and phosphorus.

Pilocarpine is a cholinergic parasympathomimetic agent. It increase secretion by the exocrine glands, and produces contraction of the iris sphincter muscle and ciliary muscle (when given topically to the eyes) by mainly three pharmacologically active, stimulating muscarinic receptors.

Table 1.2. Drug compounds currently used: Pilocarpine

TARGET	GENE NAME	GENERAL FUNCTION	SPECIFIC FUNCTION
Muscarinic acetylcholine receptor M1	CHRM1	Phosphatidylinositol phospholipase c activity	The muscarinic acetylcholine receptor mediates various cellular responses, including inhibition of adenylate cyclase, breakdown of phosphoinositides
Muscarinic acetylcholine receptor M2	CHRM2	G-protein coupled acetylcholine receptor activity	
Muscarinic acetylcholine receptor M3	CHRM3	Receptor activity	

1.5.2 CEVIMELINE

Cevimeline is a cholinergic agonist which binds to muscarinic receptors. Muscarinic agonists in sufficient dosage can increase secretion of exocrine glands, such as salivary and sweat glands and increase tone of the smooth muscle in the gastrointestinal and urinary tracts. Cevimeline binds and activates the muscarinic M1 and M3 receptors. The M1 receptors are common in secretory glands (exocrine glands such as salivary and sweat glands), and their activation results in an increase in secretion from the secretory glands. The M3 receptors are found on smooth muscles and in many glands which help to stimulate secretion in salivary glands, and their activation generally results in smooth muscle contraction and increased glandular secretions. Therefore, as saliva excretion is increased, the symptoms of dry mouth are relieved.

This compound belongs to the class of organic compounds known as azaspirodecane derivatives. These are organic compounds containing a spirodecane moiety with at least one nitrogen atom.

Cevimeline is a muscarinic agonist with parasympathomimetic activities that is used for the symptomatic treatment of dry mouth in patients with Sjögren's Syndrome.

Table 1.3. Drug compounds currently used: Cevimeline

TARGET	GENE NAME	GENERAL FUNCTION	SPECIFIC FUNCTION
Muscarinic acetylcholine receptor M1	CHRM1	Phosphatidylinositol phospholipase c activity	The muscarinic acetylcholine receptor mediates various cellular responses, including inhibition of adenylate
Muscarinic		Receptor activity	

acetylcholine receptor M3	CHRM3		cyclase,breakdown of phosphoinositides
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1.5.3 Methotrexate

Methotrexate is an antineoplastic agent used the treatment of a wide variety of cancers as well as severe psoriasis, severe rheumatoid arthritis, and juvenile rheumatoid arthritis. Methotrexate enters tissues and is converted to a methotrexate polyglutamate by folylpolyglutamate.

This compound belongs to the class of organic compounds known as glutamic acid and derivatives. These are compounds containing glutamic acid or a derivative thereof resulting from reaction of glutamic acid at the amino group or the carboxy group, or from the replacement of any hydrogen of glycine by a heteroatom. Methotrexate's mechanism of action is due to its inhibition of enzymes responsible for nucleotide synthesis including dihydrofolate reductase, thymidylate synthase, aminoimidazole caboxamide ribonucleotide transformylase (AICART), and amido phosphoribosyltransferase. Inhibition of nucleotide synthesis prevents cell division.

In rheumatoid arthritis, methotrexate polyglutamates inhibit AICART more than methotrexate. This inhibition leads to accumulation of AICART ribonucleotide, which inhibits adenosine deaminase, leading to an accumulation of adenosine triphosphate and adenosine in the extracellular space, stimulating adenosine receptors, leading to anti-inflammatory action. Methotrexate is a folate derivative that inhibits several enzymes responsible for nucleotide synthesis. This inhibition leads to suppression of inflammation as well as prevention of cell division. Because of these effects, methotrexate is often used to treat inflammation caused by arthritis or to control cell division in neoplastic diseases such as breast cancer and non-Hodgkin's lymphoma.

Methotrexate inhibits enzymes responsible for nucleotide synthesis which prevents cell division and leads to anti-inflammatory actions. It has a long duration of action and is generally given to patients once weekly.

Table 1.4. Drug compounds currently used: Methotrexate

TARGET	GENE NAME	GENERAL FUNCTION	SPECIFIC FUNCTION
Thymidylate synthase	TYMS	Thymidylate synthase activity	Contributes to the de novo mitochondrial thymidylate biosynthesis pathway.
Bifunctional purine biosynthesis protein PURH	ATIC	Protein homo-dimerization activity	Bi-functional enzyme that catalyzes 2 steps in purine biosynthesis. Promotes insulin receptor/INSR auto-phosphorylation and is involved in INSR internalization
Dihydrofolate reductase	DHFR	Nadph binding	Key enzyme in folate metabolism. Contributes to the de novo mitochondrial thymidylate biosynthesis pathway. Catalyzes an essential reaction for de novo glycine and purine synthesis

1.5.4 Hydroxychloroquine

Hydroxychloroquine is an antimalarial medication used to treat uncomplicated cases of malaria and for chemoprophylaxis in specific regions. It is a disease modifying anti-rheumatic drug (DMARD) indicated for treatment of rheumatoid arthritis and lupus erythematosus.

This compound belongs to the class of organic compounds known as 4-aminoquinolines. These are organic compounds containing an amino group attached to the 4-position of a quinoline ring system.

Hydroxychloroquine affects the function of lysosomes in humans as well as plasmodia, altering the pH of the lysosomes reduces low-affinity self-antigen presentation in autoimmune diseases. It is active against the erythrocytic forms of chloroquine-sensitive strains of *P. falciparum*, *P. malariae*, *P. vivax*, and *P. ovale*. Hydroxychloroquine is not active against the gametocytes and exoerythrocytic forms including the hypnozoite liver stage forms of *P. vivax* and *P. ovale*. Hydroxychloroquine is not effective against malaria in areas where chloroquine resistance has been reported. Hydroxychloroquine accumulation in human organelles also raise their pH, which inhibits antigen processing, prevents the alpha and beta chains of the major histocompatibility complex (MHC) class II from dimerizing, inhibits antigen presentation of the cell, and reduces the inflammatory response. Elevated pH in the vesicles may alter the recycling of MHC complexes so that only the high affinity complexes are presented on the cell surface. Self peptides bind to MHC complexes with low affinity and so they will be less likely to be presented to autoimmune T cells. Hydroxychloroquine also reduces the release of cytokines like interleukin-1 and tumor necrosis factor, possibly through inhibition of Toll-like receptors.

Table 1.5. Drug compounds currently used: Hydroxylchloroquine

TARGET	GENE NAME	GENERAL FUNCTION	SPECIFIC FUNCTION
Toll-like receptor 7	TLR7	Trans-membrane signaling receptor activity	Key component of innate and adaptive immunity. TLRs (Toll-like receptors) control host immune response against pathogens through recognition of molecular patterns specific to microorganisms.
Toll-like receptor 9	TLR9		

1.5.5 LIMITATIONS OF CURRENT TREATMENTS

Pilocarpine and Cevimeline are contraindicated in patients with uncontrolled asthma, known hypersensitivity, individuals affected by acute iritis and narrow-angle (angle closure) glaucoma as prolonged pupillary constriction and poor dilation were caused when dosed frequently over time.

Methotrexate has a narrow therapeutic index.

Due to the toxic effects of methotrexate, it is indicated for treatment of some forms of arthritis and severe psoriasis only if first line treatment has failed. Symptoms of overdose include hematologic and gastrointestinal reactions like leukopenia, thrombocytopenia, anemia, pancytopenia, bone marrow suppression, mucositis, stomatitis, oral ulceration, nausea, vomiting, gastrointestinal ulceration, and gastrointestinal bleeding.

This should be treated with glucarpidase and not be given leucovorin for 2 hours before or after glucarpidase. Prolonged usage of Hydroxychloroquine may lead to severe hypoglycemia and so diabetic patients are advised to monitor their blood glucose levels.

Patients experiencing an overdose may present with headache, drowsiness, visual disturbances, cardiovascular collapse, convulsions, hypokalemia, rhythm and conduction disorders including QT prolongation, torsades de pointes, ventricular tachycardia, and ventricular fibrillation.

This may progress to sudden respiratory and cardiac arrest. This should be treated with immediate gastric lavage and activated charcoal at a dose of at least 5 times the hydroxychloroquine dose within 30 minutes. Parenteral diazepam may be given to treat cardiotoxicity, transfusion may reduce serum concentrations of drug, patients should be monitored for at least 6 hours, fluids should be given, and ammonium chloride should be given to acidify urine and promote urinary excretion.

1.6 SYSTEMS BIOLOGY

The Trouble shooter Knowledge based drug discovery and systems biology approach will be of great help to combat these deadly pathogens. Systems biology is an emerging discipline that explains and gives better insight about the molecular interactions and the relationship between physiology and phenotype. Various statistical, mathematical and computational approaches are used to understand the biology of Sjogren's syndrome. Systems level approaches will be a great tool to understand the molecular interactions and the metabolomics of this disease. Data does not appear to be in short supply in contemporary biology. The development of high throughput technologies, in particular, has generated massive amounts of information. While these technologies produce information about the chemistry of a system, as with the sequence and structure databases, the biological status of the organism is often of little importance. Systems biology relies on a wide variety of data types. Some of these have become fairly standard, such as sequence and structure information, mass spectrometry, and microarrays.

The interaction of experiment and models requires accurate datasets to infer network structures, to create the models, and to test and distinguish the predictions from multiple models. Successive iterations of model building, prediction, experiment, and subsequent refinements of the models are the result. This repeating cycle of experimentation and

theoretical work is the engine that pushes the progress of systems biology research (Brkic Z, et al., 2013).

Consequently, while the concept of systems biology is not all that new, one reason that it has displayed renewed vigor is the impressive advance of experimental technologies. The development and spread of various approaches to high-throughput measurements have been contributing to generation of a large amount of systematic data from a wide variety of biological systems. As systems biology emerges in the post-genomic era, the emphasis is shifting from annotation of individual genes and gene products to ascertaining how DNA-protein and protein-protein interactions occur within a complex network of structural, metabolic, and regulatory pathways in cells. This goal is, of course, aligned with that pursued in “reductionist” molecular cell biology for the past two decades, in which efforts to identify and characterize pathways typically have proceeded in a component centric manner, beginning with an initial gene or protein of particular interest and attempting to ascertain other genes and/or proteins involved in the same pathway.

However, although component-centric approaches have been successful in assembling most of the available knowledge about pathways to date, they have several inherent difficulties. First is the time required: accurate models of pathway function emerge only after evidence is accumulated over many years, with the work of many researchers at many laboratories. Second, these approaches do not directly reveal how multiple pathways influence each other or reveal this crosstalk only accidentally.

Systems biology can offer an accelerated approach to this goal of identifying networks by which genes and proteins interact to carry out cellular operational and regulatory functions, using computational mining methods on high-throughput experimental data. The resulting high-level models are finding increasing utilization as tools for drug

discovery, both by small companies as well as large pharmaceutical companies.

1.7 CURRENT STATUS OF DRUG DEVELOPMENT

Early trials in SS failed to meet their primary outcomes which may in part be due to the use of inappropriate or insensitive study endpoints. Recent trials targeting B-cells, B-T cell co-stimulation and IFN signaling have shown promising results. Development of composite endpoints including patient reported outcomes and objective disease measure may provide a more holistic approach to disease assessment. The impact of these new tools on therapeutic development that benefit patients remains to be fully evaluated.

1.8 HERBAL DRUG DELIVERY

In ancient days, humans used natural herbal products as medicines for a variety of diseases. Majority of the pharmaceutical compounds that are being used nowadays are derived from plant resources. Secondary metabolites of plants are well known for their pharmacological property and are exclusive to a specific plant. They exhibit significant characteristics such as chemical diversity, macromolecular specificity and less toxicity. These properties make them used as lead molecules in drug discovery.

CHAPTER 2

LITERATURE SURVEY

In the past decade, advances in the understanding of the pathology of SS have identified a growing portfolio of potential therapeutic targets. Whilst the initial disease trigger remains unclear, innate immunity appears to play a role in the disease onset. For instance, an interferon (IFN) gene signature of IFN-induced over expressed genes has been identified in the salivary glands and peripheral blood of patients with SS (Callaghan R et al ., 2008).

IFN activation induces the cytokine B-cell activating factor (BAFF) (Daridon C et al ., 2009), which is aberrantly produced in T cells, B cells, and salivary gland epithelial cells

(Das et al ., 2020).

The activated NF- κ B signaling pathway plays an important role in pathogenesis of primary Sjögren's syndrome (pSS) (Chen et al ., 2015)

Adaptive immunity also plays a key role in SS pathogenesis. B-cell dysfunction is a hallmark of SS pathology. BAFF (B-cell activating factor) provides a key link between innate immune responses and adaptive autoimmunity initiation, although other cytokines may also be involved (Delaleu et al ., 2008).

BAFF levels are raised within the salivary glands of SS patients (Das et al ., 2020). Serum BAFF levels correlate with an increase in the disease associated auto antibodies anti-Ro/SSA, anti-La/SSB and rheumatoid factor (RF) (Delaleu et al ., 2015)

In addition, BAFF transgenic mice develop a syndrome with clinical features similar to SS (Elkon., Gharavi., Hughes., 1984). The autoantibodies anti-Ro/SSA and anti-La/SSB are common serological findings in SS. Anti-Ro/SSA is found in 50– 70% of SS patients (Emamian et al ., 2009)

Anti-La/SSB is rarely found alone but found alongside anti-Ro/SSA in 30–60% of patients]. Anti-Ro/SSA and anti-La/SSB alongside rheumatoid factors can appear up to a median of 4–6 years prior to the onset of symptoms (Fox RI et al., 1984).

The Ro antigen is a ribonucleoprotein complex containing RNAs with the two proteins Ro-52 or Ro-60 (with the number indicating their molecular weight in kD (Furugaki K et al ., 2008).

The La antigen is a 48kD transcription factor for RNA polymerase III. Other autoantigens have also been described within SS, however their relative contribution to pathogenesis and role within therapeutic research is still uncertain (Groom et al ., 2002).

Clinically, the presence of the autoantibodies anti-Ro/SSA and anti-La/SSB is associated with higher prevalence of glandular and extra glandular manifestations (Harborne., 1973).

However, whether these autoantibodies are directly pathogenic remains unclear.

Conceivably, these autoantibodies may induce IFN production by dendritic cells (Hazalin. N et al ., 2012).

IFN α in turn upregulates nuclear translocation of Ro52 which may contribute to IFN α driven apoptosis (Hernández-Molina et al ., 2011).

Increasing IFN activation in this manner may then contribute to BAFF signaling and B-cell dysfunction (Hileman., 2006).

Several genetic loci are shown to be involved in the etiology of multiple autoimmune diseases in humans and support sharing of underlying disease mechanisms across related phenotypes. Associations of certain HLA loci with autoimmune diseases have been reported extensively in SS, SLE, RA, ankylosing spondylitis, psoriasis, multiple sclerosis, and type 1 diabetes (Hernández-Molina et al ., 2011).

A growing list of non-HLA genes also has been implicated in multiple autoimmune diseases. Examples include associations of cytotoxic T-lymphocyte–associated antigen 4 (CTLA4) with AITD, type 1 diabetes mellitus (T1D), celiac disease, Wegener's granulomatosis, SLE, vitiligo, Addison's disease, and RA (Hunt et al ., 2005)

Programmed Death 1(PD-1). with RA, T1D, and SLE;31 and protein tyrosine phosphatase non receptor type 22 (PTPN22) with SLE, RA, T1D, Graves' disease, and Hashimoto's thyroiditis (Jadid et al ., 2002).

Interferon regulatory factor 5 (IRF5) and signal transducer and activator of transcription 4 (STAT4) are genes strongly associated with SLE for which there are recent data suggesting association in pSS (Raman et al ., 2008).

Murine studies also are consistent with models of multigenic inheritance, and many susceptibility loci have been identified that are shared across different autoimmune mouse models for SS and other autoimmune diseases (Ladner et al.,2005).

The utilization of herbal plants has been done since antiquated occasions and should even be viewed because of the beginning of current medication. Mixtures of plant

beginning are and still are a big wellspring of mixtures for drugs. A modest amount of each single tracheophyte i.e. the plants with both the Xylem and phloem for the transportation of water also as minerals are used as medicinal plants (Lendrem et al ., 2014).

Data has estimated that the medicinal plants were to be around 350,000 species] and almost half 1,000,000 were to be medicinal species (Luo G et al ., 2020).

From ancient occasions, plants utilized in medicines are so far utilized today for many the acute also as chronic diseases (Mariette et al ., 2003).

The experimentation technique was utilized to treat illnesses before the normalization of the successful conventions or maybe basically to feel good, to acknowledge valuable plants with helpful impacts and properties.

Till today, many higher plants are developed worldwide to urge helpful substances for bringing into the sector of medication and drug stores (Mariette et al ., 2015).

The use of bioactive mixtures detached and faraway from restorative plants as helpful specialists has been a big region in biomedical and furthermore the disclosure of novel regular items in research (Meijer et al ., 2015).

Human protein encoded by HOXA5 quality and located as bunches named A, B, C and D on four separate chromosomes, methylation of this quality directs the tumor silencer P53 and accepts fundamental works in tumorigenesis. (Oves, M.et al., 2018).

Mitogen Activated Protein Kinase 14 similarly called as P38 – which encodes MAPK 14 quality and it's additionally called pressure started serine/threonine unequivocal kinases (SAPKs). They're perceived in activated safe cell macrophages with important works in combustible cytokine selection (Tumor Necrosis Factor alpha) (Oves, M.et al., 2018).

The foremost minimal restricting energy and restraint constants of terpenoids and flavonoids obtained through docking templates are examined for his or her In vitro acetylcholinesterase inhibitory development using tacrine as standard (Prokunina et al ., 2004).

Serum and Glucocorticoid induced Kinase isoform 1 (alluded to as SGK 1) may be a positive authoritative of ENa C. SGK articulation is turned on due to various improvements and incorporates data from a couple of pathways including the insulin, mineralocorticoid and Camp flagging pathway. Apoptosis regulator BAX is understood as Bcl-2 protein 4 which encodes by BAX characteristics and that they have a spot with Bcl-2 quality family. Bcl-2 works as an apoptotic activator and it's coordinated by the tumor silencer p53 (Strandberg , et al ., 2008).

Chloroquine (CQ) is used to prevent and treat malaria and amebiasis, while hydroxychloroquine (HCQ), a less toxic metabolite of chloroquine, is used to treat rheumatic diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA) and Sjogren's syndrome. (Jorge et al.,2018).

While generally considered safe, several adverse effects of HCQ and CQ have been reported, gastrointestinal discomfort being the most common. Allergic reactions rarely occur. Even rarer adverse effects include cardiomyopathy, cardiac conduction defects, neuromyotonic, cytopenia, and skin hyperpigmentation. (Bansal et al .,2019)

CHAPTER 3

AIM AND SCOPE

3.1 AIM

To identify binding interaction between potential natural drug product and Nuclear factor kappa light chain-enhancer of activated B cells (NF- κ B inducing kinase) which is involved in Sjogren's syndrome

3.2 OBJECTIVES

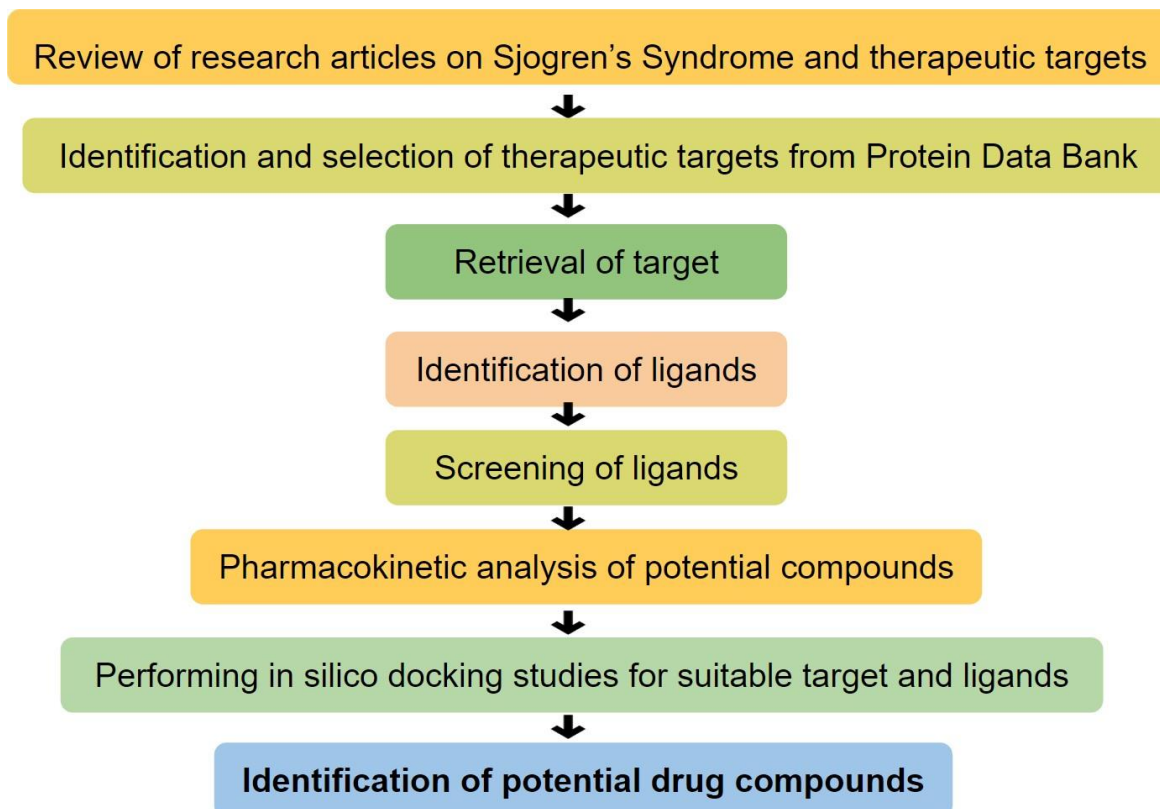
To address the problem associated with the current drug discovery and to fill the gap of in vitro and in silico results we found systems biology approach emerges as a novel approach for target identification in a network of molecules while drug discovery using computational tools is playing major role in reducing the time development of the drug and taken it to market. To perform and achieve observations the experiments were designed and outline as:

- ◆ To understand the current drug development using a system biology approach using docking studies
- ◆ To identify potential intracellular protein and retrieval of plant secondary metabolites from different databases.
- ◆ To understand Bio-molecular interactions and role of NF- κ B inducing kinase in anti-inflammatory signals
- ◆ To understand the physico-chemical properties of natural compounds that bind with the target NF- κ B inducing kinase.

CHAPTER 4

MATERIALS AND METHODOLOGY

4.1. WORKFLOW



4.2. TARGET IDENTIFICATION

Target-identification and mechanism-of-action studies have important roles in small-molecule probes and drug discovery. Biological and technological advances have resulted in the increasing use of cell-based assays to discover new biologically active small molecules. Such studies allow small-molecule action to be tested in a more disease-

relevant setting at the outset, but they require follow-up studies to determine the precise protein target or targets responsible for the observed phenotype. Target identification can be approached by direct biochemical methods, genetic interactions or computational inference. In many cases, however, combinations of approaches may be required to fully characterize on-target and off-target effects and to understand mechanisms of small-molecule action. (Schenone, et al ,2013)

Target identification is a critical step in drug discovery, as it helps to identify and validate specific molecular targets that can be modulated to achieve a therapeutic effect. The first step is to conduct disease biology research to identify potential targets that are involved in the disease's pathology. This can be done through various sources such as scientific literature, databases, and genetic studies. The target will be obtained from the PDB, and heteroatoms will be removed.

4.2.1 Protein Data Bank(PDB)

The Protein Data Bank (PDB) contains three-dimensional (3D) structures of biological substances such as DNA, RNA, and proteins. Obtaining info on biological molecules is a valuable resource for researchers and students. The Protein Data Bank (PDB) contains the 3D structures of proteins found in nature. The identification of the structure of macromolecules aids in the discovery of illness mechanisms. It aids in the understanding of how structure and biomolecules communicate.

4.2.2 Uniprot

Uniprot is used to obtain knowledge about proteins and their functions. Uniprot annotates protein sequence data in great depth, including localization, post-translational modifications, and interactions. Uniprot is a collaboration between the Swiss Institute of Bioinformatics (SIB), the European Bioinformatics Institute (EBI), and the Protein Information Resource. Uniprot connects to several other protein sequence libraries, including UniprotKB/Swissprot, UniProtKB/TrEMBL, and UniRef. The UniProtKB/TrEMBL ratio has yet to be found experimentally. UniProtKB/Swiss-Prot is an experimentally

characterised library. To prevent redundancy, protein sequences are grouped. Uniprot offers tools for sequence analysis, including BLAST, which can be used to identify identical sequences, and PROSITE, which can be used to find protein motifs and domains. (Luo et al ., 2020)

Target-based virtual screening methods depend on the availability of structural information of the target, that being either determined experimentally or derived computationally by means of homology modelling techniques. These methods aim at providing, on one hand, a good approximation of the expected conformation and orientation of the ligand into the protein cavity (docking) and, on the other hand, a reasonable estimation of its binding affinity(scoring). (Ekins et al .,2007).

4.3 LIGAND IDENTIFICATION

Ligand identification and screening of compounds using in silico methods can help to identify natural products. This approach can accelerate the drug discovery process and lead to the development of more effective treatments for obesity and other metabolic disorders. Another approach is to use virtual screening, which involves screening large databases of natural products. Identifying ligands with natural sources can be done by using various resources, such as literature review, traditional knowledge, ethnobotanical databases, or natural product databases like PubChem, ChEMBL, or ZINC. Using the MTI (Medical Text Indexer) open screening tool can also be a useful approach to identify potential ligands for a specific target protein. MTI is a natural language processing tool that can analyze and extract information from scientific literature to identify compounds that are associated with the target protein. (Swedberg ., et al 2015)

4.3.1 PubChem

PubChem is a freely accessible database of chemical molecules and their activities against biological assays.It is maintained by the National Center for Biotechnology Information, a component of the National Library of Medicine,United States National Institutes of Health. It is used to search chemicals by name, molecular formula, structure,

and other identifiers and it has information like chemical and physical properties, biological activities, safety and toxicity information, patents, literature citations and more.

4.3.2 *PhytoHub*

PhytoHub database is a freely available online source containing detailed information about dietary phytochemicals and their human and animal metabolites. For every phytochemical, this database contains information like the chemical structure, physicochemical data such as solubility and physiological charges, the main dietary sources, etc. and these are extracted from the literature by a team of invited experts. It has been designed for use in nutritional metabolomics and in the field of the health effects of plant foods and their phytochemicals. Simple queries can be raised on Phytohub by food source, phytochemical name, molecular formula, monoisotopic mass, or MS/MS fragments, as well as more advanced queries. It is built with MySQL and Perl processing chains, thus providing an intuitive web interface. For a queried monoisotopic mass or molecular formula, the database will return a list of possible metabolites, along with their physicochemical properties, spectral data and possible dietary precursors linked to food sources. For a queried food, it will return a list of metabolites likely to be present in biofluids after consumption (Giacomoni, et al ., 2013).

It also provides information on known human metabolites (also manually extracted from the literature and from online databases by the curators), in silico predicted metabolites. It also provides hyperlinks to other online databases. It is continuously curated and updated by scientists with expertise on the different families of food phytochemicals. It contains over 960 dietary phytochemicals (including 511 polyphenols) and 375 known human or animal metabolites of these compounds. For every compound PhytoHub provides a downloadable chemical structure as well as identifiers, synonyms and physicochemical properties, occurrence in foods, known metabolites with biofluid location and associated references, as well as mass spectral data with search possibilities for use in metabolomics.

Some data are compiled from diverse databases/spectral libraries or computed directly from the chemical structure, whereas data on dietary sources and known metabolites are manually extracted from the literature by experts in the field. This is a major strength of PhytoHub as it provides information that cannot be found yet on any other database (Giacomoni, et al ., 2013).

4.4 LIGAND SCREENING-ADMET DATA PREDICTION

Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties play a versatile part in drug designing steps. These properties accounted for the major reason for drug failure in clinical trial phases. In the traditional drug development process, the ADMET properties were employed at the lateral phase of the drug development process. Due to drug failure reasons, nowadays ADMET calculations were made initially before synthesizing the drug molecules in order to avoid poor bioactive drug molecules and other R&D development costs. All ADMET predictions were made by using Swiss ADME and ProTox-II, a web-based application for predicting ADME data and building drug-like library using in-silico method

4.4.1 Pharmacokinetic Analysis using SWISS-ADME

SWISSADME is a free web application developed by the Swiss Institute of Bioinformatics (SIB) for predicting the pharmacokinetic features of small compounds. It specifically uses computational models to forecast critical ADME features of substances, such as Lipophilicity(LogP), Water solubility, Absorption, Distribution, Metabolism and Excretion.

These predictions can help with drug discovery and development by predicting how a molecule will behave in the body and whether it has the potential to be an effective medicine. However, it is important to highlight that these predictions are simply approximations and should be validated by more experimental research. (Patil, et al ., 2022).

SWISSADME predicts a wide range of ADME-related properties, including permeability, blood-brain barrier penetration, solubility, and others. To anticipate the properties of the

molecules, it employs a variety of computer algorithms such as ML and SVM.

4.4.2 Toxicity Analysis using OSIRIS

OSIRIS (Online Simple Interface for Research on the Toxicity of Chemicals) is a toxicity analysis software tool for small compounds. OSIRIS predicts probable toxicity endpoints based on a compound's chemical structure using a combination of expert rules and quantitative structure-activity relationship (QSAR) models.

OSIRIS can be a useful tool in early-stage drug discovery for identifying potential toxicities and prioritizing compounds for further testing. However, it should be noted that OSIRIS predictions are not a substitute for experimental assays, and experimental validation is required to confirm anticipated toxicity endpoints and assure the safety of proposed drug candidates.

To use OSIRIS, a user enters the chemical structure of the compound of interest and then submits the compound for analysis. OSIRIS then generates a report that includes a summary of the important chemical properties that contribute to the prediction as well as the projected toxicity endpoints for each substance.

4.5 IN SILICO DOCKING STUDIES

Docking is a computational method used in drug discovery to predict how a small molecule, typically a drug candidate, will bind to a target protein. The goal of docking is to predict the most energetically favorable binding orientation of the ligand within the binding pocket of the protein. (Das, et al ., 2020)

The basic idea behind docking is to predict the best orientation of a tiny molecule within a target protein's binding site. The procedure entails producing small molecule conformations and calculating the energy of interaction between the ligand and the protein. The projected binding pose is then chosen based on the tiny molecule's most energetically favorable conformation. (Weng et al., 2021).

Identification of binding sites and its analysis of interactions between ligands and target

Drug designing docking is a powerful tool in drug discovery, as it allows researchers to screen large libraries of compounds quickly and efficiently, and to prioritize the most promising candidates for further development. Docking involves identification of binding sites and the analysis of interactions between ligands and target.

The process of drug designing docking typically involves the following steps:

Preparation of the protein structure: The target protein structure is obtained from experimental techniques or from computational methods, and is prepared by removing any unwanted water molecules, ions, and other ligands that may interfere with docking.

Preparation of ligand structures: The small molecule drug candidates are prepared by optimizing their geometry and assigning partial charges, to allow them to interact with the protein in a meaningful way.

Docking: The ligands are docked onto the protein structure using computational algorithms that predict the most favorable orientation and conformation of the ligand in the protein binding site.

Scoring: The predicted ligand-protein complexes are scored based on their predicted binding energy or other parameters that are indicative of the strength of the interaction.

Analysis and selection: The ligand-protein complexes are analysed to identify the most promising drug candidates, which can be further optimized and tested in vitro and in vivo for their efficacy and safety.

Docking is frequently used to identify prospective drug candidates as a first stage in the drug discovery process. It can also be used to improve existing pharmaceuticals or to discover new applications for existing drugs.

However, it is important to note that drug designing docking is just one step in the drug discovery process, and experimental validation is still required to confirm the efficacy and safety of potential drug candidate

4.6 CDOCKER

Cdocker is a set-up of computerized docking devices like AutoDock. It is intended to foresee how little particles, for example, substrates or medication competitors, tie to a receptor of realized 3D construction. It plays out the docking of the ligand to a bunch of networks portraying the objective protein. The molecular docking and visualization studies were performed with the help of the CDocker program in Discovery Studio v3. 5 (Accelrys, USA).(Singh, et al., 2016).

CHARMm targets the study of biological systems such as proteins, peptides, small molecule ligands, nucleic acids, lipids and carbohydrates.

The CDocker is a flexible docking module. It uses protein site features, referred to as hot spots, consisting of two types states (polar and apolar). The ligand poses are placed into the polar and apolar receptor interactions site. A polar hotspot is preferred by a polar ligand atom (e.g., a hydrogen bond donor or acceptor), and a apolar hotspot is preferred by an apolar atom (e.g., a carbon atom). The protocol allows the user to specify several modes for generating ligand conformations for docking. Further, it allows to identify interacting residues of the receptor with bound ligand as well as 2D diagrams of docking to examine the docking association between the medication (ligand) and the medication target (receptor) in-silico.

A major emphasis in these studies is to compare the relative performance and accuracy of various grid-based approximations to explicit all-atom force field calculations. In these docking studies, the protein is kept rigid while the ligands are treated as fully flexible and

a final minimization step is used to refine the docked poses. A docking success rate of 74% is observed when an explicit all-atom representation of the protein (full force field) is used, while a lower accuracy of 66-76% is observed for grid-based methods. All docking experiments considered a 41-member protein-ligand validation set. A significant improvement in accuracy (76 vs. 66%) for the grid-based docking is achieved if the explicit all-atom force field is used in a final minimization step to refine the docking poses. Statistical analysis shows that even lower-accuracy grid-based energy representations can be effectively used when followed with full force field minimization. The results of these grid-based protocols are statistically indistinguishable from the detailed atomic dockings and provide up to a sixfold reduction in computation time. For the test case examined here, improving the docking accuracy did not necessarily enhance the ability to estimate binding affinities using the docked structures.(Wu, G., et al, 2003)

Protein-ligand docking is a commonly used method for lead identification and refinement. While traditional structure-based docking methods represent the receptor as a rigid body, recent developments have been moving toward the inclusion of protein flexibility.(Gagnon et al., 2016)

Proteins exist in an inter-converting ensemble of conformational states, but effectively and efficiently searching the conformational space available to both the receptor and ligand remains a well-appreciated computational challenge. To this end, Flexible CDOCKER method is used as an extension of the family of complete docking solutions available within CHARMM. This method integrates atomically detailed side chain flexibility with grid-based docking methods, maintaining efficiency while allowing the protein and ligand configurations to explore their conformational space simultaneously. This is in contrast to existing approaches that use induced-fit like sampling, such as Glide or Autodock, where the protein or the ligand space is sampled independently in an iterative fashion. Presented here are developments to the CHARMM docking methodology to incorporate receptor flexibility and improvements to the sampling protocol as demonstrated with re-docking trials on a subset of the CCDC/Astex set. These developments within CDOCKER achieve docking accuracy competitive with or exceeding

the performance of other widely utilized docking programs.(Gagnon et al., 2016).

4.6.1 Preparing the protein

Protein Data Bank (PDB) documents can have an assortment of potential issues that should be revised before they can be utilized in Cdocker. This includes added waters, more than one atom, chain breaks, substitute areas and so on. The water particles and all heteroatoms must be removed. The receptor document is all hetero-utilized via particle types which assists with recognizing the various kinds of molecules.

alternatively;

The target preparation can be done using Swiss Model web tool SWISS-PDB Viewer. The hetero atoms [HETATM] bound that are not a part of the native protein are removed and energy minimized by the steepest method. After this, the optimized protein targets can be checked for molecular docking to identify the best activity.

4.6.2 Preparing the ligand

A library of small molecules is created in compatible file format(sdf file) is created by listing all screened compounds retrieved after filtering using SWISS-ADME and OSIRIS.

4.6.3 Docking procedure using CDOCKER

The 3D Structure of the target is opened from the files explorer in suitable file format. It shows the active sites in the branch window. The suitable binding sites and residues are defined and displayed parameters are set to display the target and the small molecules library and the rest are default, CDOCKER generates 10 poses for every ligand in the library of which the best poses can be determined based on the scores and 2D diagrams

CHAPTER 5

RESULTS AND DISCUSSION

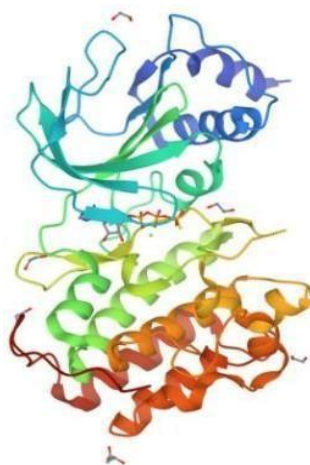
5.1 TARGET IDENTIFICATION

The target is obtained from Protein Data Bank (PDB), UniProt. The PDB ID is 4DN5; Crystal Structure of NF- κ B-inducing Kinase (NIK)

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) is a protein complex. The activated NF- κ B signaling pathway plays an important role in pathogenesis of Sjogren's syndrome. These findings therefore demonstrate that the I κ B α -mediated feedback regulation of NF- κ B has an essential role in controlling T-cell development and functions, provide mechanistic insight into the development of Sjögren's Syndrome (Hu et al, 2007).

5.1.1 3D STRUCTURE OF THE TARGET

Table 5.1: Uniprot ID and structure of selected Target protein

PROTEIN NAME	UNIPROT ID	STRUCTURE	TARGETED PROTEIN NAME
Nuclear factor kappa-light- chain- enhancer of activated B cells	4DN5		Crystal Structure of NF- κ B-inducing Kinase (NIK)

5.1.2 TARGET PROTEIN INSIGHTS FROM PDB

From the protein Data bank RCSB PDB Homepage the selected targeted protein was retrieved. PDB provides the detail of structure and whole summary of the target protein

5.1.3 4DN5 Crystal structure of NF- κ B inducing kinase

(Nuclear Factor Kappa-light-chain-enhancer Of Activated B Cells)

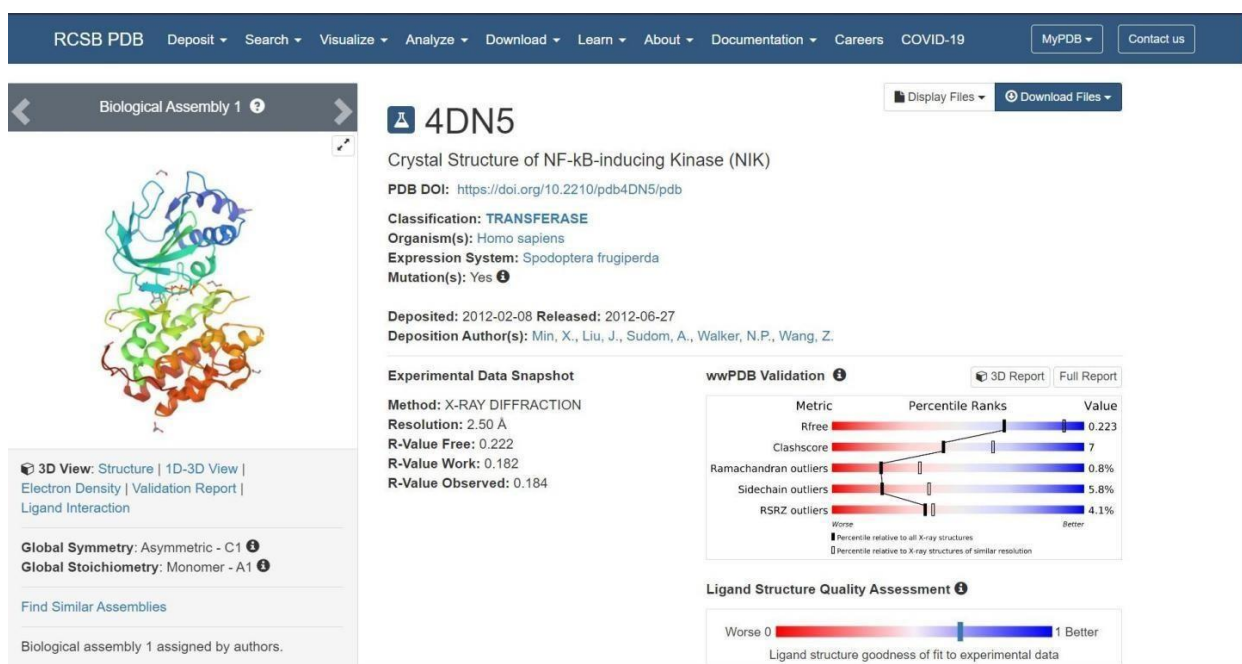


Fig:5.1: Target protein retrieval from protein database bank

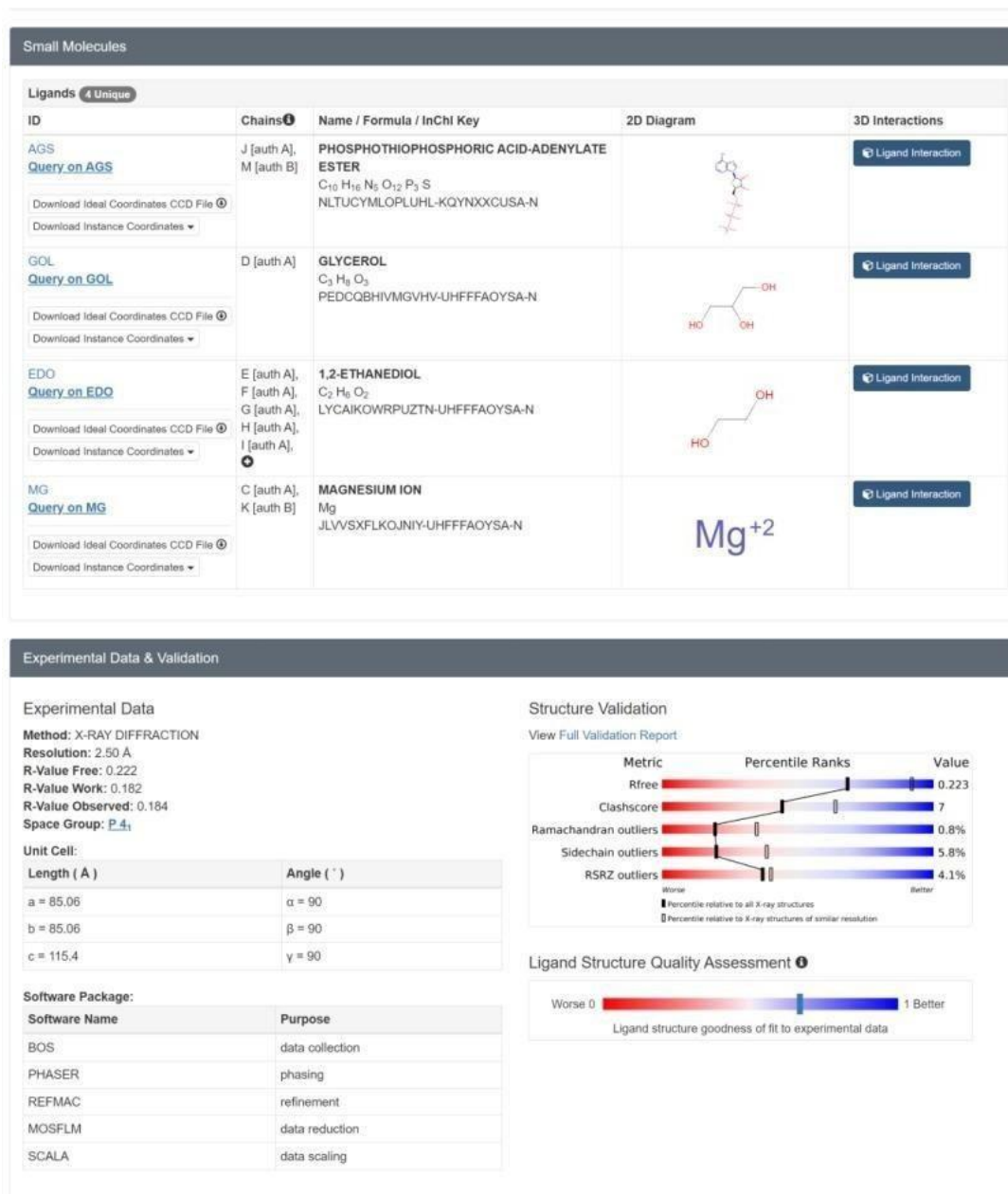


Fig:5.2: Ligands and its binding affinity

RCSB PDB Deposit Search Visualize Analyze Download Learn About Documentation Careers COVID-19 MyPDB Contact us

Small Molecules

Ligands 1 Unique

ID	Chains	Name / Formula / InChI Key	2D Diagram	3D Interactions
NAG Query on NAG Download Ideal Coordinates CCD File Download Instance Coordinates	K [auth F]	2-acetamido-2-deoxy-beta-D-glucopyranose C ₈ H ₁₅ N O ₆ OVRNDRQMDRJTHS-FMDGEEDCSA-N		Ligand Interaction

Experimental Data & Validation

Experimental Data

Method: X-RAY DIFFRACTION
Resolution: 3.15 Å
R-Value Free: 0.232
R-Value Work: 0.187
R-Value Observed: 0.189
Space Group: [P3₁21](#)

Unit Cell:

Length (Å)	Angle (°)
a = 138.71	α = 90
b = 138.71	β = 90
c = 179.09	γ = 120

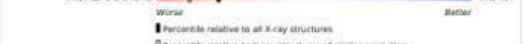
Software Package:

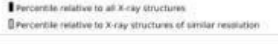
Software Name	Purpose
BUSTER	refinement

[View more in-depth experimental data](#)

Structure Validation

[View Full Validation Report](#)

Metric	Percentile Ranks	Value
Rfree		0.242
Clashscore		8
Ramachandran outliers		1.1%
Sidechain outliers		15.1%
RSRZ outliers		6.3%

Worse  Better
■ Percentile relative to all X-ray structures
□ Percentile relative to X-ray structures of similar resolution

Ligand Structure Quality Assessment

Worse 0  1 Better
Ligand structure goodness of fit to experimental data

Fig 5.3: Ligands and its binding affinity

5.2 LIGAND IDENTIFICATION

5.2.1 PHYTOHUB SMALL MOLECULES

74 small molecules were retrieved as possible ligands from Phytohub.

Table 5.2: Small molecules retrieved from Phytohub

S No	Source	Molecule	Canonical SMILES	Formula
1	Pepper	Brassicasterol	<chem>OC1CCC2(C(=CCC3C2CCC2(C3CCC2C(C=CC(C(C)C)C)C)C1)C</chem>	C ₂₈ H ₄₆ O
2		Campesterol	<chem>OC1CCC2(C(=CCC3C2CCC2(C3CCC2C(CCC(C(C)C)C)C)C1)C</chem>	C ₂₈ H ₄₈ O
3		Carene	<chem>CC1=CCC2C(C1)C2(C)C</chem>	C ₁₀ H ₁₆
4		Caryophyllen-beta	<chem>CC1=CCCC(=C)C2C(CC1)C(C2)(C)C</chem>	C ₁₅ H ₂₄
5		Cineol	<chem>CC12CCC(CC1)C(O2)(C)C</chem>	C ₁₀ H ₁₈ O
6		Limonene	<chem>CC1=CCC(CC1)C(=C)C</chem>	C ₁₀ H ₁₆
7		Phylloquinone	<chem>CC(CCCC(CCCC(C)C)C)CCCC(=CCC1=</chem>	C ₃₁ H ₄₆ O ₂

			<chem>C(C)C(=O)c2c(C1=O)cccc2)C</chem>	
8		Piperine	<chem>O=C(N1CCCCC1)C=CC=Cc1ccc2c(c1)OCO2</chem>	<chem>C17H19NO3</chem>
9		Rotundone	<chem>CC(=C)C1CCC(C2=C(C1)C(C)CC2=O)C</chem>	<chem>C15H22O</chem>
10		Sabinene	<chem>C=C1CCC2(C1C2)C(C)C</chem>	<chem>C10H16</chem>
11		Sitosterol-beta	<chem>CCCCCCCCCCCCCCCCCCCCC(=O)OCC(OC(=O)CCCCC=CC=CCCC=CCC=CCC)COC(=O)CCCCCCC=C=CCC=CCCCCCC</chem>	<chem>C59H102O6</chem>
11		Stigmasterol	<chem>CCC(C(C)C)C=CC(C1CCC2C1(C)CCC1C2CC=C2C1(C)CCC(C2)O)C</chem>	<chem>C29H48O</chem>
12	Buttercup	4-Hydroxyglucobras- sacin	<chem>OCC1OC(SC(=NOS(=O)(=O)O)Cc2c[nH]c3c2c(O)ccc3)C(C(C1O)O)O</chem>	<chem>C16H20N2O10S2</chem>
13		4-Methoxyglucobr	<chem>OCC1OC(SC(=NOS(=O)(=O)O)Cc2c[nH]c</chem>	<chem>C17H22N2O10S2</chem>

		assicin	<chem>3c2c(OC)ccc3)C(C(C1O)O)O</chem>	
14		Glucolyssin	<chem>OCC1OC(SC(=NOS(=O)(=O)[O-])CCCCCS(=O)C)C(C(C1O)O)O</chem>	C13H24NO 10S3-
15	Buttercup	Glucobrassicinapin	<chem>C=CCCCC(=NOS(=O)(=O)O)SC1OC(CO)C(C(C1O)O)O</chem>	C12H21NO 9S2
16		Glucobrassicin	<chem>OCC1OC(SC(=NOS(=O)(=O)O)Cc2c[nH]c3c2cccc3)C(C(C1O)O)O</chem>	C16H20N2 O9S2
17		Glucoiberin	<chem>OCC1OC(SC(=NOS(=O)(=O)O)CCCS(=O)C)C(C(C1O)O)O</chem>	C11H21NO 10S3
18		Glucoiberberin	<chem>CSCCCC(=NOS(=O)(=O)[O-])SC1OC(CO)C(C(C1O)O)O</chem>	C11H20NO 9S3-
19		Gluconapin	<chem>C=CCCC(=NOS(=O)(=O)O)SC1OC(CO)C(C(C1O)O)O</chem>	C11H19NO 9S2
20		Gluconapoleiferin	<chem>C=CCC(CC(=NOS(=O)(=O)O)SC1OC(CO</chem>	C12H21NO 10S2

			<chem>)C(C(C1O)O)O)O</chem>	
21		Gluconasturtiin	<chem>OCC1OC(SC(=NOS(=O)(=O)O)CCc2cccc2)C(C(C1O)O)O</chem>	C15H21NO 9S2
22		Neoglucobrassicin	<chem>OCC1OC(SC(=NOS(=O)(=O)O)Cc2cn(c3c2cccc3)OC)C(C(C1O)O)O</chem>	C17H22N2 O10S2
23		Phenethyl isothiocyanate	<chem>S=C=NCCc1cccc1</chem>	C9H9NS
24		Progoitrin	<chem>C=CC(CC(=NOS(=O)(=O)O)SC1OC(CO)C(C(C1O)O)O)O</chem>	C11H19NO 10S2
25	Carrot	(+)-Lariciresinol	<chem>OCC1C(COC1c1ccc(c(c1)OC)O)Cc1ccc(c(c1)OC)O</chem>	C20H24O6
26		3-O-Caffeoylquinic acid	<chem>O=C(OC1CC(O)(CC(C1O)O)C(=O)O)C=Cc1ccc(c(c1)O)O</chem>	C16H18O9
27		3-O-p-Coumaroylquinic acid	<chem>O=C(OC1CC(O)(CC(C1O)O)C(=O)O)C=Cc1ccc(cc1)O</chem>	C16H18O8
28		5-Feruloylquinic acid	<chem>COc1cc(C=CC(=O)OC2CC(O)(CC(C2O)O)C(=O)O)ccc1O</chem>	C17H20O9

29		Caffeic acid	<chem>OC(=O)C=Cc1ccc(c(c1)O)O</chem>	C9H8O4
30		Diosgenin	<chem>CC1CCC2(OC1)OC1C(C2C)C2(C(C1)C1CC=C3C(C1CC2)(C)CCC(C3)O)C</chem>	C27H42O3
31		Falcarindiol	<chem>CCCCCCCC=CC(C#CC#CC(C=C)O)O</chem>	C17H24O2
32		Falcarindiol acetate	<chem>CCCCCCCC=CC(C#CC#CC(OC(=O)C)C=C)O</chem>	C19H26O3
33		Falcarinol	<chem>CCCCCCCC=CCC#CC#CC(C=C)O</chem>	C17H24O
34		Falcarinolone	<chem>CCCCCCCC=CC(C#CC#CC(=O)C=C)O</chem>	C17H22O2
35		Falcarinone	<chem>CCCCCCCC=CCC#CC#CC(=O)C=C</chem>	C17H22O
36		Lutein	<chem>CC(=CC=CC=C(C=C)C=C(C=CC1=C(C)C(C(CC1(C)C)O)C)C=CC=C(C=CC1C(=C)C(CC1(C)C)O)C)C</chem>	C40H56O2
37		Quinic acid	<chem>OC1C(O)CC(CC1O)(O)C(=O)O</chem>	C7H12O6

38		Umbelliferone	<chem>Oc1ccc2c(c1)oc(=O)cc2</chem>	C9H6O3
39		(+)-Catechin	<chem>Oc1cc2OC(c3ccc(c(c3)O)O)C(Cc2c(c1)O)O</chem>	C15H14O6
40	Apple	(-)-Epicatechin	<chem>Oc1cc2OC(c3ccc(c(c3)O)O)C(Cc2c(c1)O)O</chem>	C15H14O6
41		3-O-p-Coumaroylquinic acid	<chem>O=C(OC1CC(O)(CC(C1O)O)C(=O)O)C=Cc1ccc(cc1)O</chem>	C16H18O8
42		Avicularin	<chem>OCC1OC(C(C1O)O)Oc1c(oc2c(c1=O)c(O)cc(c2)O)c1ccc(c(c1)O)O</chem>	C20H18O11
43		Carvomenthenol	<chem>CC1=CCC(CC1)C(O)(C)C</chem>	C10H18O
44		Coumaroylquinic acid (p-)	<chem>O=C(OC1CC(O)(CC(C1O)O)C(=O)O)C=Cc1ccc(cc1)O</chem>	C16H18O8
45		Farnesol	<chem>OCC=C(CCC=C(CC=C(C)C)C)C</chem>	C15H26O
46		Gallic acid	<chem>OC(=O)c1cc(O)c(c(c1)O)O</chem>	C7H6O5

47		Maslinic Acid	<chem>OC1CC2(C)C(C(C1O)(C)C)CCC1(C2CC=C2C1(C)CCC1(C2CC(C)(C)CC1)C(=O)O)C</chem>	C30H48O4
48		Phloridzin	<chem>OCC1OC(Oc2cc(O)c(c2C(=O)CCc2ccc(c2)O)O)C(C(C1O)O)O</chem>	C21H24O10
49	Apple	Rhamnitol	<chem>OCC(C(C(C(O)C)O)O)O</chem>	C6H14O5
50		Ursolic Acid	<chem>CC1CCC2(C(C1C)C1=CCC3C(C1(CC2)C)(C)CCC1C3(C)CCC(C1(C)C)O)C(=O)O</chem>	C30H48O3
51		Xylose	<chem>OC1COC(C(C1O)O)O</chem>	C5H10O5
52	Fennel	4-Hydroxybenzoic acid 4-O-glucoside	<chem>OCC1OC(Oc2ccc(cc2)C(=O)O)C(C(C1O)O)O</chem>	C13H16O8
53		Estragole	<chem>COc1ccc(cc1)CC=C</chem>	C10H12O
54		Falcarinol	<chem>CCCCCCCC=CCC#CC#CC(C=C)O</chem>	C17H24O
55		Ferule acid	<chem>COc1cc(C=CC(=O)O)O</chem>	C10H10O4

			)ccc1O	
56	Cumin	Cuminaldehyde	<chem>O=Cc1ccc(cc1)C(C)C</chem>	C10H12O
57		Terpinene-gamma	<chem>CC1=CCC(=CC1)C(C)C</chem>	C10H16
58	Parsley	Apigenin	<chem>Oc1ccc(cc1)c1cc(=O)c2c(o1)cc(cc2O)O</chem>	C15H10O5
59		Bergapten	<chem>COC1C2CCC(=O)OC2C1CCO2</chem>	C12H8O4
60		Myrcene	<chem>C=CC(=C)CCC=C(C)C</chem>	C10H16
61	Orange	Hesperetin	<chem>COC1CCC(CC1O)C1CC(=O)c2c(O1)cc(cc2O)O</chem>	C16H14O6
62		Proline betaine	<chem>[O-]C(=O)C1CCC[N+](C)C1</chem>	C7H13NO2
63		Quercetin	<chem>Oc1cc(O)c2c(c1)oc(c(c2=O)O)c1ccc(c(c1)O)O</chem>	C15H10O7
64		Sinapic acid	<chem>COC1CC(C=CC(=O)O)CC(C1O)OC</chem>	C11H12O5
65		Synephrine	<chem>CNCC(c1ccc(cc1)O)O</chem>	C9H13NO2

66		Valencene	<chem>CC(=C)C1CCC2=CC</chem> <chem>CC(C2(C1)C)C</chem>	C15H24
67	Olive	16-B1- phytoprostane	<chem>CCC(C=CC1=C(CCC</chem> <chem>CCCCC(=O)O)C(=O)</chem> <chem>CC1)O</chem>	C18H28O4
68		Cinnamic acid	<chem>OC(=O)C=Cc1ccccc</chem> <chem>1</chem>	C9H8O2
69		Homovanillic acid	<chem>COc1cc(ccc1O)CC(=</chem> <chem>O)O</chem>	C9H10O4
70		Homovanillyl alcohol	<chem>OCCc1ccc(c(c1)OC)</chem> <chem>O</chem>	C9H12O3
71		Hydroxytyrosol	<chem>OCCc1ccc(c(c1)O)O</chem>	C8H10O3
72		Secoisolariciresi nol	<chem>OCC(C(Cc1ccc(c(c1)</chem> <chem>OC)O)CO)Cc1ccc(c(</chem> <chem>c1)OC)O</chem>	C20H26O6
73		Tyrosol	<chem>OCCc1ccc(cc1)O</chem>	C8H10O2
74		Vanillic acid	<chem>COc1cc(ccc1O)C(=O</chem> <chem>)O</chem>	C8H8O4

5.3 SCREENING OF LIGANDS

It is important to screen the small molecules obtained from various natural sources in order to determine the ligands with the potential to bind with the chosen target. SWISS ADME and OSIRIS tools are used to perform ADMET Analysis and screen all 74 compounds retrieved from Phytohub database.

Source	Molecule	Formula	MW	#Rotatable bonds	#H-bond acceptors	#H-bond donors	Consensus Log P	GI absorption	Lipinski #violations	Bioavailability Score
carrot	(+)-Lariciresinol	C20H24O6	360.4	6	6	3	2.38	High	0	0.55
	Caffeic acid	C9H8O4	180.16	2	4	3	0.93	High	0	0.56
apple	(+)-Catechin	C15H14O6	290.27	1	6	5	0.85	High	0	0.55
	(-)-Epicatechin	C15H14O6	290.27	1	6	5	0.85	High	0	0.55
	Gallic acid	C7H6O5	170.12	1	5	4	0.21	High	0	0.56
	Malonic Acid	C3H4O4	104.06	1	4	3	5.24	High	1	0.56
	Ferulic acid	C10H10O4	194.18	3	4	2	1.36	High	0	0.85
fennel	Apigenin	C15H10O5	270.24	1	5	3	2.11	High	0	0.55
orange	Hesperetin	C16H14O6	302.28	2	6	3	1.91	High	0	0.55
	Quercetin	C15H10O7	302.24	1	7	5	1.23	High	0	0.55
	Sinapic acid	C11H12O5	224.21	4	5	2	1.31	High	0	0.56
olive	16-B1-phytoprostane	C18H28O4	308.41	11	4	2	3.34	High	0	0.85
	Homovanillic acid	C9H10O4	182.17	3	4	2	0.88	High	0	0.85
	Hydroxytyrosol	C8H10O3	154.16	2	3	3	0.56	High	0	0.55
	Secoisolariciresinol	C20H26O6	362.42	9	6	4	2.5	High	0	0.55
	Vanillic acid	C8H8O4	168.15	2	4	2	1.08	High	0	0.85

Fig 5.4: Screening of small molecules based on lipinski's rule

5.3.1 ADMET ANALYSIS USING SWISS-ADME

The SWISS ADME Tool is used to perform ADMET Analysis and screen all 74 compounds retrieved from Phytohub database out of which 16 compounds were filtered as potential ligands based on Lipinski's Rule with violations marked in red

5.3.1.1 Lariciresinol

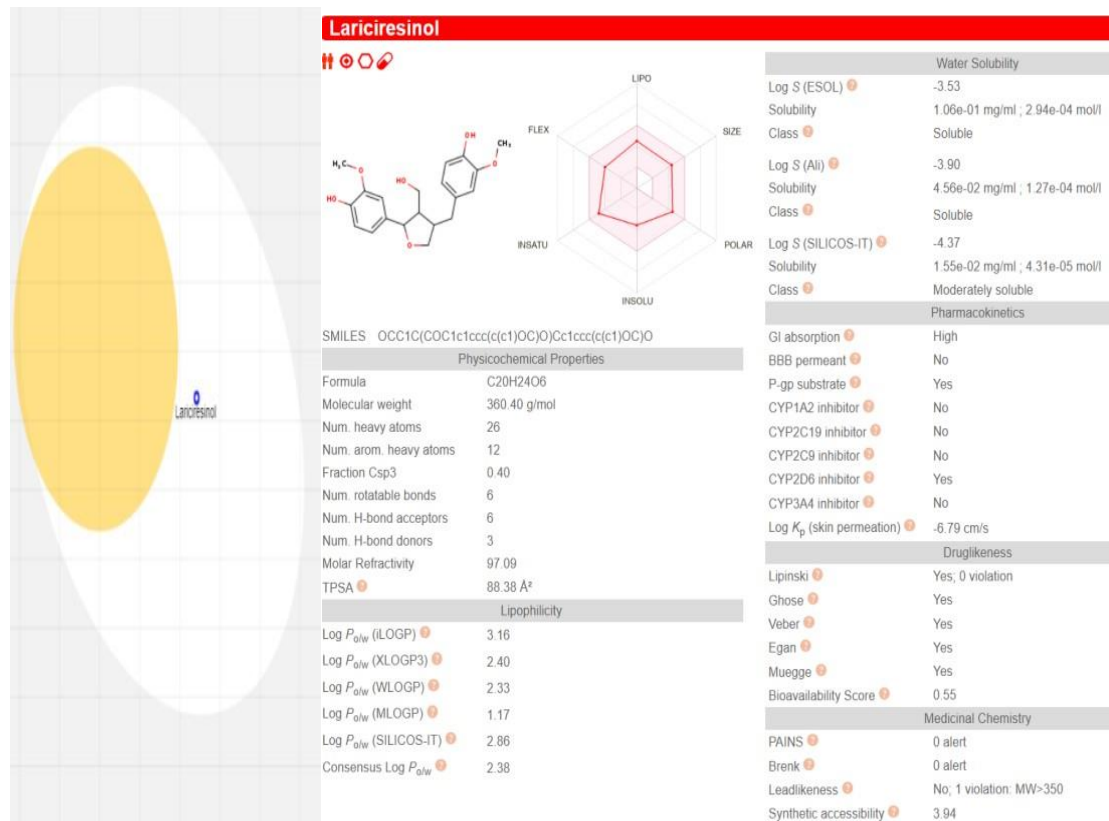


Fig 5.5: ADMET Analysis and Boiled egg structure of Lariciresinol

Lariciresinol has 3 H-bond donors, 6 H-bond acceptors, 6 rotatable H-bonds. The molar refractivity is 97.09 and the TPSA score is 88.38. It has high GI Absorption. The bioavailability score is 0.55. It has no lipinski violations.

The boiled egg structure shows the molecule as a blue point predicted to be substrate of the P-glycoprotein (PGP+) thereby actively pumped up from the brain or to the gastrointestinal lumen and located in the white ellipse which represents compounds with high probability to be passively absorbed by the gastrointestinal tract

5.3.1.2 Caffeic acid

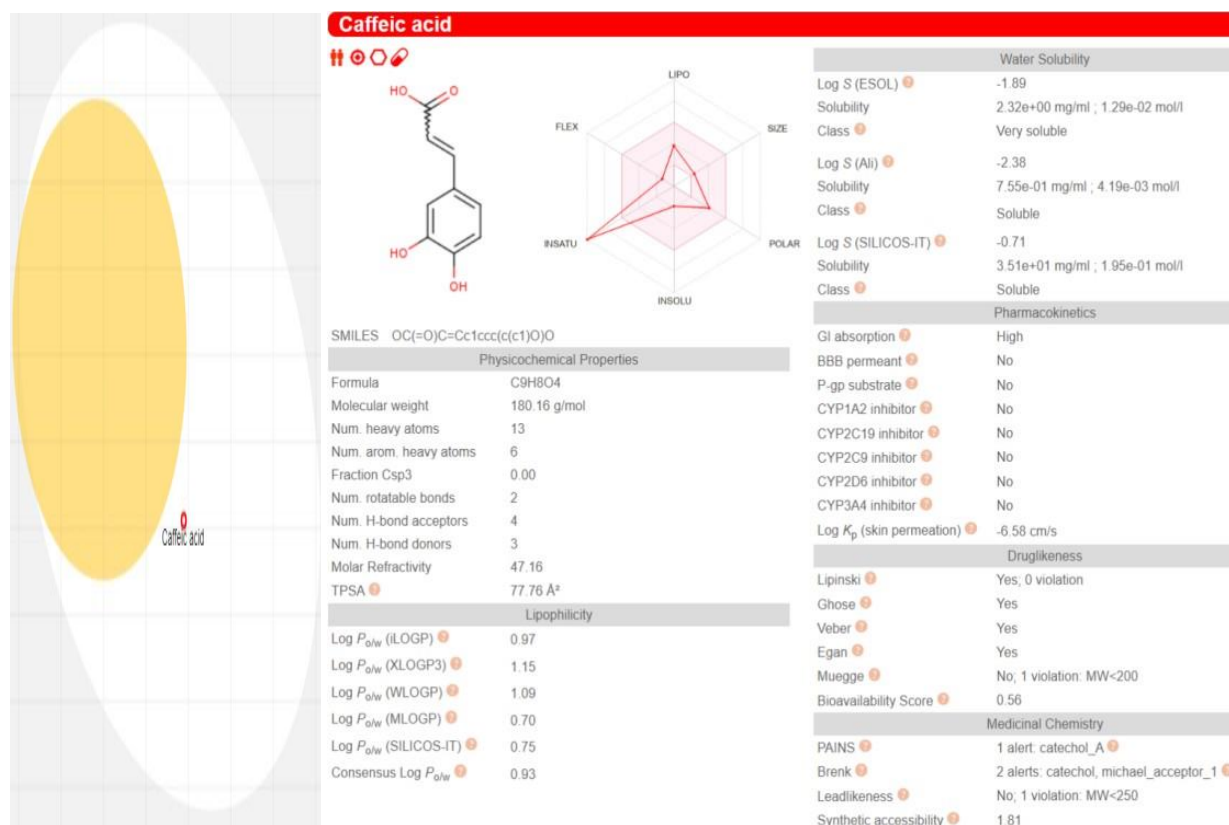


Fig 5.6: ADMET Analysis and Boiled egg structure of Caffeic acid

Caffeic acid has 3 H-bond donors, 4 H-bond acceptors, 2 rotatable H-bonds. The molar refractivity is 47.16 and the TPSA score is 77.76. It has high GI Absorption. The bioavailability score is 0.56. It has no lipinski violations.

The boiled egg structure shows the molecule as a red point predicted to be a non-substrate of the P-glycoprotein (PGP-) and located in the white ellipse which represents compounds with high probability to be passively absorbed by the gastrointestinal tract.

5.3.1.3 Catechin

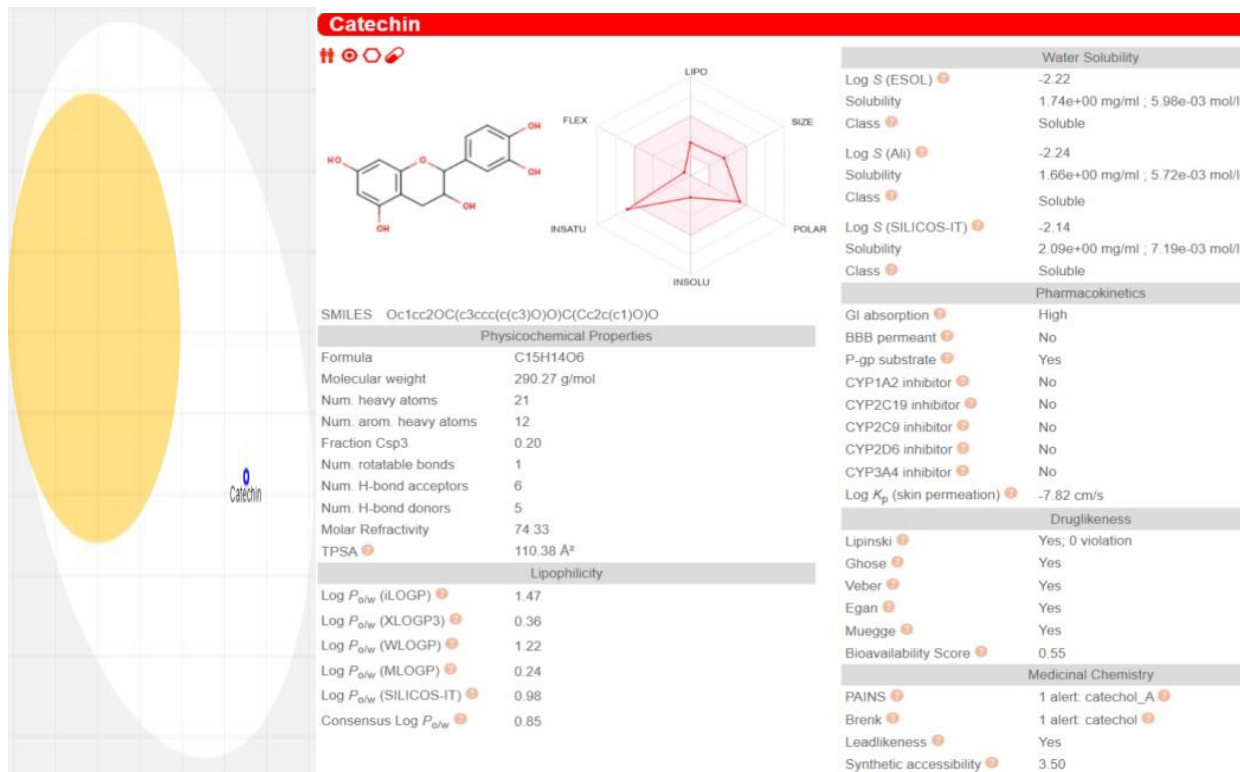


Fig 5.7: ADMET Analysis and boiled egg structure of Catechin

Catechin has 5 H-bond donors, 6 H-bond acceptors, 1 rotatable H-bond. The molar refractivity is 74.33 and the TPSA score is 110.36. It has high GI Absorption. The bioavailability score is 0.55. It has no lipinski violations.

The boiled egg structure shows the molecule as a blue point predicted to be a substrate of the P-glycoprotein (PGP+) thereby actively pumped up from the brain or to the gastrointestinal lumen and located in the white ellipse which represents compounds with high probability to be passively absorbed by the gastrointestinal tract.

5.3.1.4 Epicatechin

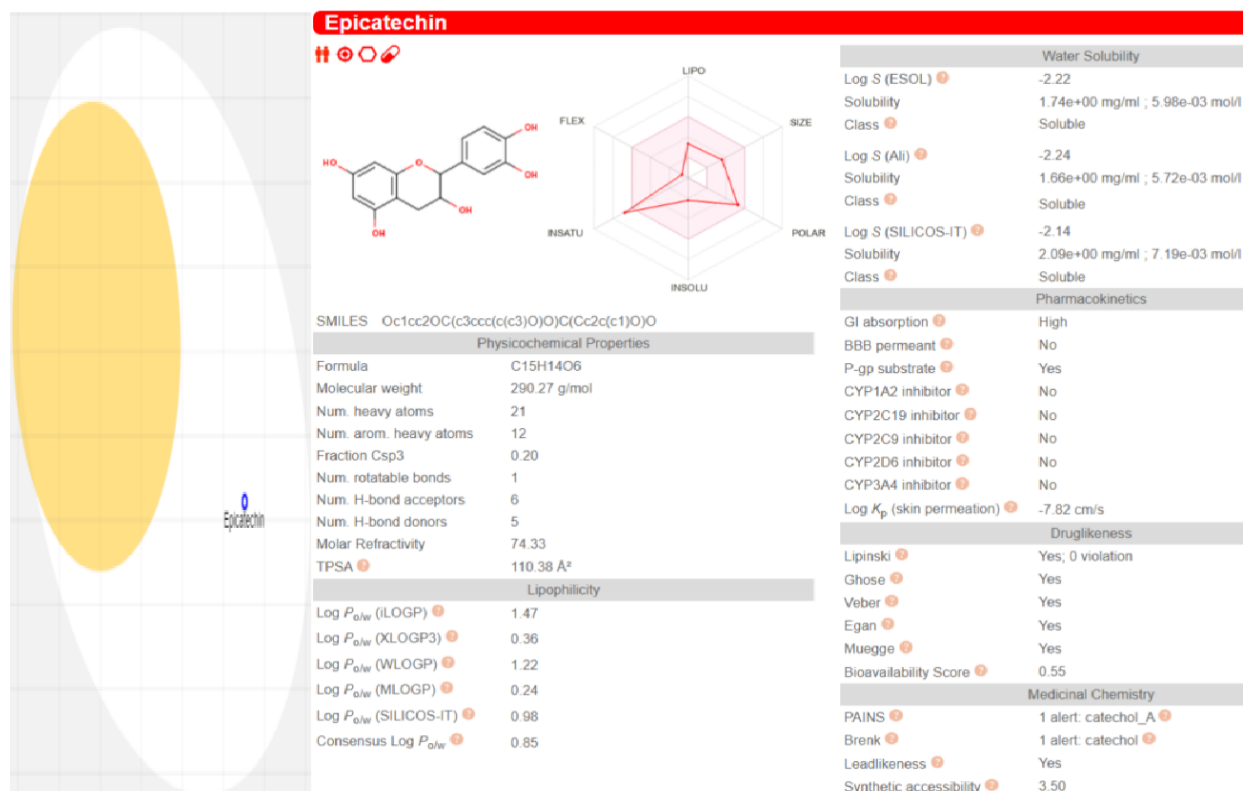


Fig 5.8: ADMET Analysis and Boiled egg structure of Epicatechin

Epicatechin has 5 H-bond donors, 6 H-bond acceptors, 1 rotatable H-bonds. The molar refractivity is 74.33 and the TPSA score is 110.38. It has high GI Absorption. The bioavailability score is 0.55. It has no lipinski violations.

The boiled egg structure shows the molecule as a blue point predicted to be substrate of the P-glycoprotein (PGP+) thereby actively pumped up from the brain or to the gastrointestinal lumen and located in the white ellipse which represents compounds with high probability to be passively absorbed by the gastrointestinal tract

5.3.1.5 Gallic acid

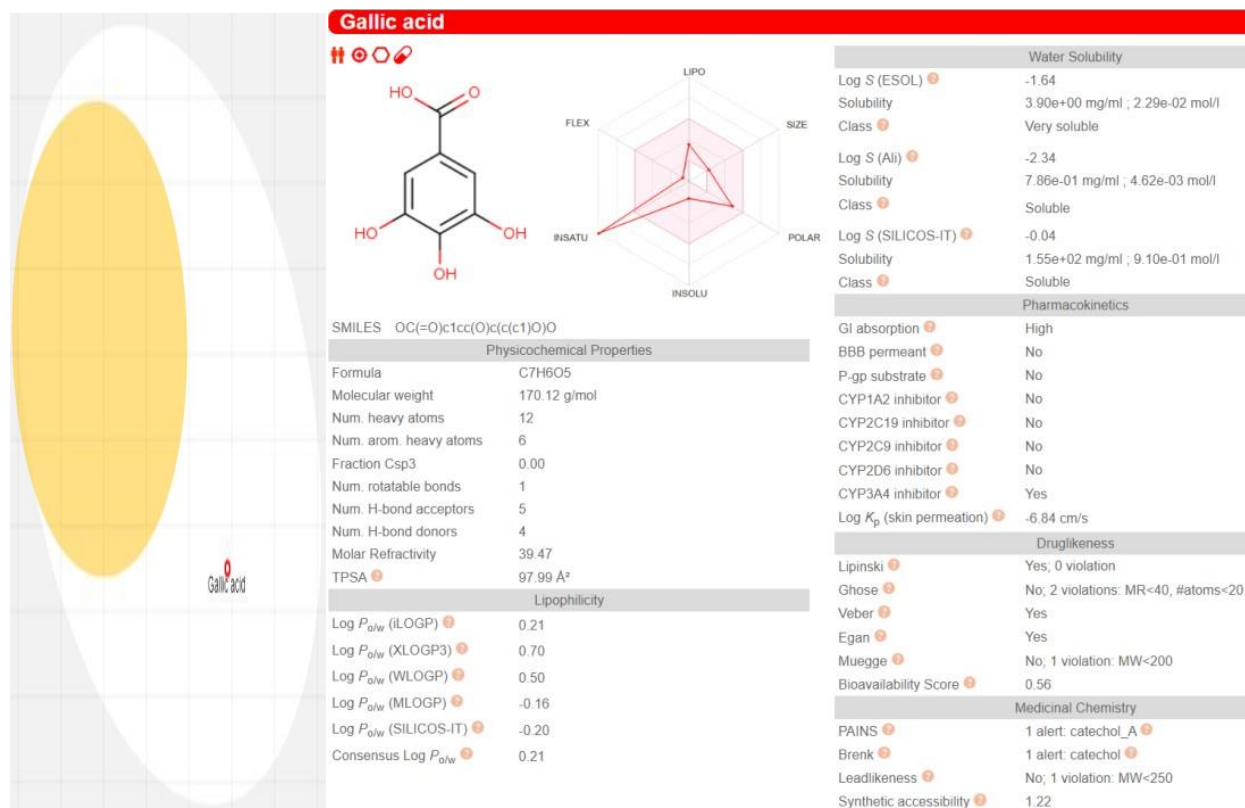


Fig 5.9: ADMET Analysis and Boiled egg structure of Gallic acid

Gallic acid has 4 H-bond donors, 5 H-bond acceptors, 1 rotatable H-bond. The molar refractivity is 39.47 and the TPSA score is 97.99. It has high GI Absorption. The bioavailability score is 0.56. It has no lipinski violations.

The boiled egg structure shows the molecule as a red point predicted to be a non-substrate of the P-glycoprotein (PGP-) and located in the white ellipse which represents compounds with high probability to be passively absorbed by the gastrointestinal tract.

5.3.1.6 Maslinic acid

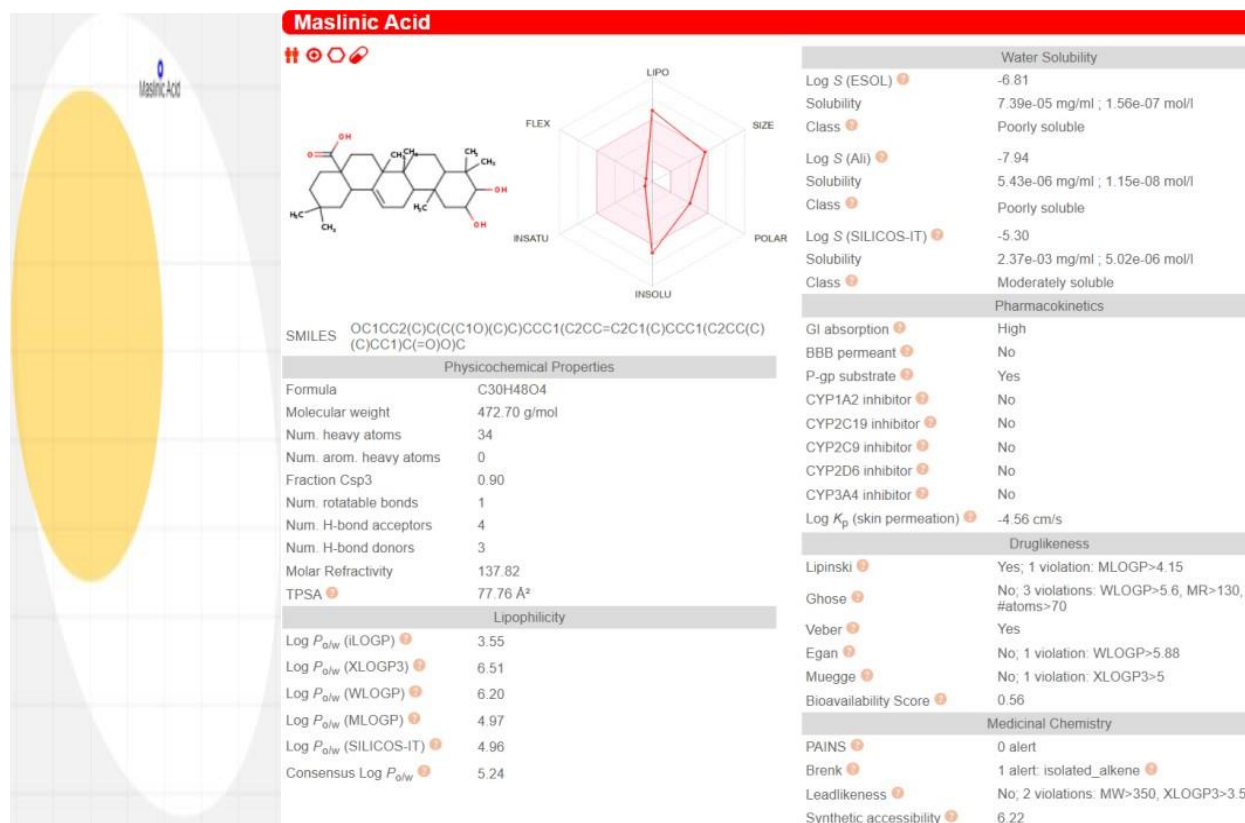


Fig 5.10: ADMET Analysis and Boiled egg structure of Maslinic acid

Maslinic acid has 3 H-bond donors, 4 H-bond acceptors, 1 rotatable H-bonds. The molar refractivity is 137.82 and the TPSA score is 77.76. It has high GI Absorption. The bioavailability score is 0.56. It has only 1 lipinski violation.

The boiled egg structure shows the molecule as a blue point predicted to be substrate of the P-glycoprotein (PGP+) thereby actively pumped up from the brain or to the gastrointestinal lumen and located in the white ellipse which represents compounds with high probability to be passively absorbed by the gastrointestinal tract

5.3.1.7 Ferulic acid

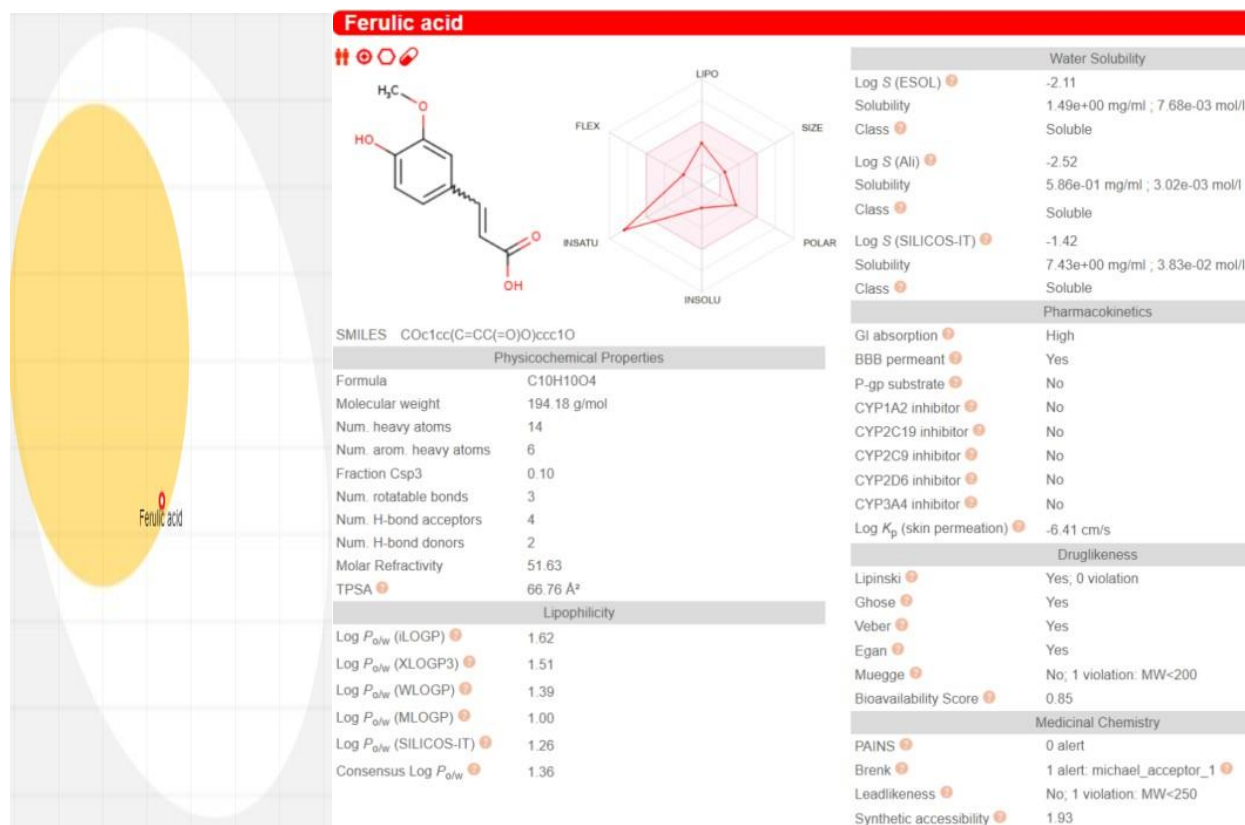


Fig 5.11: ADMET Analysis and Boiled egg structure of Ferulic acid

Ferulic acid has 2 H-bond donors, 4 H-bond acceptors, 3 rotatable H-bond. The molar refractivity is 51.63 and the TPSA score is 66.76. It has high GI Absorption. The bioavailability score is 0.85. It has no lipinski violations.

The boiled egg structure shows the molecule as a red point predicted to be a non-substrate of the P-glycoprotein (PGP-) and located in the yellow ellipse (i.e. the yolk) which represents compounds with high probability to permeate through the BBB to access the CNS.

5.3.1.8 Apigenin

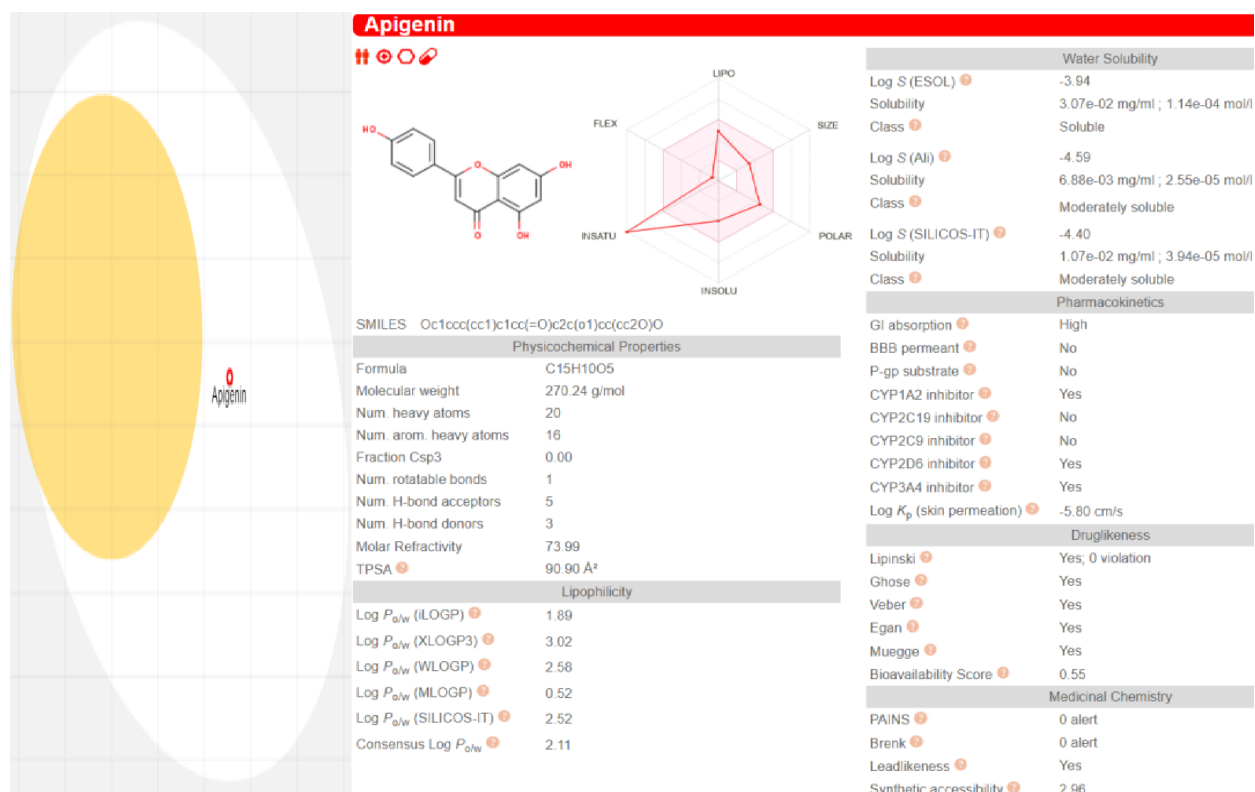


Fig 5.12: ADMET Analysis and Boiled egg structure of Apigenin

Apigenin has 3 H-bond donors, 5 H-bond acceptors, 1 rotatable H-bond. The molar refractivity is 73.99 and the TPSA score is 90.90. It has high GI Absorption. The bioavailability score is 0.55. It has no lipinski violations.

The boiled egg structure shows the molecule as a red point predicted to be a non-substrate of the P-glycoprotein (PGP-) and located in the white ellipse which represents compounds with high probability to be passively absorbed by the gastrointestinal tract.

5.3.1.9 Hesperetin

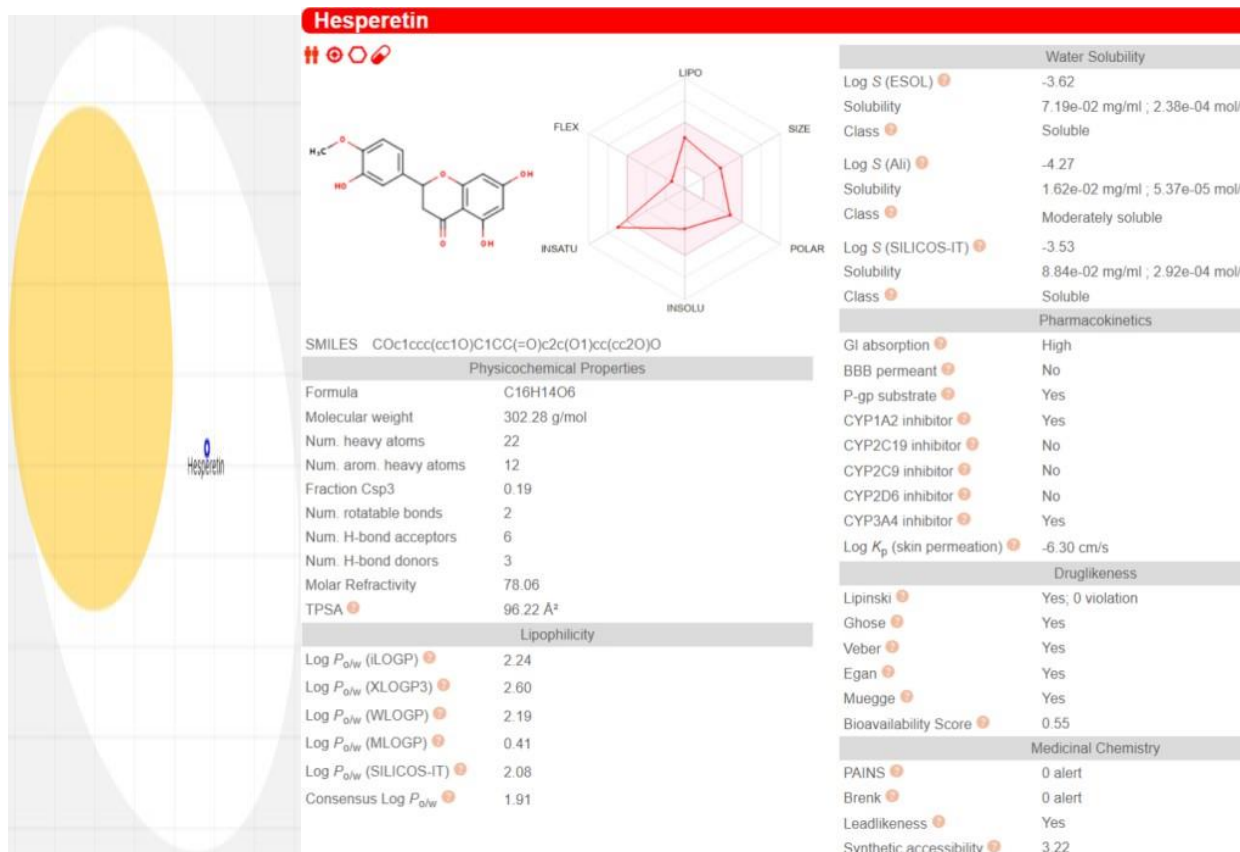


Fig 5.13: ADMET Analysis and Boiled egg structure of Hesperitin

Hesperitin has 3 H-bond donors, 6 H-bond acceptors, 2 rotatable H-bonds. The molar refractivity is 78.06 and the TPSA score is 96.22. It has high GI Absorption. The bioavailability score is 0.55. It has no lipinski violations.

The boiled egg structure shows the molecule as a blue point predicted to be substrate of the P-glycoprotein (PGP+) thereby actively pumped up from the brain or to the gastrointestinal lumen and located in the white ellipse which represents compounds with high probability to be passively absorbed by the gastrointestinal tract

5.3.1.10 Quercetin

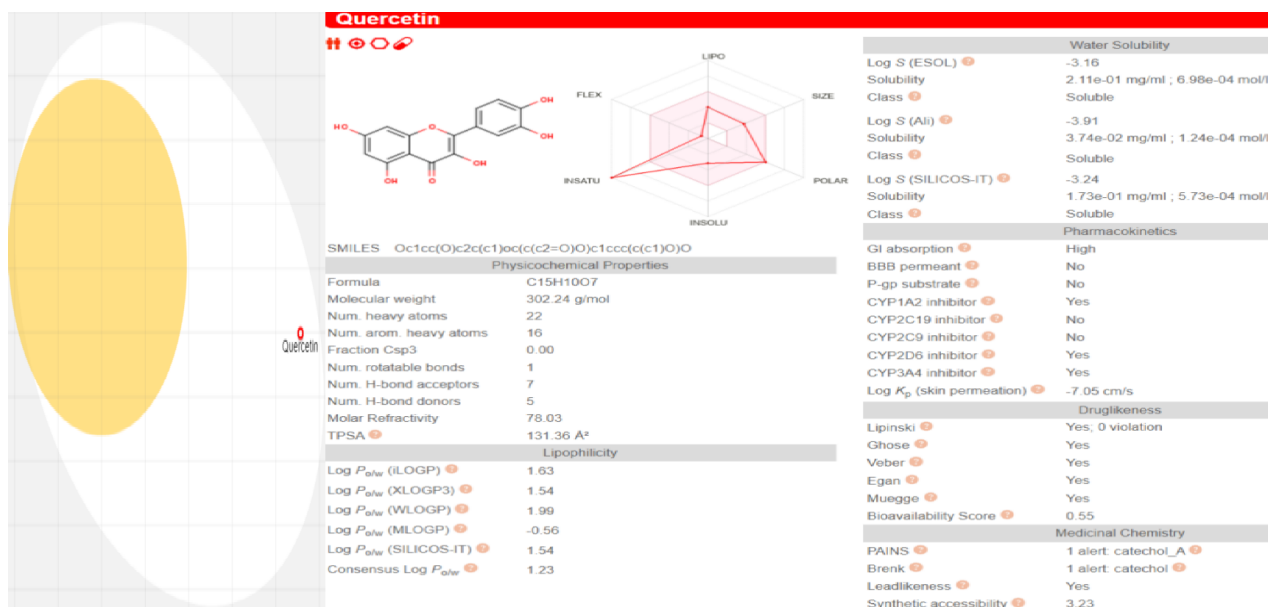


Fig 5.14: ADMET Analysis and Boiled egg structure of Quercetin

Quercetin has 5 H-bond donors, 7 H-bond acceptors, 1 rotatable H-bond. The molar refractivity is 78.03 and the TPSA score is 131.36. It has high GI Absorption. The bioavailability score is 0.55. It has no lipinski violations.

The boiled egg structure shows the molecule as a red point predicted to be a non-substrate of the P-glycoprotein (PGP-) and located in the white ellipse which represents compounds with high probability to be passively absorbed by the gastrointestinal tract.

5.3.1.11 Sinapic acid

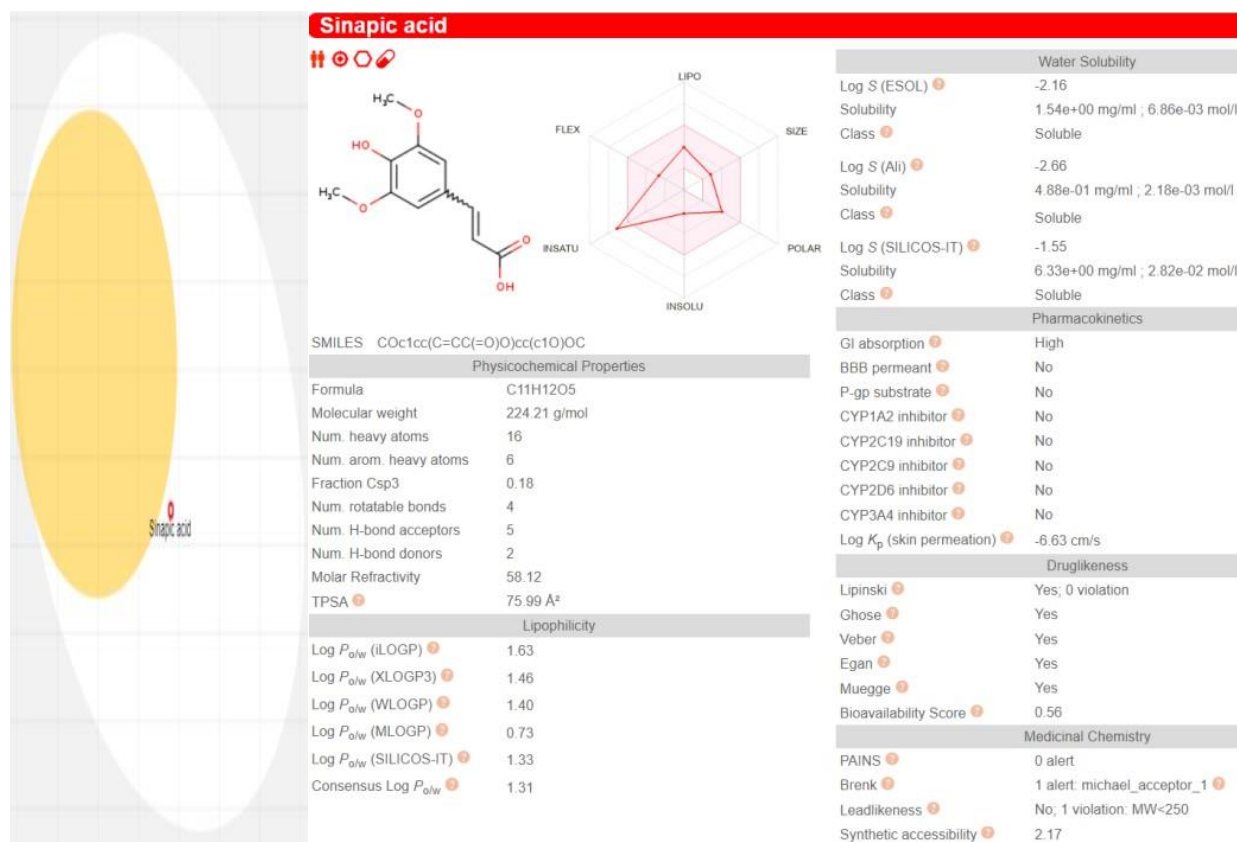


Fig 5.15: ADMET Analysis and Boiled egg structure of Sinapic acid

Sinapic acid has 2 H-bond donors, 5 H-bond acceptors, 4 rotatable H-bonds. The molar refractivity is 58.12 and the TPSA score is 75.99. It has high GI Absorption. The bioavailability score is 0.56. It has no lipinski violations.

The boiled egg structure shows the molecule as a red point predicted to be a non-substrate of the P-glycoprotein (PGP-) and located in the white ellipse which represents compounds with high probability to be passively absorbed by the gastrointestinal tract.

5.3.1.12 16-B1-phytoprostane

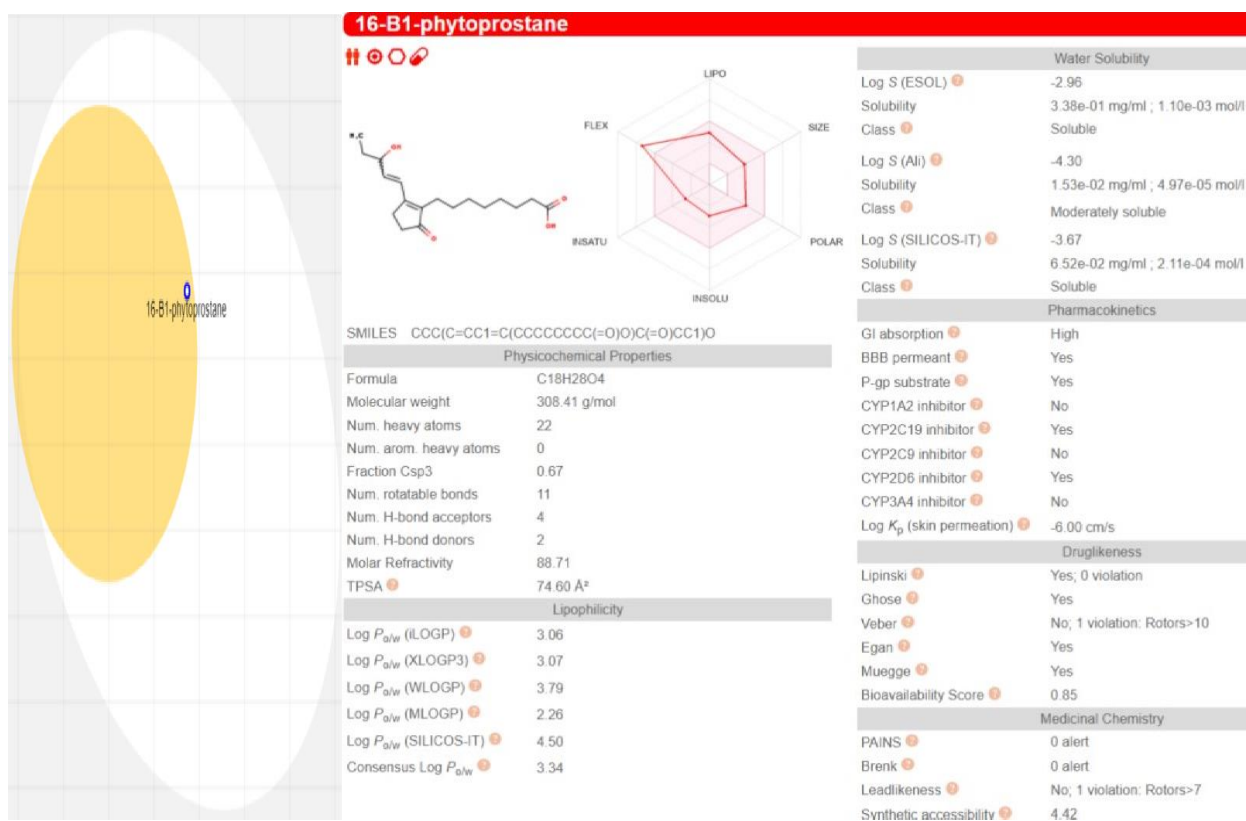


Fig 5.16: ADMET Analysis and Boiled egg structure of 16-1B phytoprostane

16-1B phytoprostane or Octanoic acid has 2 H-bond donors, 4 H-bond acceptors, 11 rotatable H-bonds. The molar refractivity is 88.71 and the TPSA score is 74.62. It has high GI Absorption. The bioavailability score is 0.85. It has no lipinski violations.

The boiled egg structure shows the molecule as a blue point predicted to be substrate of the P-glycoprotein (PGP+) thereby actively pumped up from the brain or to the gastrointestinal lumen and located in the yellow ellipse (i.e. the yolk) which represents compounds with high probability to permeate through the BBB to access the CNS.

5.3.1.13 Homovanillic acid

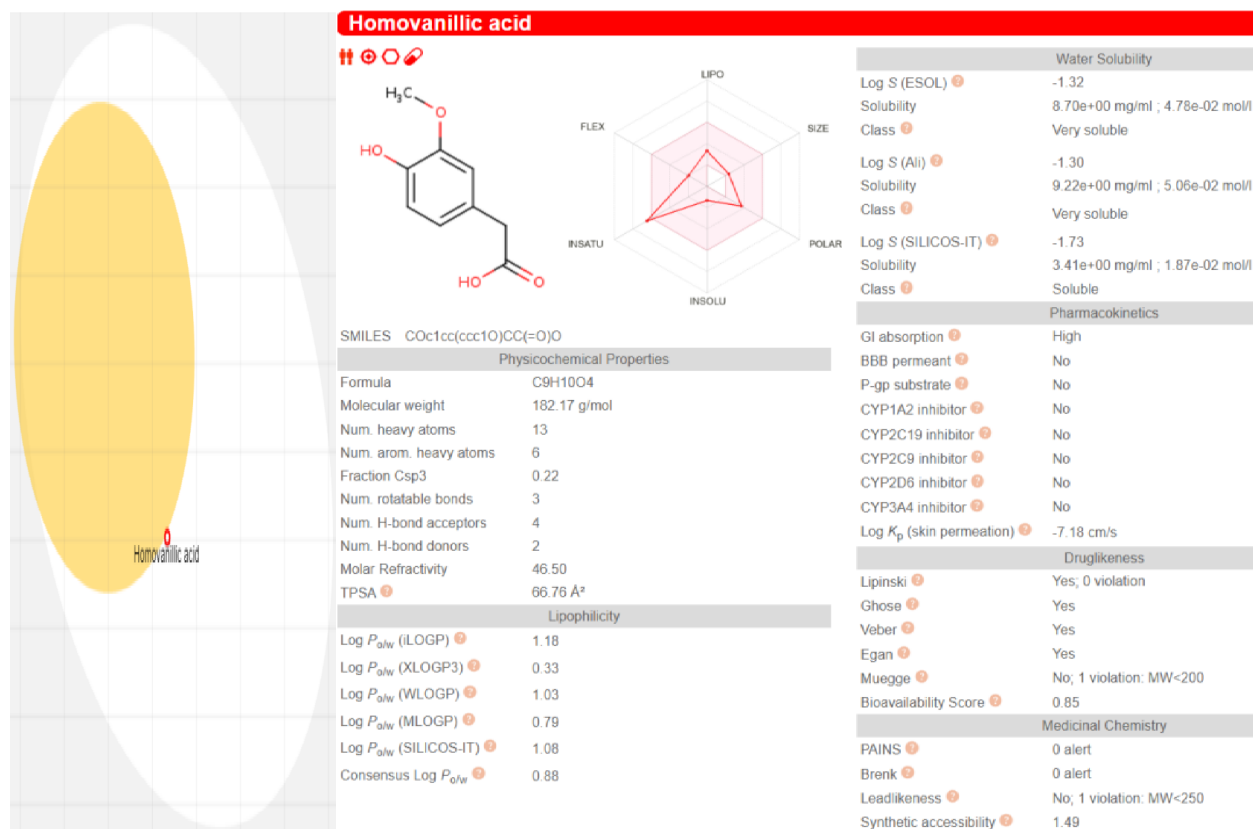


Fig 5.17: ADMET Analysis and Boiled egg structure of Homovanillic acid

Homovanillic acid has 2 H-bond donors, 4 H-bond acceptors, 3 rotatable H-bonds. The molar refractivity is 46.50 and the TPSA score is 66.76. It has high GI Absorption. The bioavailability score is 0.85. It has no lipinski violations.

The boiled egg structure shows the molecule as a red point predicted to be a non-substrate of the P-glycoprotein (PGP-) and located in the white ellipse which represents compounds with high probability to be passively absorbed by the gastrointestinal tract.

5.3.1.14 Hydroxytyrosol

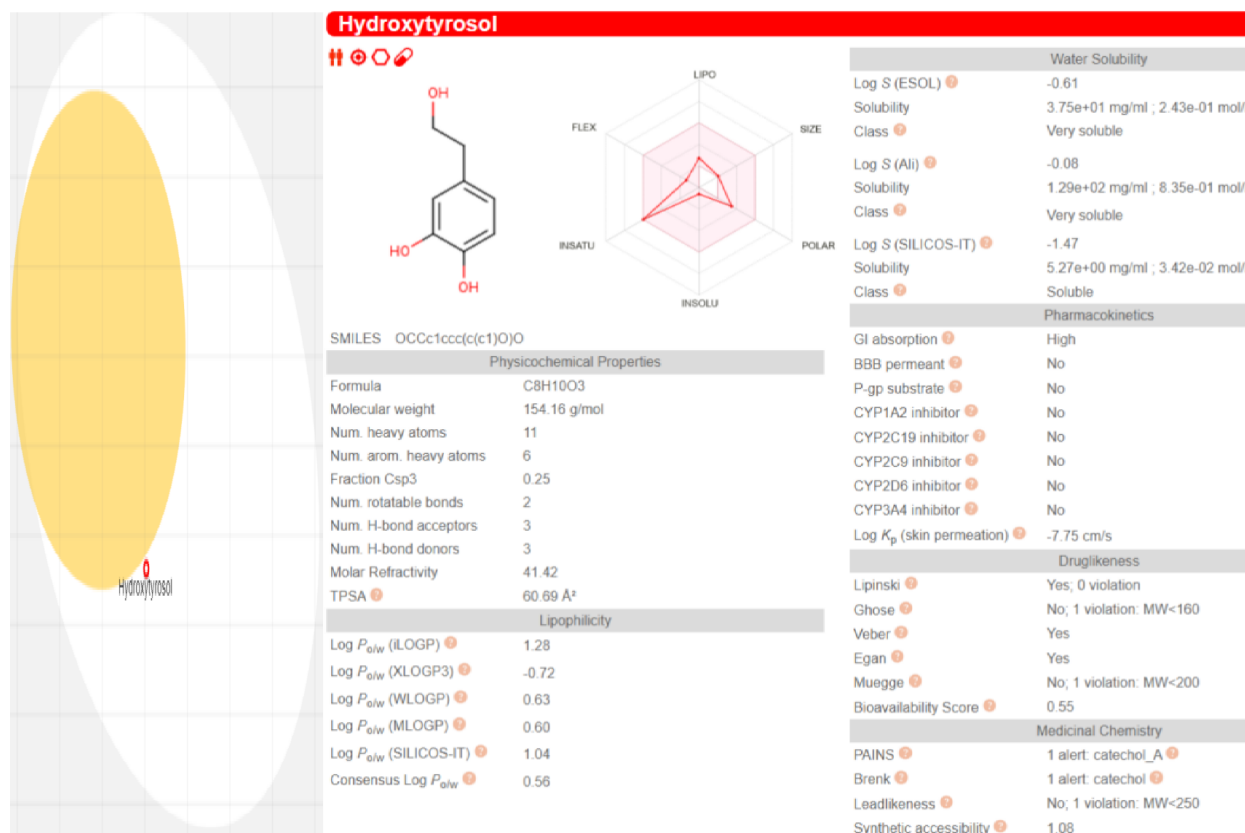


Fig 5.18: ADMET Analysis and Boiled egg structure of Hydroxytyrosol

Hydroxytyrosol has 3 H-bond donors, 3 H-bond acceptors, 2 rotatable H-bonds. The molar refractivity is 41.42 and the TPSA score is 60.69. It has high GI Absorption. The bioavailability score is 0.55. It has no lipinski violations.

The boiled egg structure shows the molecule as a red point predicted to be a non-substrate of the P-glycoprotein (PGP-) and located in the white ellipse which represents compounds with high probability to be passively absorbed by the gastrointestinal tract.

5.3.1.15 Secoisolariciresinol

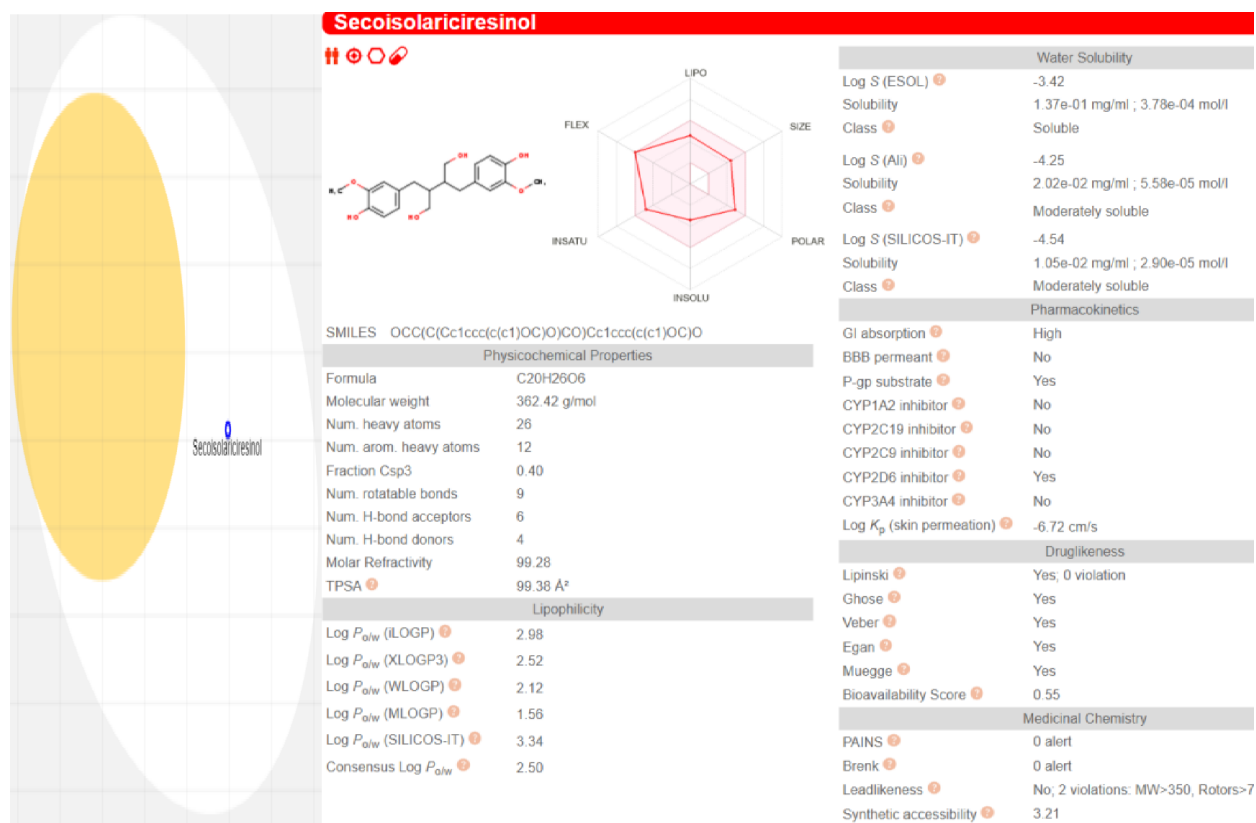


Fig 5.19: ADMET Analysis and Boiled egg structure of Secoisolariciresinol

Secoisolariciresinol has 4 H-bond donors, 6 H-bond acceptors, 9 rotatable H-bonds. The molar refractivity is 99.28 and the TPSA score is 99.36. It has high GI Absorption. The bioavailability score is 0.55. It has no Lipinski violations.

The boiled egg structure shows the molecule as a blue point predicted to be substrate of the P-glycoprotein (PGP+) thereby actively pumped up from the brain or to the gastrointestinal lumen and located in the white ellipse which represents compounds with high probability to be passively absorbed by the gastrointestinal tract.

5.3.1.16 Vanillic acid

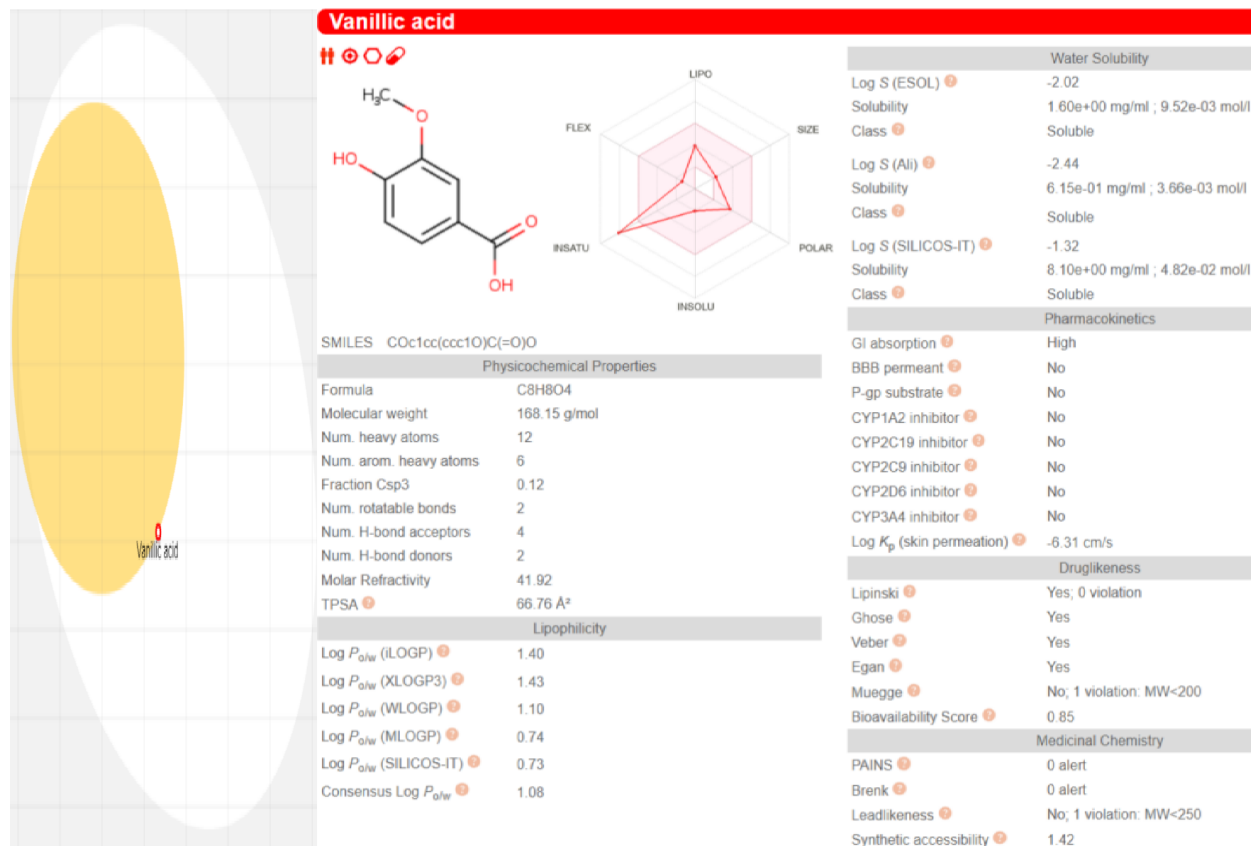


Fig 5.20: ADMET Analysis and Boiled egg structure of Vanillic acid

Vanillic acid has 2 H-bond donors, 4 H-bond acceptors, 2 rotatable H-bonds. The molar refractivity is 41.92 and the TPSA score is 66.76. It has high GI Absorption. The bioavailability score is 0.85. It has no lipinski violations.

The boiled egg structure shows the molecule as a red point predicted to be a non-substrate of the P-glycoprotein (PGP-) and located in the white ellipse which represents compounds with high probability to be passively absorbed by the gastrointestinal tract.

Table 5.3: SWISS-ADME properties of small molecules

Compound Properties	S No	1	2	3	4	5
	Molecule	Lariciresinol	Caffeic acid	Catechin	Epicatechin	Gallic acid
Physico-chemical Properties	Canonical SMILES	<chem>OCC1C(COC1c1ccc(c(c1)OC)O)Cc1ccc(c(c1)OC)O</chem>	<chem>OC(=O)C=Cc1ccc(c(c1)O)O</chem>	<chem>Oc1cc2OC(c3ccc(c(c3)O)O)C(Cc2c(c1)O)O</chem>	<chem>Oc1cc2OC(c3ccc(c(c3)O)O)C(Cc2c(c1)O)O</chem>	<chem>OC(=O)c1cc(O)c(c(c1)O)O</chem>
	Formula	C ₂₀ H ₂₄ O ₆	C ₉ H ₈ O ₄	C ₁₅ H ₁₄ O ₆	C ₁₅ H ₁₄ O ₆	C ₇ H ₆ O ₅
	MW	360.4	180.16	290.27	290.27	170.12
	#Heavy atoms	26	13	21	21	12
	#Aromatic heavy atoms	12	6	12	12	6
	Fraction Csp ³	0.4	0	0.2	0.2	0
	#Rotatable bonds	6	2	1	1	1
	#H-bond acceptors	6	4	6	6	5
	#H-bond donors	3	3	5	5	4
	MR	97.09	47.16	74.33	74.33	39.47
	TPSA	88.38	77.76	110.38	110.38	97.99
Lipophilicity	iLOGP	3.16	0.97	1.47	1.47	0.21
	XLOGP3	2.4	1.15	0.36	0.36	0.7
	WLOGP	2.33	1.09	1.22	1.22	0.5
	MLOGP	1.17	0.7	0.24	0.24	-0.16
	Silicos-IT Log P	2.86	0.75	0.98	0.98	-0.2

Compound Properties	S No	1	2	3	4	5
	Molecule	Lariciresinol	Caffeic acid	Catechin	Epicatechin	Gallic acid
	Consensus Log P	2.38	0.93	0.85	0.85	0.21
Water Solubility	ESOL Log S	-3.53	-1.89	-2.22	-2.22	-1.64
	ESOL Solubility (mg/ml)	0.11	2.32	1.74	1.74	3.9
	ESOL Solubility (mol/l)	0	0.01	0.01	0.01	0.02
	ESOL Class	Soluble	Very soluble	Soluble	Soluble	Very soluble
	Ali Log S	-3.9	-2.38	-2.24	-2.24	-2.34
	Ali Solubility (mg/ml)	0.05	0.76	1.66	1.66	0.79
	Ali Solubility (mol/l)	0	0	0.01	0.01	0
	Ali Class	Soluble	Soluble	Soluble	Soluble	Soluble
	Silicos-IT LogSw	-4.37	-0.71	-2.14	-2.14	-0.04
	Silicos-IT Solubility (mg/ml)	0.02	35.1	2.09	2.09	155
	Silicos-IT Solubility (mol/l)	0	0.2	0.01	0.01	0.91
	Silicos-IT class	Moderately soluble	Soluble	Soluble	Soluble	Soluble

Compound Properties	S No	1	2	3	4	5
	Molecule	Lariciresinol	Caffeic acid	Catechin	Epicatechin	Gallic acid
Pharmacokinetics	GI absorption	High	High	High	High	High
	BBB permeant	No	No	No	No	No
	Pgp substrate	Yes	No	Yes	Yes	No
	CYP1A2 inhibitor	No	No	No	No	No
	CYP2C19 inhibitor	No	No	No	No	No
	CYP2C9 inhibitor	No	No	No	No	No
	CYP2D6 inhibitor	Yes	No	No	No	No
	CYP3A4 inhibitor	No	No	No	No	Yes
	log Kp (cm/s)	-6.79	-6.58	-7.82	-7.82	-6.84
Drug-likeness	Lipinski #violations	0	0	0	0	0
	Ghose #violations	0	0	0	0	2
	Veber #violations	0	0	0	0	0
	Egan #violations	0	0	0	0	0
	Muegge #violations	0	1	0	0	1
	Bioavailability Score	0.55	0.56	0.55	0.55	0.56

Compound Properties	S No	1	2	3	4	5
	Molecule	Lariciresinol	Caffeic acid	Catechin	Epicatechin	Gallic acid
Medicinal Chemistry	PAINS #alerts	0	1	1	1	1
	Brenk #alerts	0	2	1	1	1
	Leadlikeness #violations	1	1	0	0	1
	Synthetic Accessibility	3.94	1.81	3.5	3.5	1.22

Compound Properties	S No	6	7	8	9	10
	Molecule	Maslinic Acid	Ferulic acid	Apigenin	Hesperetin	Quercetin
Physico-chemical Properties	Canonical SMILES	<chem>OC1CC2(C)C(C(C1O)(C)C)CCC1(C2CC=C2C1(C)CCC1(C2CC(C)(C)CC1)C(=O)O)C</chem>	<chem>COc1cc(C=CC(=O)O)c cc1O</chem>	<chem>Oc1ccc(cc1)c1cc(=O)c2c(o1)cc(cc2O)O</chem>	<chem>COc1ccc(cc1O)C1CC(=O)c2c(O1)cc(cc2O)O</chem>	<chem>Oc1cc(O)c2c(c1)oc(c(c2=O)O)c1ccc(c(c1)O)O</chem>
	Formula	C30H48O4	C10H10O4	C15H10O5	C16H14O6	C15H10O7
	MW	472.7	194.18	270.24	302.28	302.24
	#Heavy atoms	34	14	20	22	22
	#Aromatic heavy atoms	0	6	16	12	16
	Fraction Csp3	0.9	0.1	0	0.19	0
	#Rotatable bonds	1	3	1	2	1

Compound Properties	S No	6	7	8	9	10
	Molecule	Maslinic Acid	Ferulic acid	Apigenin	Hesperetin	Quercetin
	#H-bond acceptors	4	4	5	6	7
	#H-bond donors	3	2	3	3	5
	MR	137.82	51.63	73.99	78.06	78.03
	TPSA	77.76	66.76	90.9	96.22	131.36
Lipophilicity	iLOGP	3.55	1.62	1.89	2.24	1.63
	XLOGP3	6.51	1.51	3.02	2.6	1.54
	WLOGP	6.2	1.39	2.58	2.19	1.99
	MLOGP	4.97	1	0.52	0.41	-0.56
	Silicos-IT Log P	4.96	1.26	2.52	2.08	1.54
	Consensus Log P	5.24	1.36	2.11	1.91	1.23
Water Solubility	ESOL Log S	-6.81	-2.11	-3.94	-3.62	-3.16
	ESOL Solubility (mg/ml)	0	1.49	0.03	0.07	0.21
	ESOL Solubility (mol/l)	0	0.01	0	0	0
	ESOL Class	Poorly soluble	Soluble	Soluble	Soluble	Soluble
	Ali Log S	-7.94	-2.52	-4.59	-4.27	-3.91
	Ali Solubility (mg/ml)	0	0.59	0.01	0.02	0.04
	Ali Solubility (mol/l)	0	0	0	0	0
	Ali Class	Poorly soluble	Soluble	Moderately soluble	Moderately soluble	Soluble

Compound Properties	S No	6	7	8	9	10
	Molecule	Maslinic Acid	Ferulic acid	Apigenin	Hesperetin	Quercetin
	Silicos-IT LogSw	-5.3	-1.42	-4.4	-3.53	-3.24
	Silicos-IT Solubility (mg/ml)	0	7.43	0.01	0.09	0.17
	Silicos-IT Solubility (mol/l)	0	0.04	0	0	0
	Silicos-IT class	Moderately soluble	Soluble	Moderately soluble	Soluble	Soluble
Pharmacokinetics	GI absorption	High	High	High	High	High
	BBB permeant	No	Yes	No	No	No
	Pgp substrate	Yes	No	No	Yes	No
	CYP1A2 inhibitor	No	No	Yes	Yes	Yes
	CYP2C19 inhibitor	No	No	No	No	No
	CYP2C9 inhibitor	No	No	No	No	No
	CYP2D6 inhibitor	No	No	Yes	No	Yes
	CYP3A4 inhibitor	No	No	Yes	Yes	Yes
	log Kp (cm/s)	-4.56	-6.41	-5.8	-6.3	-7.05
	Lipinski #violations	1	0	0	0	0

Compound Properties	S No	6	7	8	9	10
	Molecule	Maslinic Acid	Ferulic acid	Apigenin	Hesperetin	Quercetin
Drug-Likeness	Ghose #violations	3	0	0	0	0
	Veber #violations	0	0	0	0	0
	Egan #violations	1	0	0	0	0
Drug-likeness	Muegge #violations	1	1	0	0	0
	Bioavailability Score	0.56	0.85	0.55	0.55	0.55
Medicinal Chemistry	PAINS #alerts	0	0	0	0	1
	Brenk #alerts	1	1	0	0	1
	Leadlikeness #violations	2	1	0	0	0
	Synthetic Accessibility	6.22	1.93	2.96	3.22	3.23

Compound Properties	S No	11	12	13	14	15	16
	Molecule	Sinapic acid	16-B1-phytoprostane	Homovanillic acid	Hydroxytyrosol	Secoisolariciresinol	Vanillic acid
Physicochemical Properties	Canonical SMILES	<chem>COc1cc(C=CC(=O)O)cc(c1O)OC</chem>	<chem>CCC(C=CC1=C(CCCC1)CCCC(=O)O</chem>	<chem>COc1cc(ccc1O)CC(=O)O</chem>	<chem>OCCc1ccc(c(c1)O)O</chem>	<chem>OCC(C(Cc1ccc(c(c1)OC)O)CO)</chem>	<chem>COc1cc(ccc1O)C(=O)O</chem>

Compound Properties	S No	11	12	13	14	15	16
	Molecule	Sinapic acid	16-B1-phytoprostane	Homovanillic acid	Hydroxytyrosol	Secoisolaricresinol	Vanillic acid
			<chem>O)C(=O)CC1)O</chem>			<chem>Cc1ccc(c(c1)OC)O</chem>	
	Formula	C ₁₁ H ₁₂ O ₅	C ₁₈ H ₂₈ O ₄	C ₉ H ₁₀ O ₄	C ₈ H ₁₀ O ₃	C ₂₀ H ₂₆ O ₆	C ₈ H ₈ O ₄
	MW	224.21	308.41	182.17	154.16	362.42	168.15
	#Heavy atoms	16	22	13	11	26	12
	#Aromatic heavy atoms	6	0	6	6	12	6
	Fraction Csp ³	0.18	0.67	0.22	0.25	0.4	0.12
	#Rotatable bonds	4	11	3	2	9	2
	#H-bond acceptors	5	4	4	3	6	4
	#H-bond donors	2	2	2	3	4	2
	MR	58.12	88.71	46.5	41.42	99.28	41.92
	TPSA	75.99	74.6	66.76	60.69	99.38	66.76
Lipophilicity	iLOGP	1.63	3.06	1.18	1.28	2.98	1.4
	XLOGP3	1.46	3.07	0.33	-0.72	2.52	1.43
	WLOGP	1.4	3.79	1.03	0.63	2.12	1.1
	MLOGP	0.73	2.26	0.79	0.6	1.56	0.74
	Silicos-IT Log P	1.33	4.5	1.08	1.04	3.34	0.73
	Consensus Log P	1.31	3.34	0.88	0.56	2.5	1.08

Compound Properties	S No	11	12	13	14	15	16
	Molecule	Sinapic acid	16-B1-phytoprostane	Homovanillic acid	Hydroxytyrosol	Secoisolariciresinol	Vanillic acid
Water Solubility	ESOL Log S	-2.16	-2.96	-1.32	-0.61	-3.42	-2.02
	ESOL Solubility (mg/ml)	1.54	0.34	8.7	37.5	0.14	1.6
	ESOL Solubility (mol/l)	0.01	0	0.05	0.24	0	0.01
	ESOL Class	Soluble	Soluble	Very soluble	Very soluble	Soluble	Soluble
	Ali Log S	-2.66	-4.3	-1.3	-0.08	-4.25	-2.44
	Ali Solubility (mg/ml)	0.49	0.02	9.22	129	0.02	0.62
	Ali Solubility (mol/l)	0	0	0.05	0.84	0	0
	Ali Class	Soluble	Moderately soluble	Very soluble	Very soluble	Moderately soluble	Soluble
	Silicos-IT LogSw	-1.55	-3.67	-1.73	-1.47	-4.54	-1.32
	Silicos-IT Solubility (mg/ml)	6.33	0.07	3.41	5.27	0.01	8.1
	Silicos-IT Solubility (mol/l)	0.03	0	0.02	0.03	0	0.05
	Silicos-IT class	Soluble	Soluble	Soluble	Soluble	Moderately soluble	Soluble

Compound Properties	S No	11	12	13	14	15	16
	Molecule	Sinapic acid	16-B1-phytoprostane	Homovanillic acid	Hydroxytyrosol	Secoisolariciresinol	Vanillic acid
Pharmacokinetics	GI absorption	High	High	High	High	High	High
	BBB permeant	No	Yes	No	No	No	No
	Pgp substrate	No	Yes	No	No	Yes	No
	CYP1A2 inhibitor	No	No	No	No	No	No
	CYP2C19 inhibitor	No	Yes	No	No	No	No
	CYP2C9	No	No	No	No	No	No
	CYP2D6 inhibitor	No	Yes	No	No	Yes	No
	CYP3A4 inhibitor	No	No	No	No	No	No
	log Kp (cm/s)	-6.63	-6	-7.18	-7.75	-6.72	-6.31
Drug-likeness	Lipinski #violations	0	0	0	0	0	0
	Ghose #violations	0	0	0	1	0	0
	Veber #violations	0	1	0	0	0	0
	Egan #violations	0	0	0	0	0	0
	Muegge #violations	0	0	1	1	0	1
	Bioavailability Score	0.56	0.85	0.85	0.55	0.55	0.85
	PAINS #alerts	0	0	0	1	0	0

Compound Properties	S No	11	12	13	14	15	16
	Molecule	Sinapic acid	16-B1-phytoprostane	Homovanillic acid	Hydroxytyrosol	Secoisolariciresinol	Vanillic acid
Medicinal Chemistry	Brenk #alerts	1	0	0	1	0	0
	Leadlikeness #violations	1	1	1	1	2	1
	Synthetic Accessibility	2.17	4.42	1.49	1.08	3.21	1.42

Apart from Lipinski's Rule there are several other properties that can predict the behaviour of a compound. These properties include but are not limited to factors like;

- ◆ Less than 10 rotatable H-bonds (Veber's rule),
- ◆ High GI Absorption,
- ◆ Topological polar surface area value within 60-140,
- ◆ Molar refractivity range 40-130,
- ◆ Solubility, etc.,

The 16 compounds were screened and found to have either one or no violations.

5.3.2 TOXICITY ANALYSIS USING OSIRIS

The OSIRIS tool estimates the probable toxicity of small compounds based on their chemical structures. It employs a collection of more than 80 rules to identify potentially harmful functional groups and structural traits. This tool predicts a variety of toxicity outcomes, including mutagenicity, tumorigenicity, reproductive toxicity, and irritant. Prediction results are valued and color coded. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red. Whereas a green color indicates drug-conform behaviour.

Source	Compound	Mutagenic	Tumorigenic	Irritant	Reproductive effective	cLogP	Solubility	MolWeight	TPSA	Druglikeness	Drug score
carrot	(+)-Lariciresinol	no	no	no	no	2.28	-2.48	360	88.38	0.96	0.74
	Caffeic acid	no	no	no	no	0.78	-1.41	180	77.76	1.62	0.89
apple	(+)-Catechin	no	no	no	no	1.51	-1.76	290	110.3	1.92	0.87
	(-)-Epicatechin	no	no	no	no	1.51	-1.76	290	110.3	1.92	0.87
	Gallic acid	yes	no	no	yes	0.11	-0.74	170	97.99	0.12	0.27
fennel	Maslinic Acid	no	no	no	no	5.21	-5.73	472	77.76	-2.03	0.21
	Ferulic acid	no	no	no	no	1.06	-1.72	194	66.76	1.12	0.84
parsley	Apigenin	yes	no	no	no	2.34	-2.86	270	86.99	1.21	0.47
orange	Hesperetin	no	no	no	no	2.09	-2.66	302	96.22	1.68	0.82
	Quercetin	yes	yes	no	no	1.49	-2.49	302	127.4	1.6	0.3
	Sinapic acid	no	no	no	no	0.99	-1.74	224	75.99	1.97	0.9
olive	16-B1-phytoprostane	no	no	no	no	4.05	-3.37	308	74.6	-11.6	0.38
	Homovanillic acid	no	no	no	no	0.73	-1.23	182	66.76	0.17	0.75
	Hydroxytyrosol	no	no	no	no	0.8	-1.02	154	60.69	-1.3	0.59
	Secoisolariciresinol	no	no	no	no	2.52	-2.54	362	99.38	-2.37	0.46
	Vanillic acid	yes	no	no	no	0.73	-1.35	168	66.76	-1.31	0.35

Fig no:5.21 Toxicity screening using OSIRIS

5.3.2.1 Lariciresinol

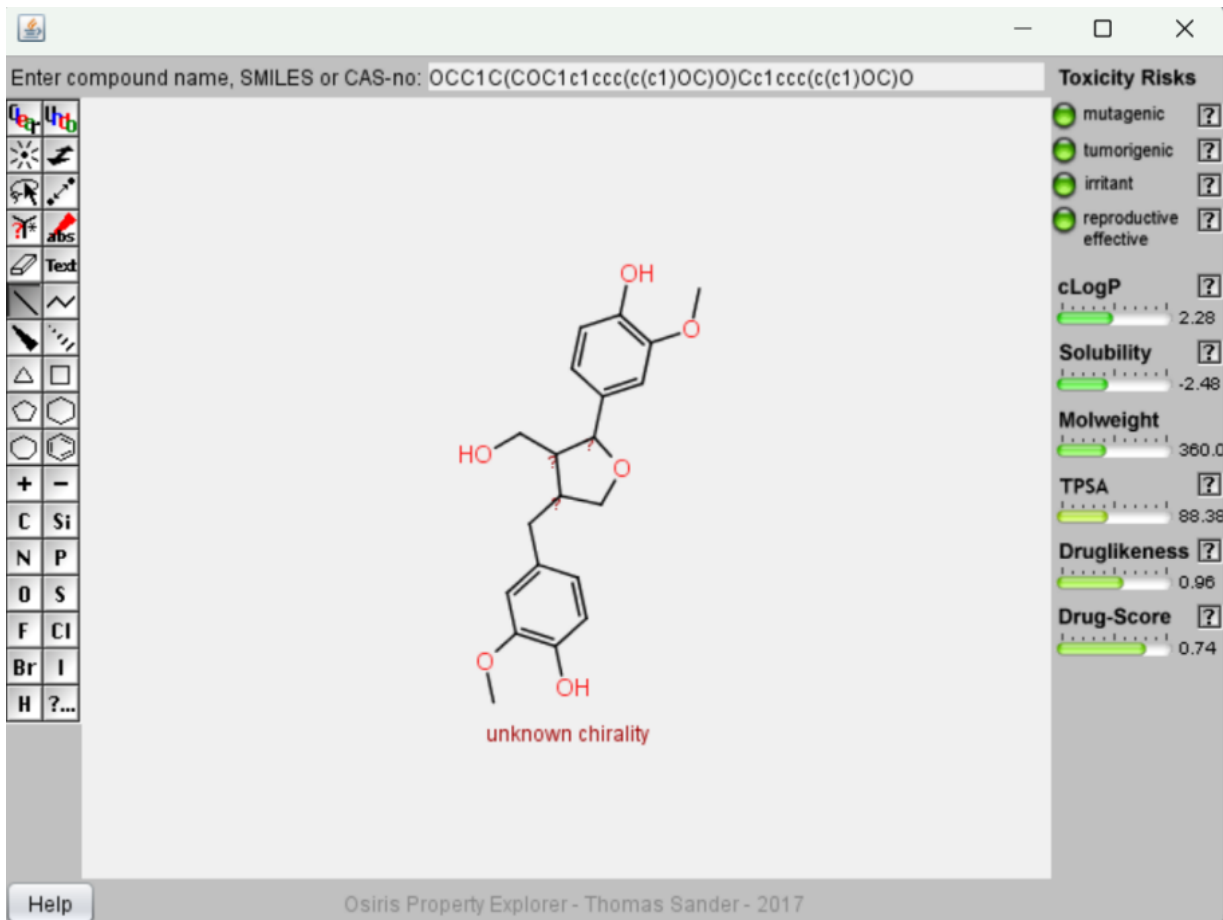


Fig no:5.22 Toxicity analysis of Lariciresinol using OSIRIS Software

Lariciresinol has no mutagenic, tumourigenic, irritant or reproductive effective properties.

The molecular weight is 360

It has a Consensus LogP value of 2.28

The Solubility of Lariciresinol is at -2.48 and the TPSA value is 88.38

The Druglikeness and Drug scores are 0.96 and 0.74 respectively

5.3.2.2 Caffeic acid

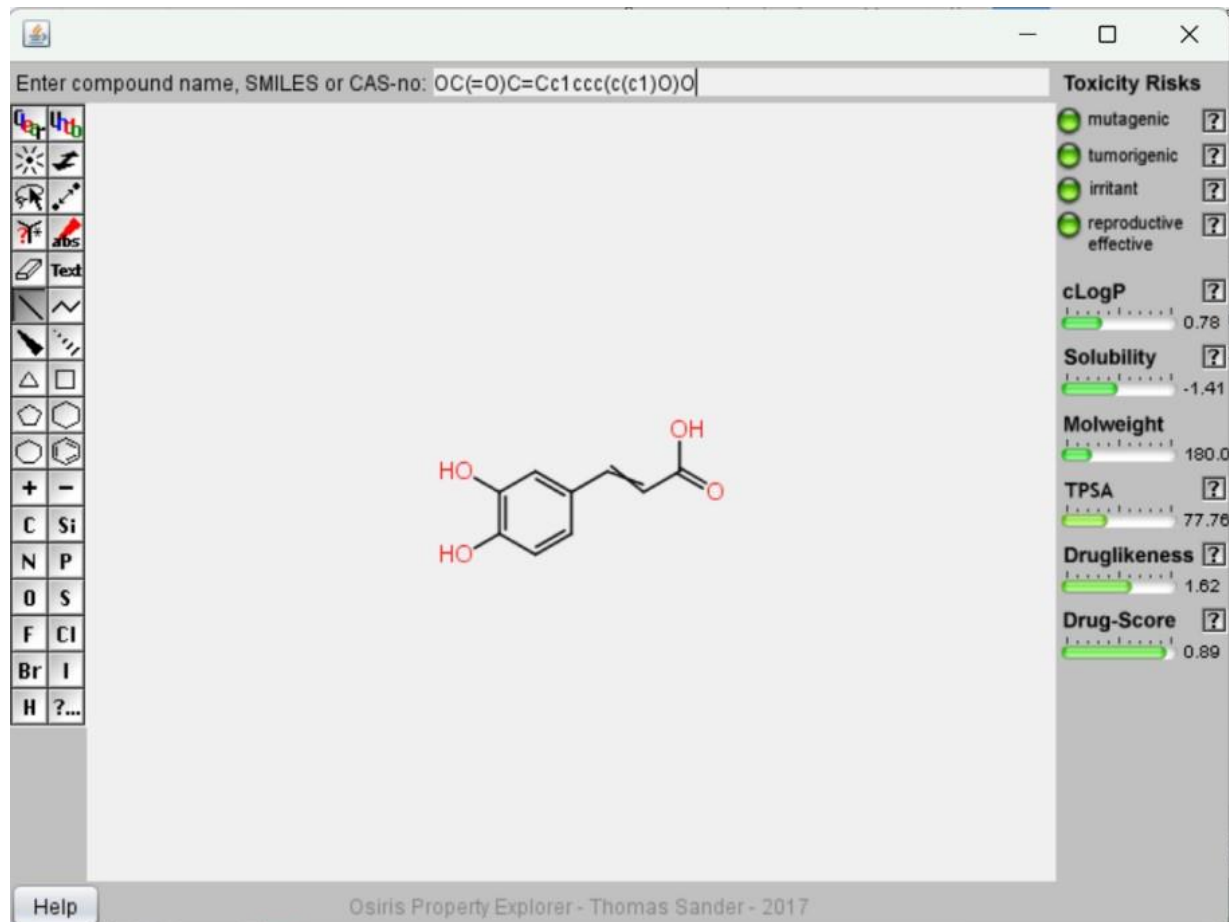


Fig no:5.23. Toxicity analysis of Caffeic acid using OSIRIS Software

Caffeic acid has no mutagenic, tumourigenic, irritant or reproductive effective properties.

The molecular weight is 180

It has a Consensus LogP value of 0.78

The Solubility of Caffeic acid is at -1.41 and the TPSA value is 77.76

The Druglikeness and Drug scores are 1.62 and 0.89 respectively

5.3.2.3 Catechin

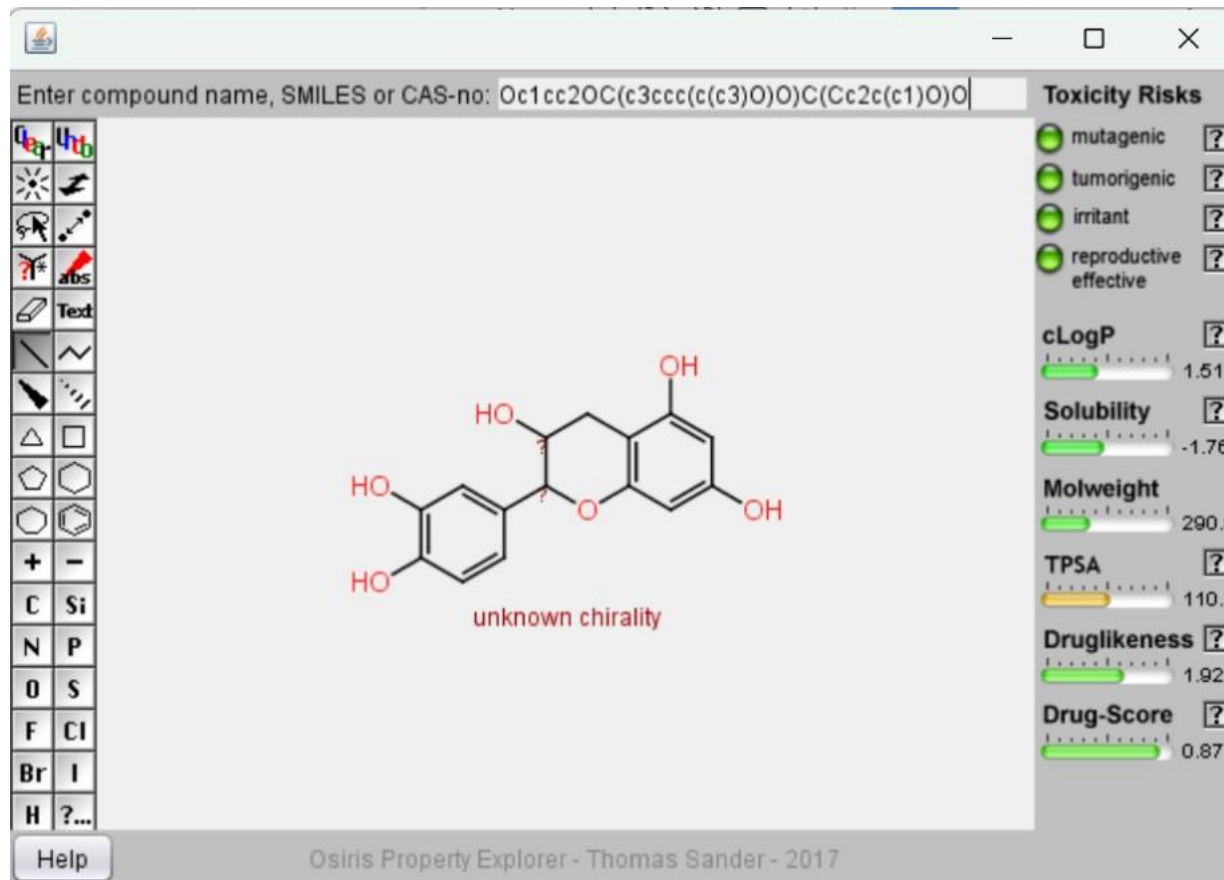


Fig no:5.24. Toxicity analysis of Catechin using OSIRIS Software

Catechin has no mutagenic, tumourigenic, irritant or reproductive effective properties.

The molecular weight is 290

It has a Consensus LogP value of 1.51

The Solubility of Catechin is at -1.76 and the TPSA value is 110.3

The Druglikeness and Drug scores are 1.92 and 0.87 respectively

5.3.2.4 Epicatechin

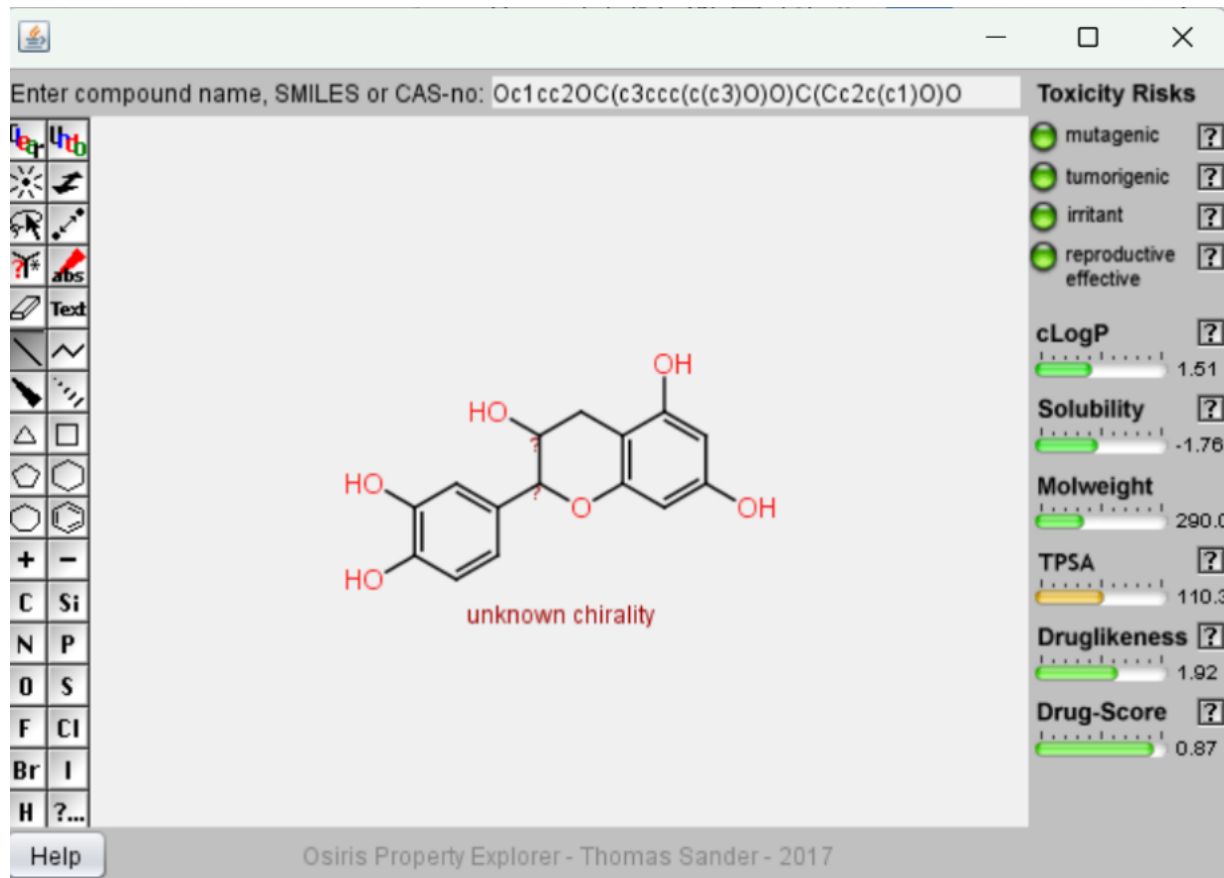


Fig no:5.25 Toxicity analysis of Epicatechin using OSIRIS Software

Epicatechin has no mutagenic, tumourigenic, irritant or reproductive effective properties.

The molecular weight is 290

It has a Consensus LogP value of 1.51

The Solubility of Epicatechin is at -1.76 and the TPSA value is 110.3

The Druglikeness and Drug scores are 1.92 and 0.87 respectively

5.3.2.5 Gallic acid

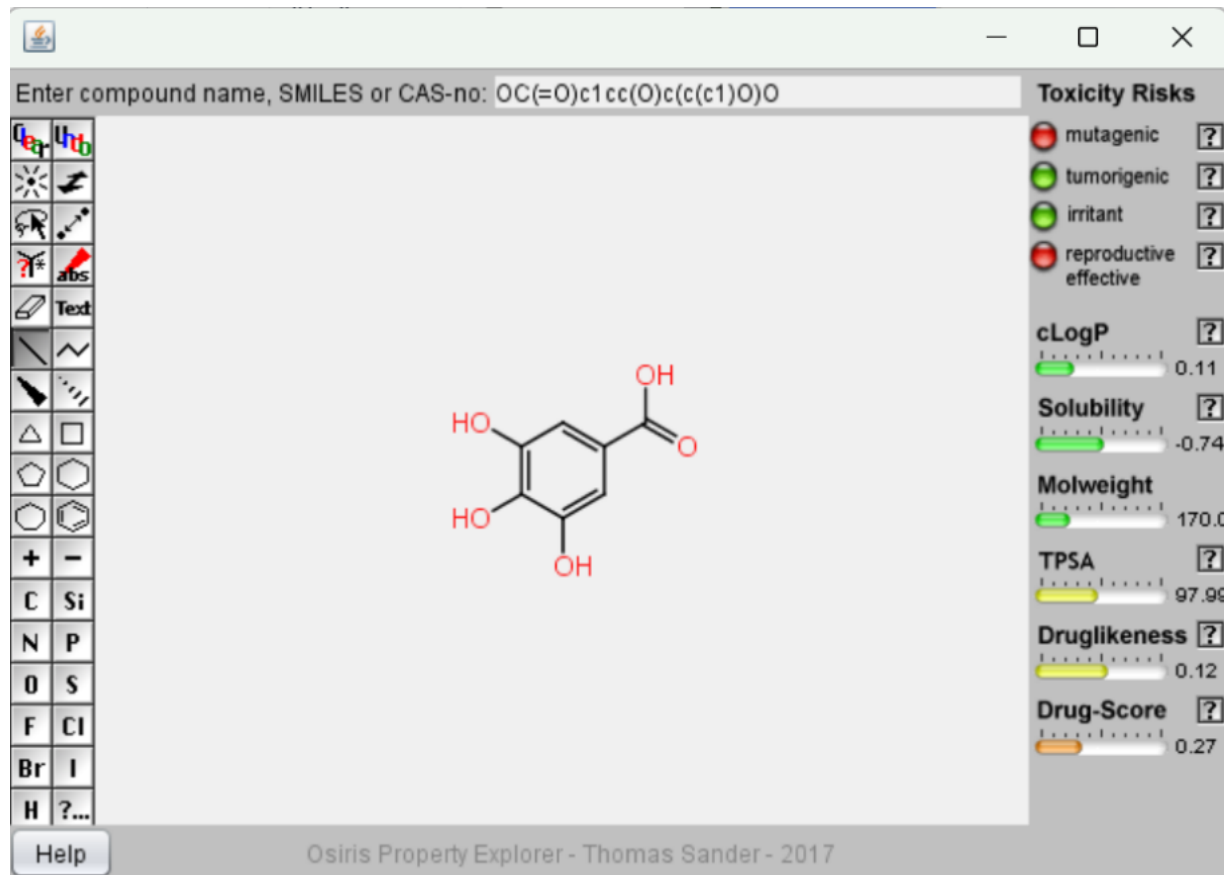


Fig no:5.26 Toxicity analysis of Gallic acid using OSIRIS Software

Gallic acid is mutagenic and reproductive effective but has no tumourigenic, irritant properties.

The molecular weight is 170

It has a Consensus LogP value of 0.11

The Solubility of Gallic acid is at -0.74 and the TPSA value is 97.99

The Druglikeness and Drug scores are 0.12 and 0.27 respectively

5.3.2.6 Maslinic Acid

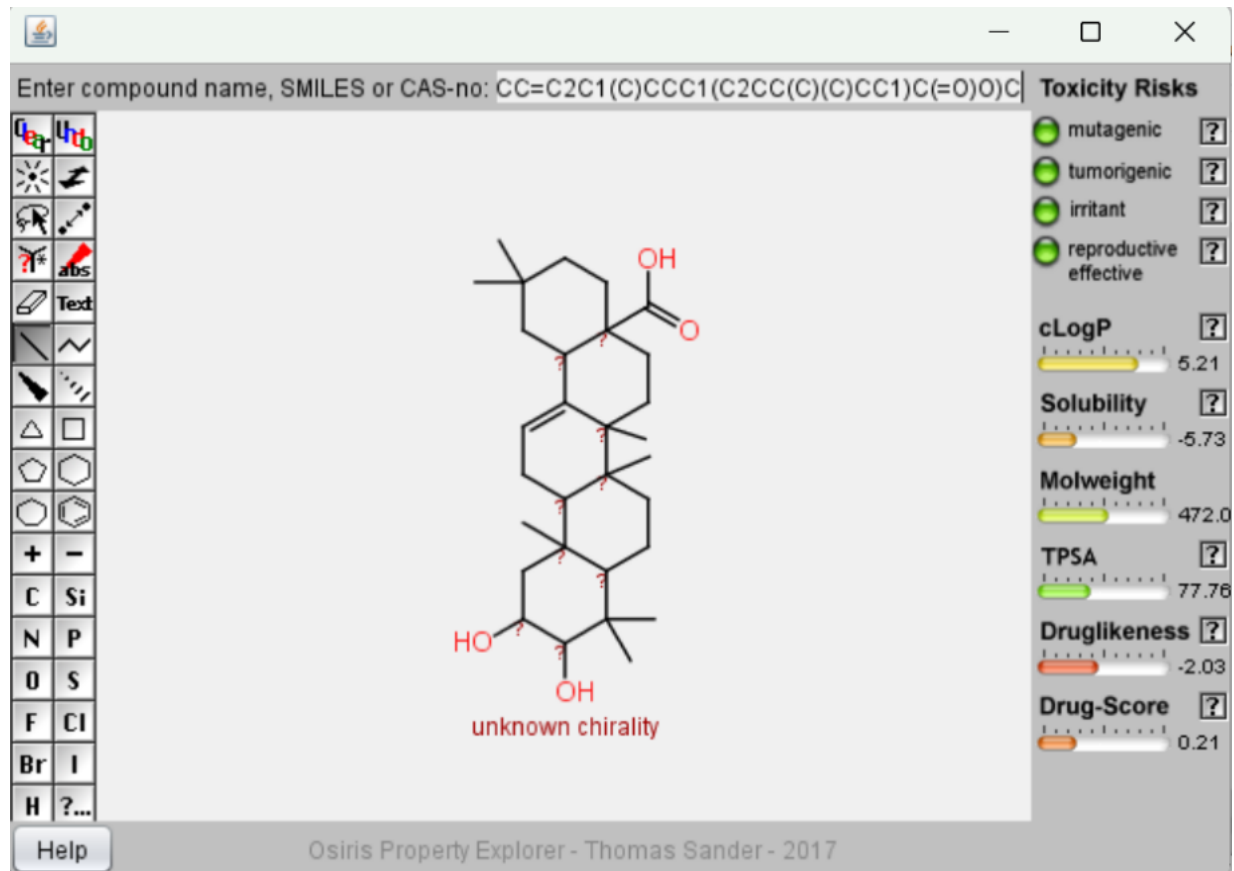


Fig no:5.27. Toxicity analysis of Maslinic acid using OSIRIS Software

Maslinic Acid has no mutagenic, tumourigenic, irritant or reproductive effective properties.

The molecular weight is 472

It has a Consensus LogP value of 5.21

The Solubility of Maslinic Acid is at -5.73 and the TPSA value is 77.76

The Druglikeness and Drug scores are -2.03 and 0.21 respectively

5.3.2.7 Ferulic acid

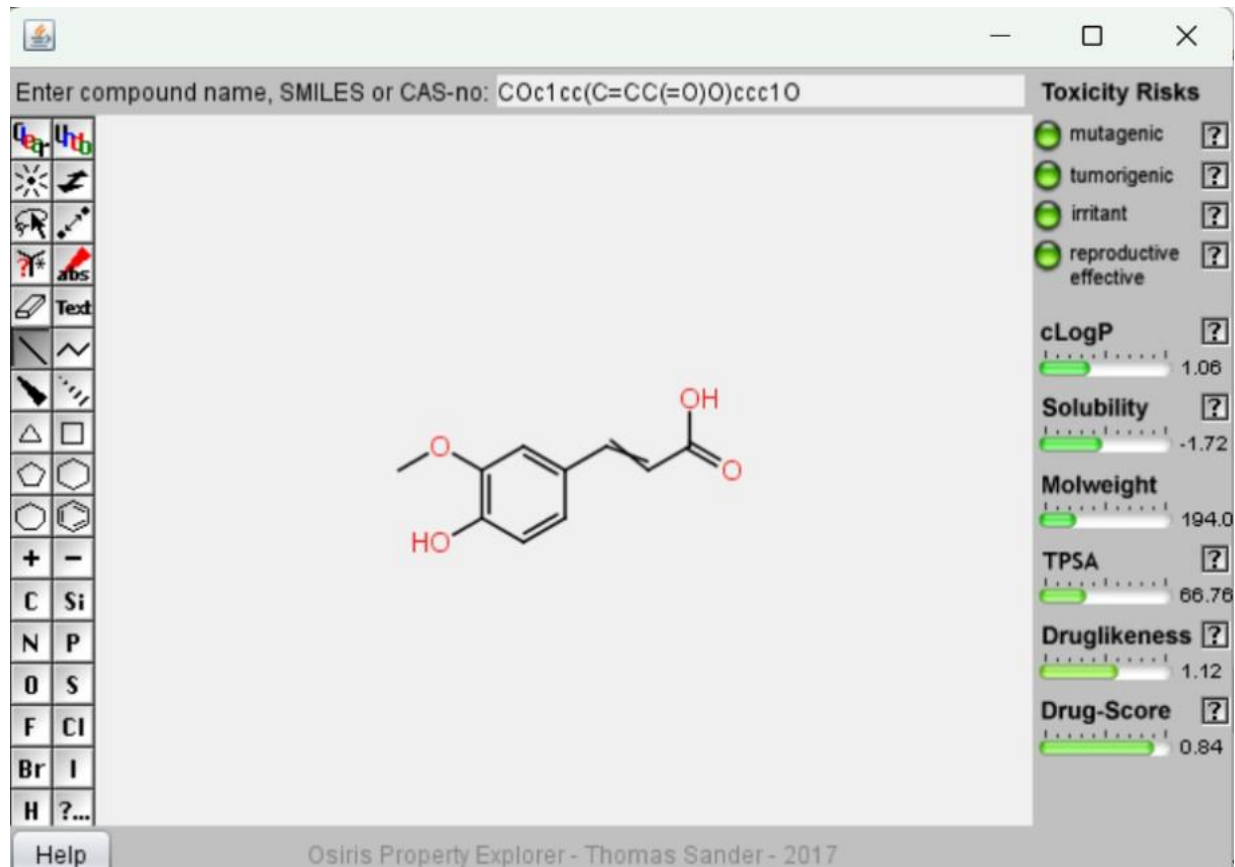


Fig no:5.28 Toxicity analysis of Ferulic acid using OSIRIS Software

Ferulic acid has no mutagenic, tumourigenic, irritant or reproductive effective properties.

The molecular weight is 194

It has a Consensus LogP value of 1.06

The Solubility of Ferulic acid is at -1.72 and the TPSA value is 66.76

The Druglikeness and Drug scores are 1.12 and 0.84 respectively

5.3.2.8 Apigenin

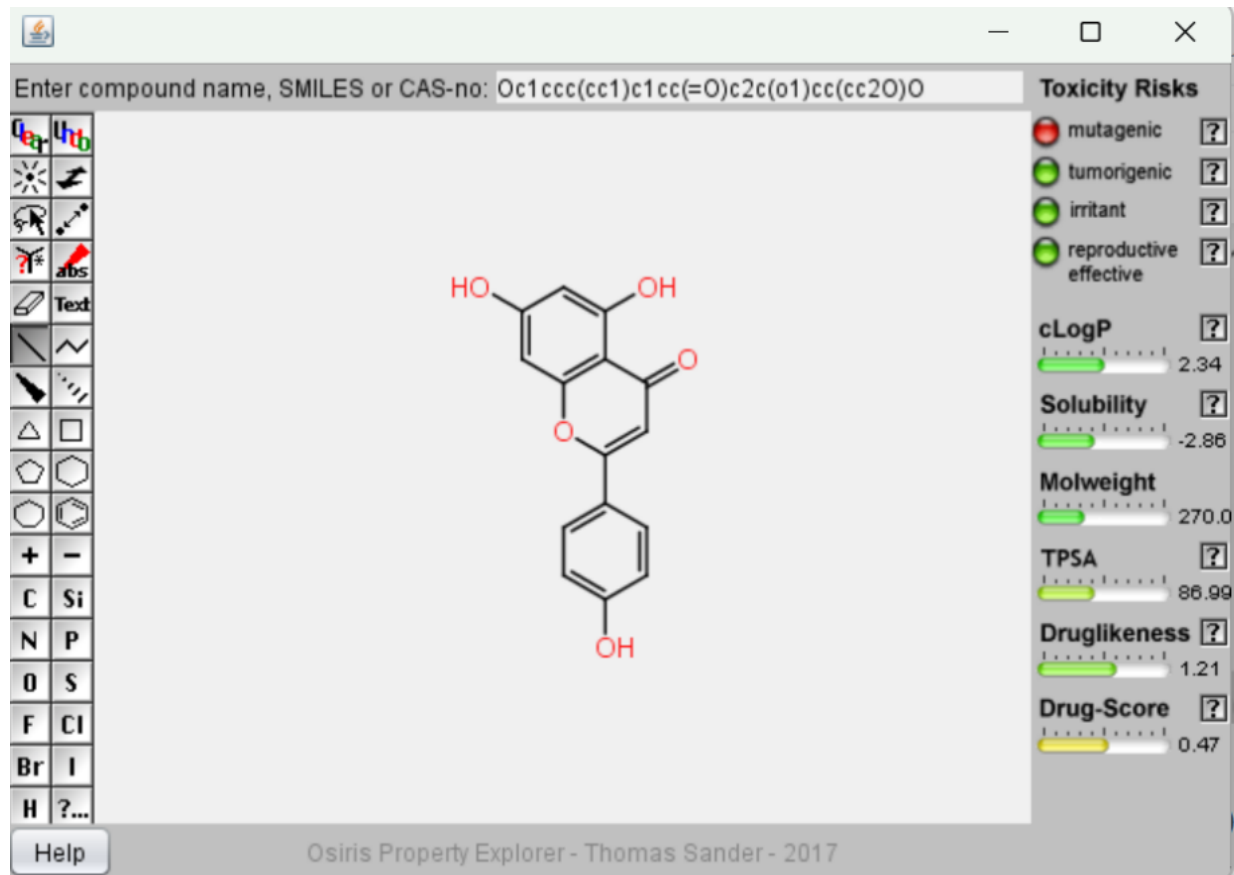


Fig no:5.29. Toxicity analysis of using OSIRIS Software

Apigenin is mutagenic but has no tumourigenic, irritant or reproductive effective properties.

The molecular weight is 270

It has a Consensus LogP value of 2.34

The Solubility of Apigenin is at -2.86 and the TPSA value is 86.99

The Druglikeness and Drug scores are 1.21 and 0.47 respectively

5.3.2.9 Hesperetin

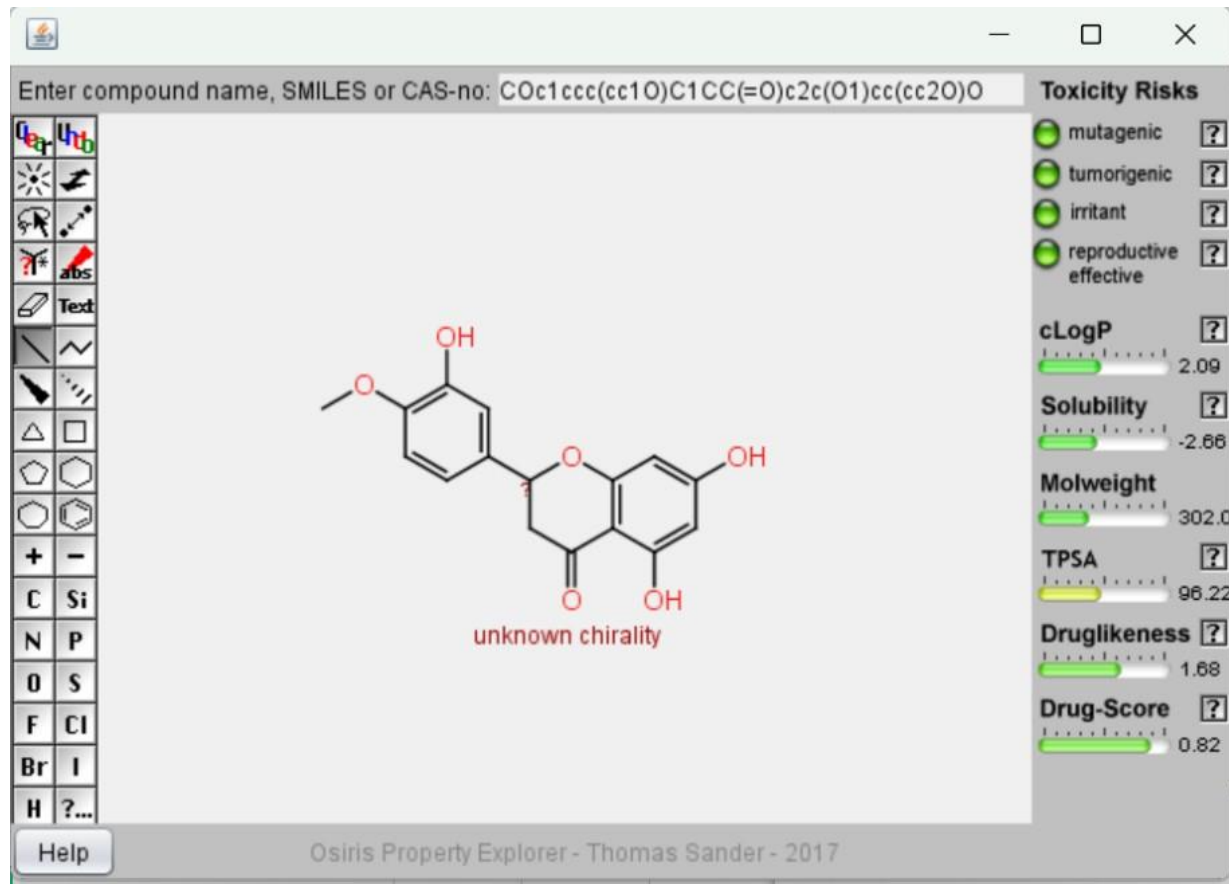


Fig no:5.30. Toxicity analysis of using OSIRIS Software

Hesperetin has no mutagenic, tumourigenic, irritant or reproductive effective properties.

The molecular weight is 302

It has a Consensus LogP value of 2.09

The Solubility of Hesperetin is at -2.66 and the TPSA value is 96.22

The Druglikeness and Drug scores are 1.68 and 0.82 respectively.

5.3.2.10 Quercetin

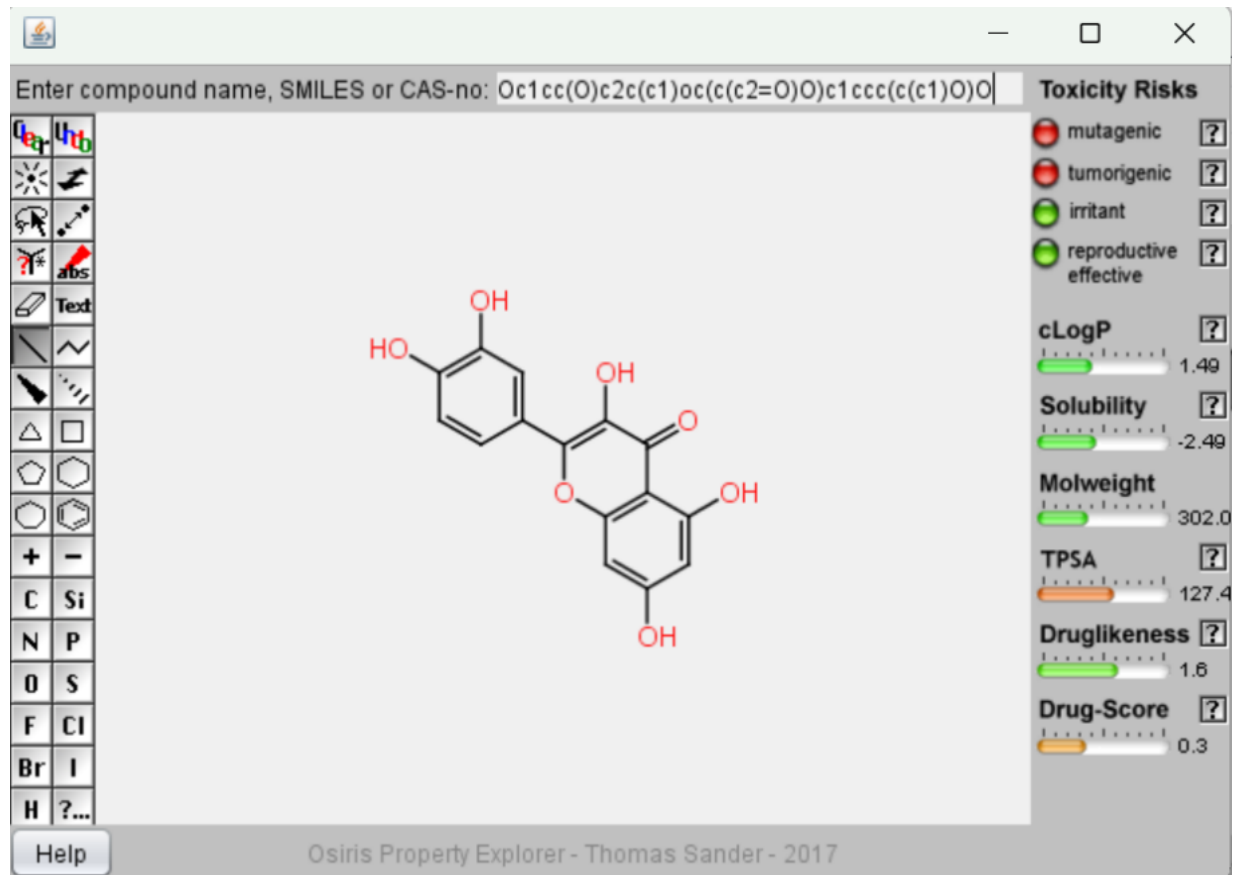


Fig no:5.31. Toxicity analysis of using OSIRIS Software

Quercetin is both mutagenic, tumourigenic but it has no irritant or reproductive effective properties.

The molecular weight is 302

It has a Consensus LogP value of 1.49

The Solubility of Quercetin is at -2.49 and the TPSA value is 127.4

The Druglikeness and Drug scores are 1.6 and 0.3 respectively

5.3.2.11 Sinapic acid

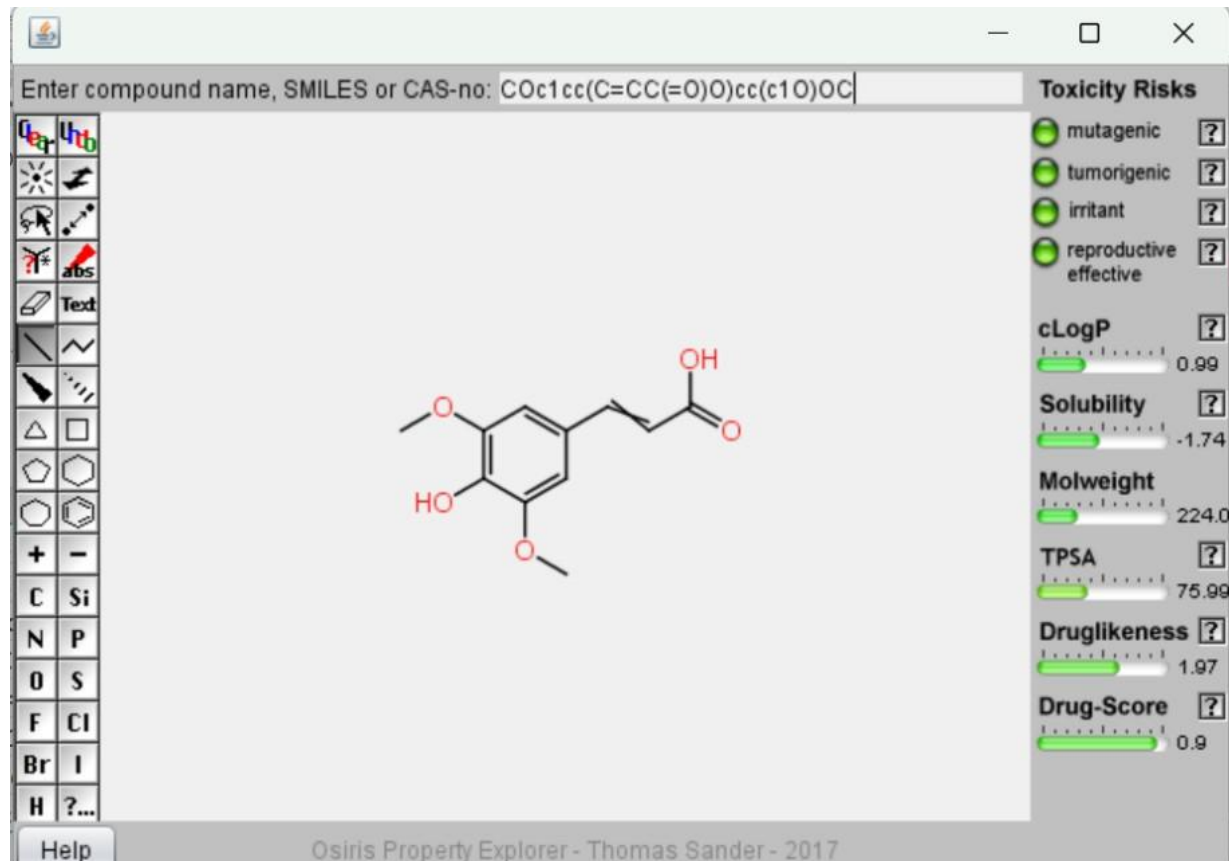


Fig no:5.32. Toxicity analysis of Sinapic acid using OSIRIS Software

Sinapic acid has no mutagenic, tumourigenic, irritant or reproductive effective properties.

The molecular weight is 224

It has a Consensus LogP value of 0.99

The Solubility of Sinapic acid is at -1.74 and the TPSA value is 75.99

The Druglikeness and Drug scores are 1.97 and 0.9 respectively

5.3.2.12 16-B1-phytoprostane

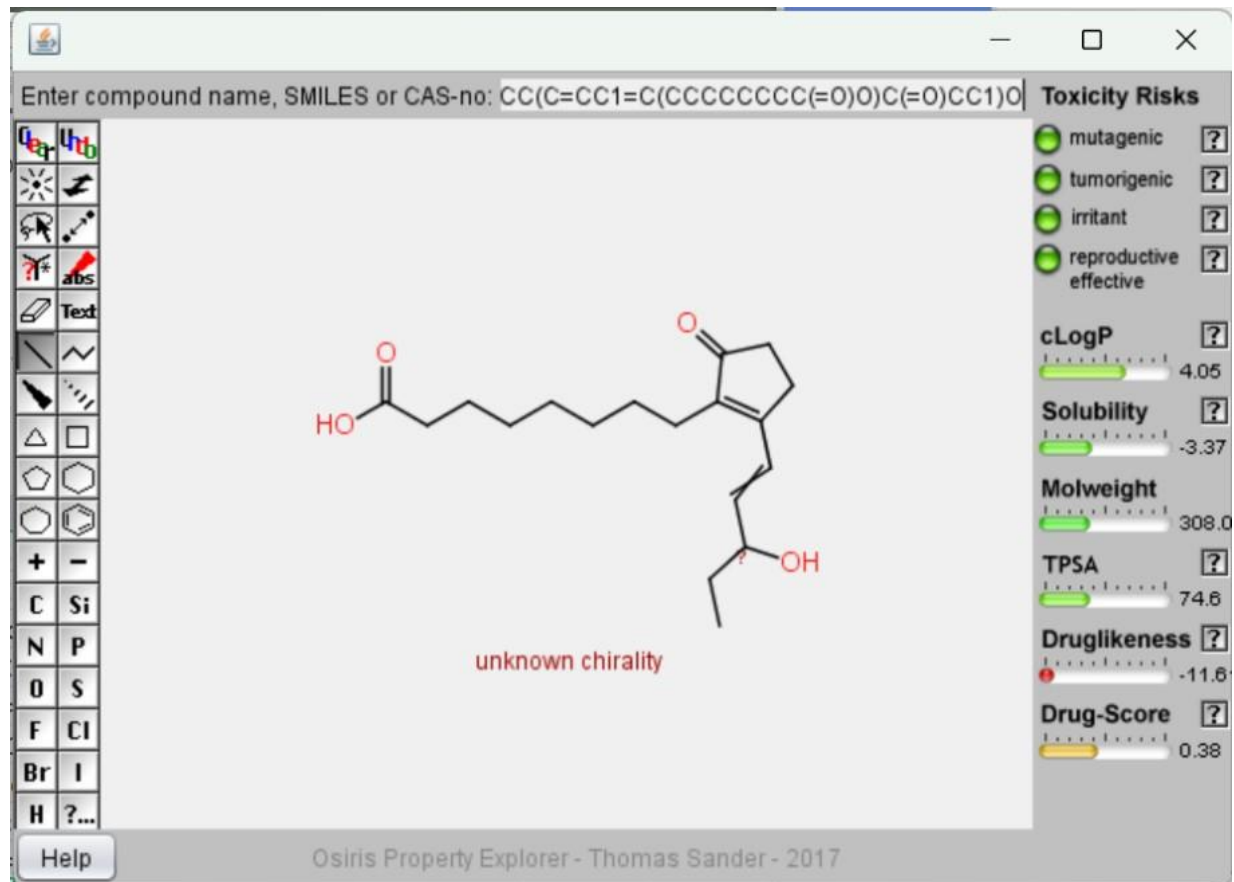


Fig no:5.33. Toxicity analysis of 16-B1-phytoprostane using OSIRIS Software

16-B1-phytoprostane has no mutagenic, tumourigenic, irritant or reproductive effective properties.

The molecular weight is 308

It has a Consensus LogP value of 4.05

The Solubility of 16-B1-phytoprostane is at -3.37 and the TPSA value is 74.6

The Druglikeness and Drug scores are -11.6 and 0.38 respectively

5.3.2.13 Homovanillic acid

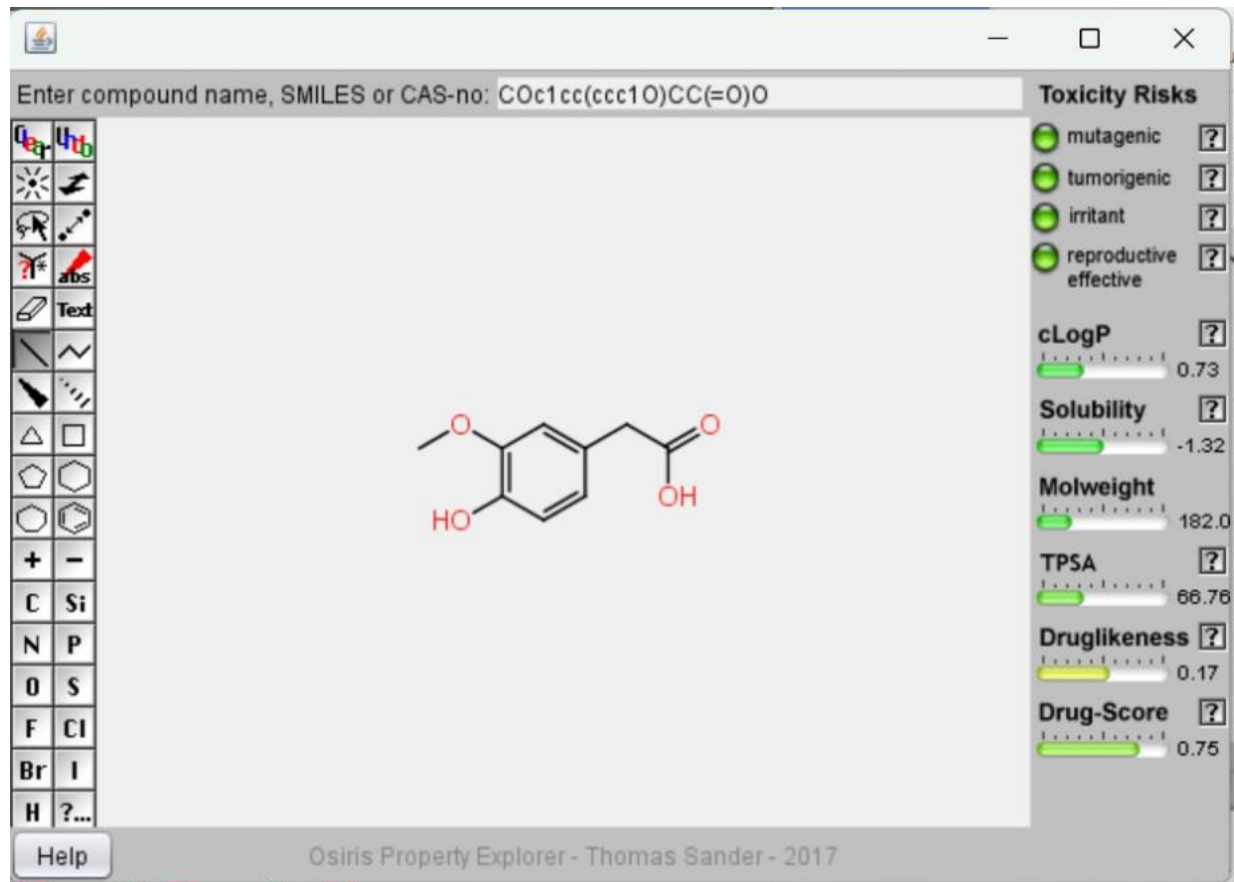


Fig no:5.34. Toxicity analysis of Homovanillic acid using OSIRIS Software

Homovanillic acid has no mutagenic, tumourigenic, irritant or reproductive effective properties.

The molecular weight is 182

It has a Consensus LogP value of 0.73

The Solubility of Homovanillic acid is at -1.23

and the TPSA value is 66.76

The Druglikeness and Drug scores are 0.17 and 0.75 respectively

5.3.2.14 Hydroxytyrosol

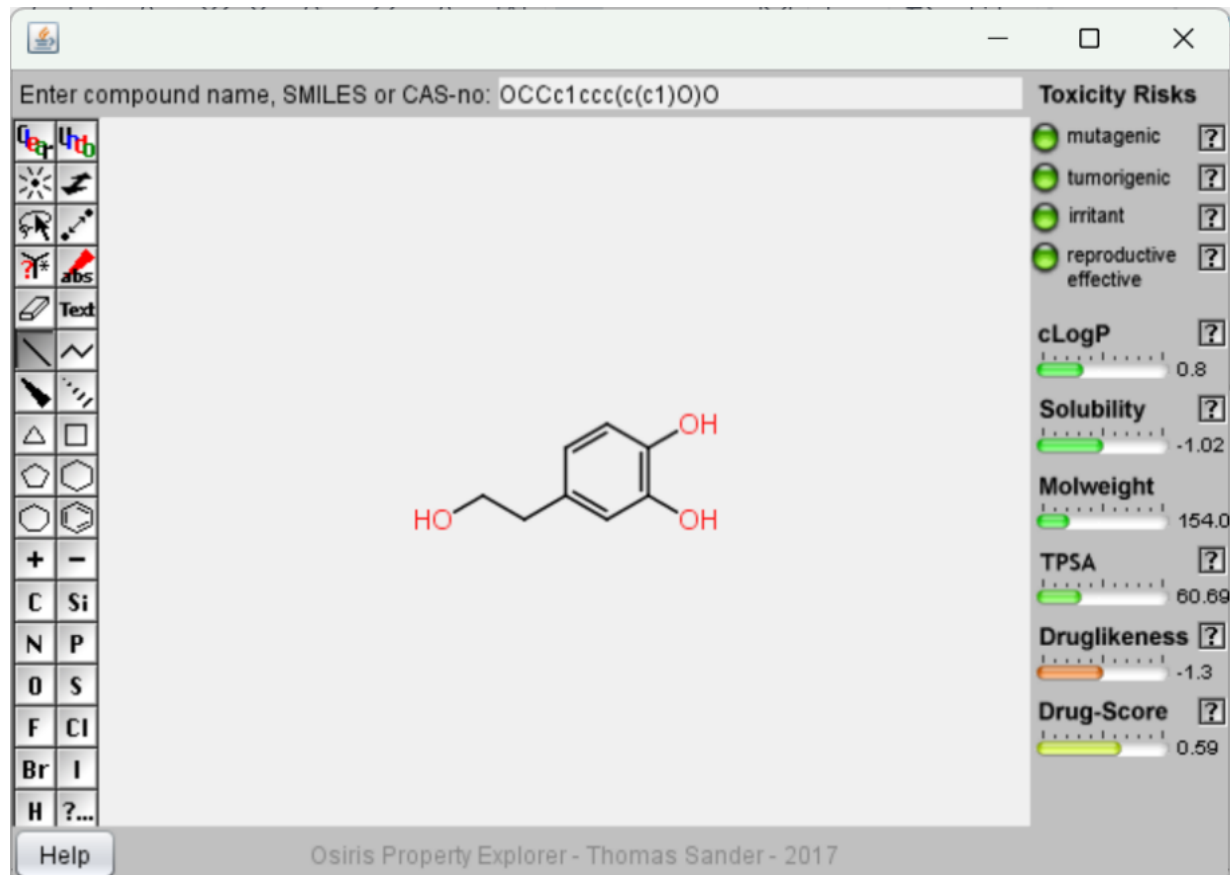


Fig no:5.35. Toxicity analysis of Hydroxytyrosol using OSIRIS Software

Hydroxytyrosol has no mutagenic, tumourigenic, irritant or reproductive effective properties.

The molecular weight is 154

It has a Consensus LogP value of 0.8

The Solubility of Hydroxytyrosol is at -1.02

and the TPSA value is 60.69

The Druglikeness and Drug scores are -1.3 and 0.59 respectively

5.3.2.15 Secoisolariciresinol

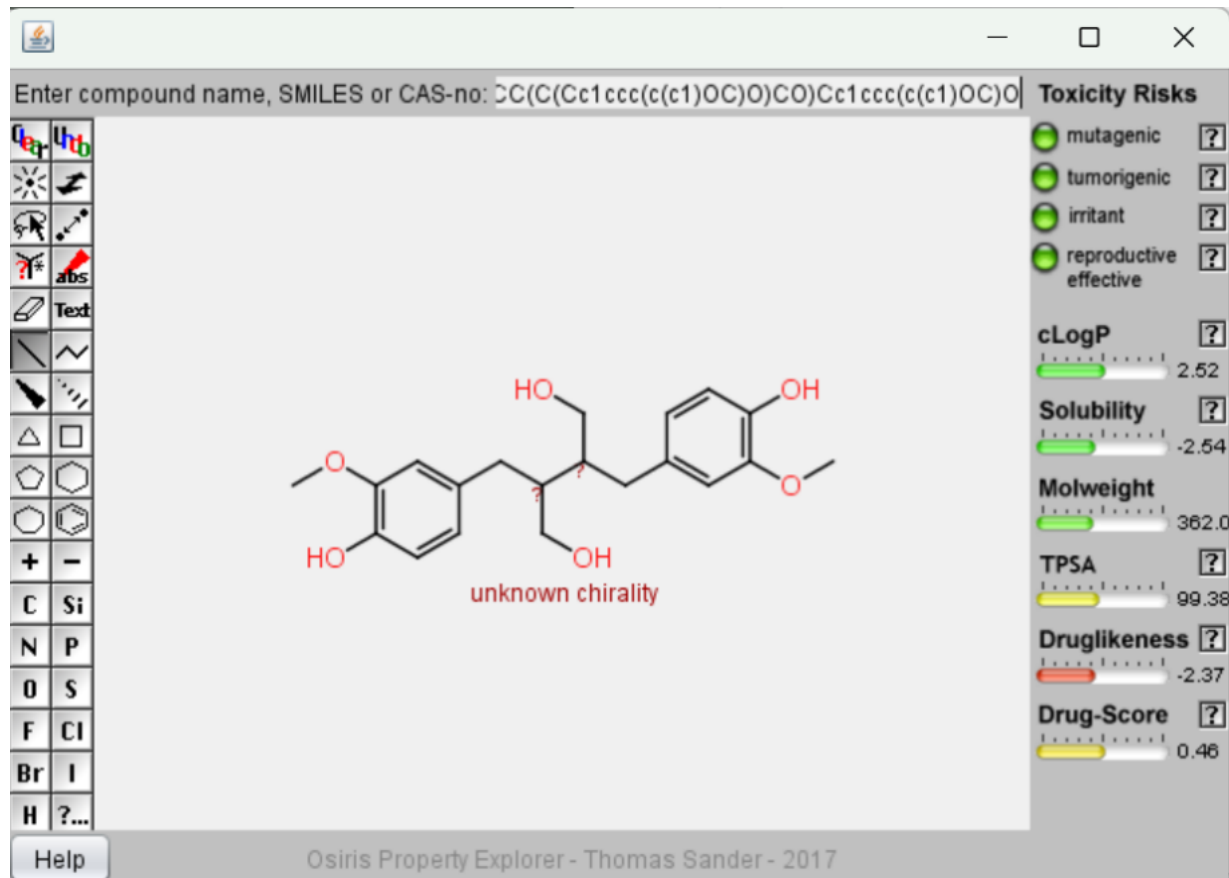


Fig no:5.36. Toxicity analysis of Secoisolariciresinol using OSIRIS Software

Secoisolariciresinol has no mutagenic, tumourigenic, irritant or reproductive effective properties.

The molecular weight is 362

It has a Consensus LogP value of 2.52

The Solubility of Secoisolariciresinol is at -2.54

and the TPSA value is 99.38

The Druglikeness and Drug scores are -2.37 and 0.46 respectively

5.3.2.16 Vanillic acid

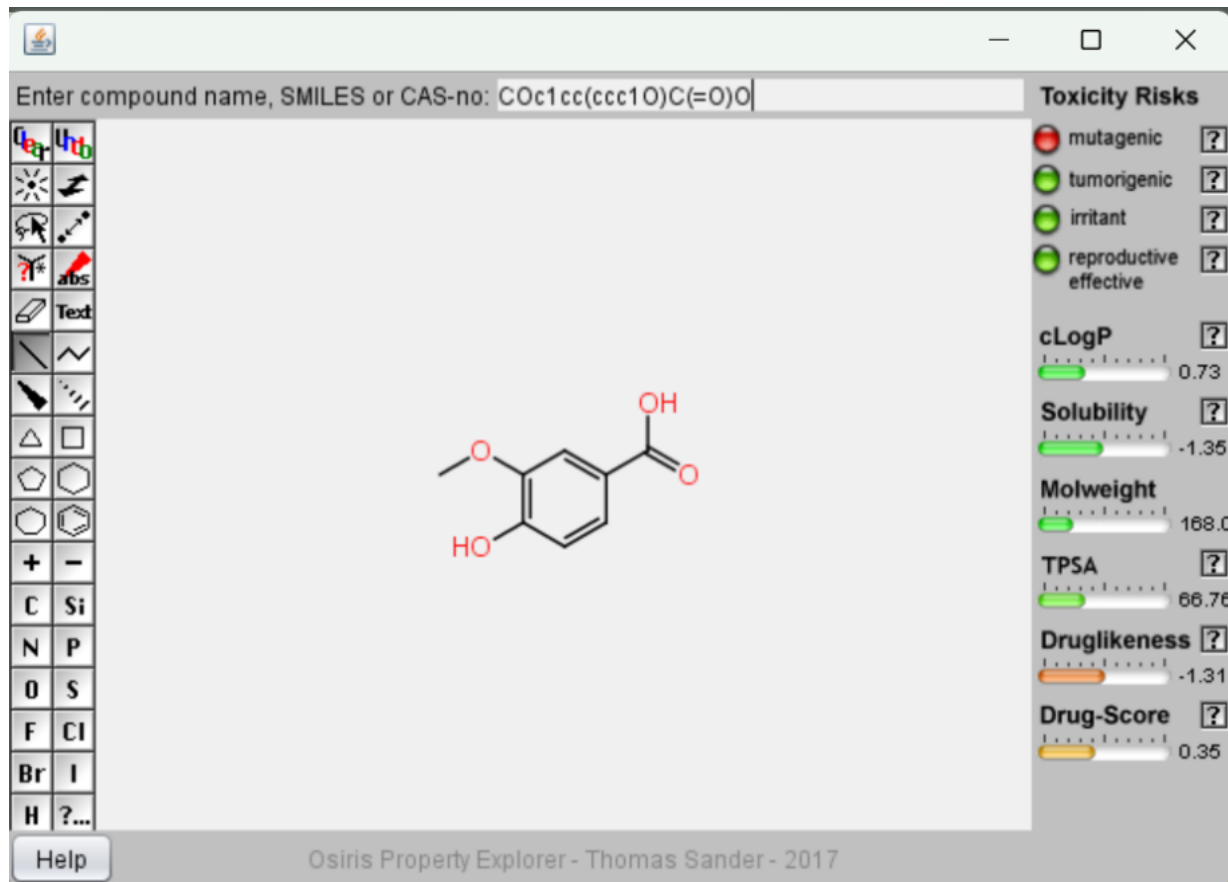


Fig no:5.37. Toxicity analysis of Vanillic acid using OSIRIS Software

Vanillic acid is mutagenic but has no tumourigenic, irritant or reproductive effective properties.

The molecular weight is 168

It has a Consensus LogP value of 0.73

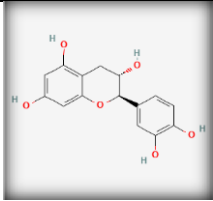
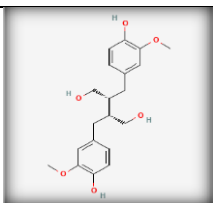
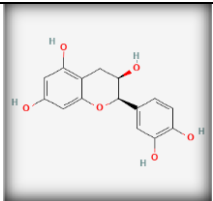
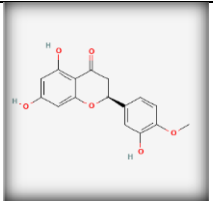
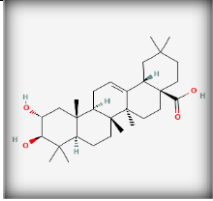
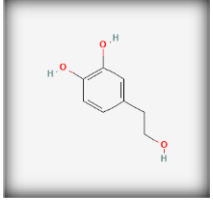
The Solubility of Vanillic acid is at -1.35

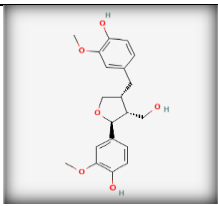
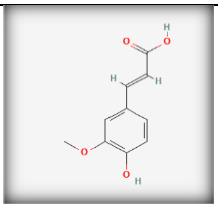
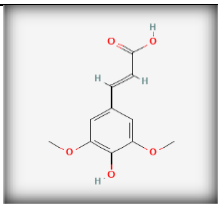
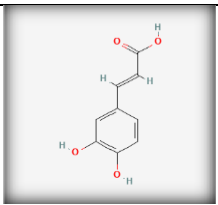
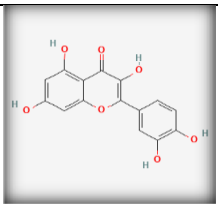
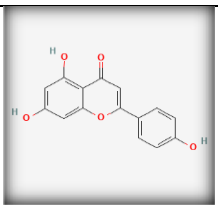
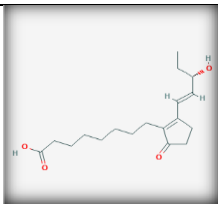
and the TPSA value is 66.76

The Druglikeness and Drug scores are -1.31 and 0.35 respectively.

5.4 SMALL MOLECULES RESULTS

Table 5.4: Ligand 2D structure

S No	SOURCE	COMPOUND	2D STRUCTURE	MW	CDOCKER ENERGY
1	Carrot	Catechin		290.27	25.0142
2	Olive	Secoisolariciresinol		362.4	23.5985
3	Apple	Epicatechin		290.27	26.2351
4	Orange	Hesperetin		302.28	23.5698
5	Apple	Maslinic Acid		472.7	-66.828
6	Olive	Hydroxytyrosol		154.16	23.8195

7	Carrot	Lariciresinol		360.4	12.7119
8	Fennel	Ferulic acid		194.18	23.4146
9	Orange	Sinapic acid		224.21	22.8871
10	Carrot	Caffeic Acid		180.16	28.8848
11	Orange	Quercetin		302.23	31.633
12	Parsley	Apigenin		270.24	25.1104
13	Olive	Octanoic acid		308.4	5.00472

The 2D structures of all 13 docked ligands show covalent bonds and cdocker scores for corresponding poses. By default pose1 optimized with the highest energies.

5.5 DOCKING RESULTS

Table 5.5: Docking results

S No	COMPOUND	MW	CDOCKER ENERGY	CDOCKER INTERACTION ENERGY	HBONDS AMINO ACID	NON H - BOND	UNFAVOURABLE BOND
1	Catechin	290.27	25.0142	36.9595	6	1	0
2	Secoisolariciresinol	362.4	23.5985	37.3265	5	5	0
3	Epicatechin	290.27	26.2351	32.4396	6	6	0
4	Hesperetin	302.28	23.5698	36.6268	6	7	0
5	Maslinic Acid	472.7	-66.828	35.5992	4	7	0
6	Hydroxytyrosol	154.16	23.8195	24.5037	4	5	0
7	Lariciresinol	360.4	12.7119	38.5165	7	6	0
8	Ferulic acid	194.18	23.4146	29.5706	5	5	0
9	Sinapic acid	224.21	22.8871	31.543	4	8	0
10	Caffeic Acid	180.16	28.8848	30.1588	5	5	0
11	Quercetin	302.23	31.633	37.2415	6	7	0
12	Apigenin	270.24	25.1104	30.8246	3	8	0
13	Octanoic acid	308.4	5.00472	36.5614	5	7	0

The ligands with more number of H-bonds, highest cdocker and cdocker interaction energy scores for each phytochemical are chosen as they have better binding affinity. Additionally favourable bonds and interactions like pi-alkyl, amide-pi stacks, alkyl groups support the stability of the molecule. Ligands with unfavourable bonds or bumps are not suitable drug compounds.

5.6 INTERACTIONS OF DRUG COMPOUNDS WITH TARGET

5.6.1 Catechin

Catechin is found in fresh tea leaves, carrots, apricots, broad beans, apples, blackberries, strawberries, cherries, pears, and raspberries

It has anti-tumor activity and is currently used for the suppression of inflammation resulting from cell injury and treatment of diseases such as IBD, lung injury, and inflammation-induced carcinogenesis.

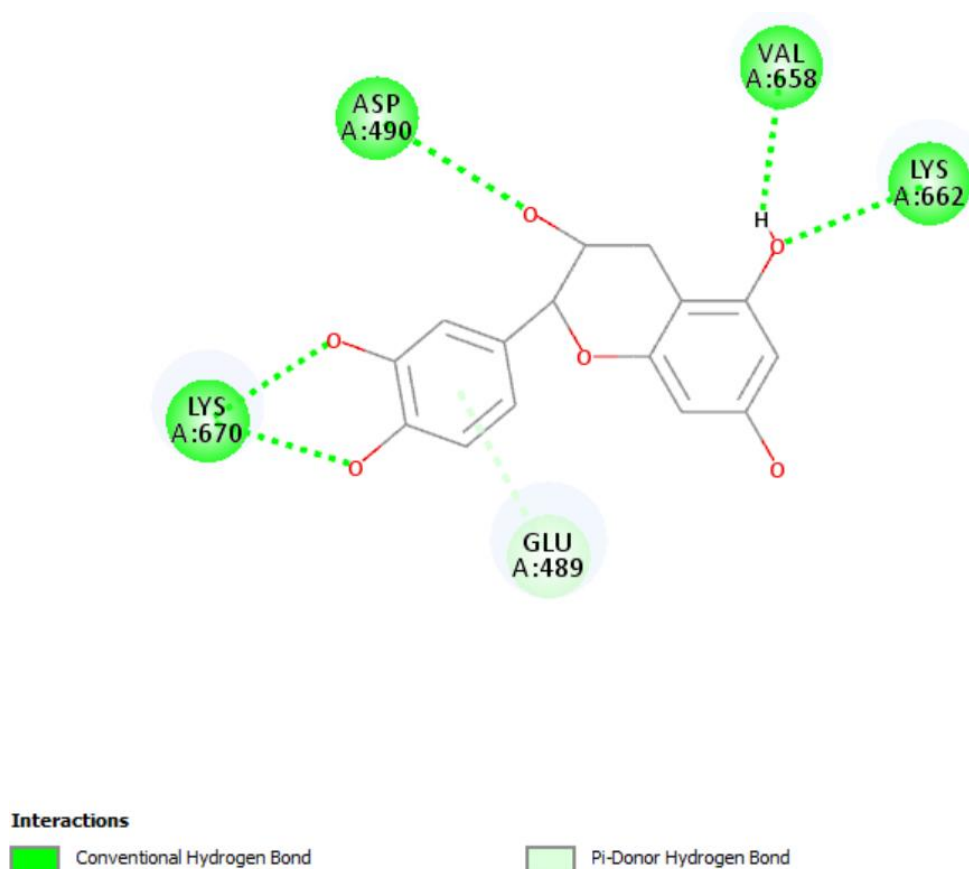


Fig no:5.38.Interaction of Catechin with target

Catechin has 5 conventional hydrogens bonding with LYS A:670, ASP A:490, VAL A:658 and LYS A:662. It also has 1 Pi-Hydrogen bond with GLU A:489

5.6.2 Secoisolariciresinol

Secoisolariciresinol is naturally found in flax seeds, walnuts, rye and olives. It is associated with a decrease in serum total cholesterol, LDL-C, and oxidative stress, and an increase in HDL-C levels

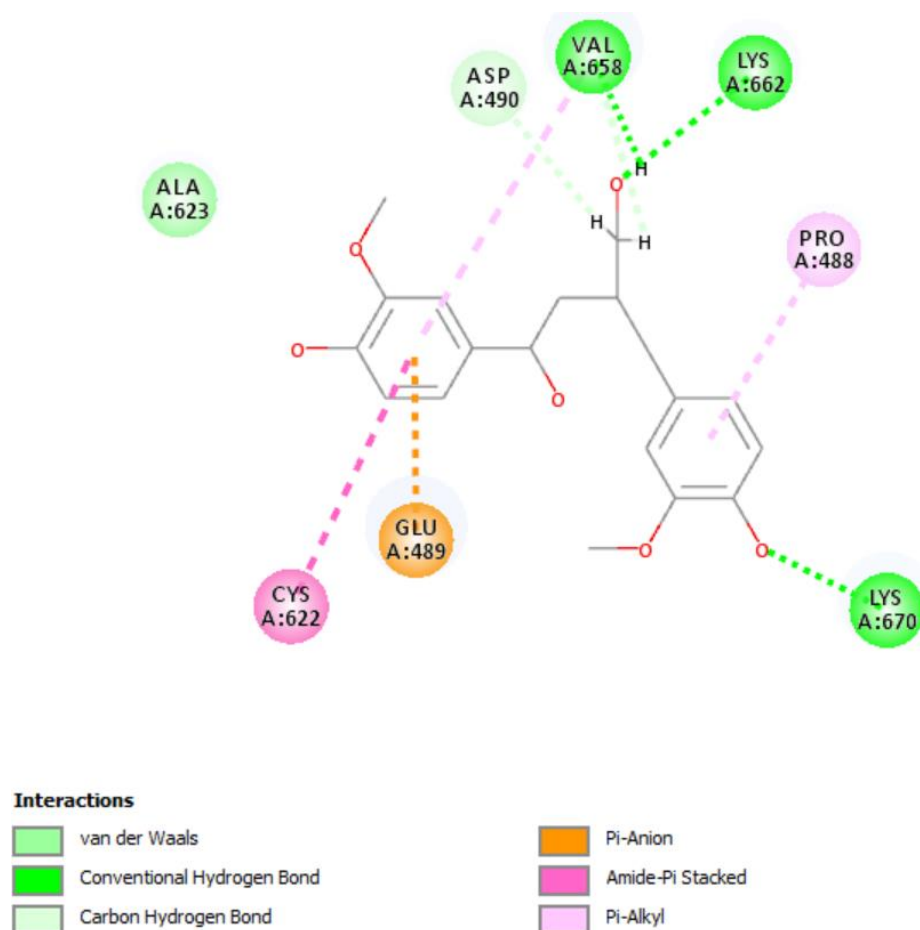


Fig no:5.39. Interaction of Secoisolariciresinol with target

Secoisolariciresinol has 3 conventional hydrogen bonds with VAL A:658, LYS A:662 and LYS A:670. It has 1 carbon hydrogen bond with ASP A:490 as well as van der Waals interaction with ALA A:623. It has a Pi-anion bond with GLY A:489 and a Pi-alkyl bond with PRO A:488. It also has an Amide-Pi stacked with CYS A:622.

5.6.3 Epicatechin

Epicatechin is found in apples, blackberries, broad beans, cherries, black grapes, pears, raspberries, and chocolate.

It promotes blood flow and metabolic rate while simultaneously regulating blood sugar levels and improves muscle growth and strength, it is also a potent antioxidant that helps reduce cholesterol levels and improve cardiovascular and brain functioning.

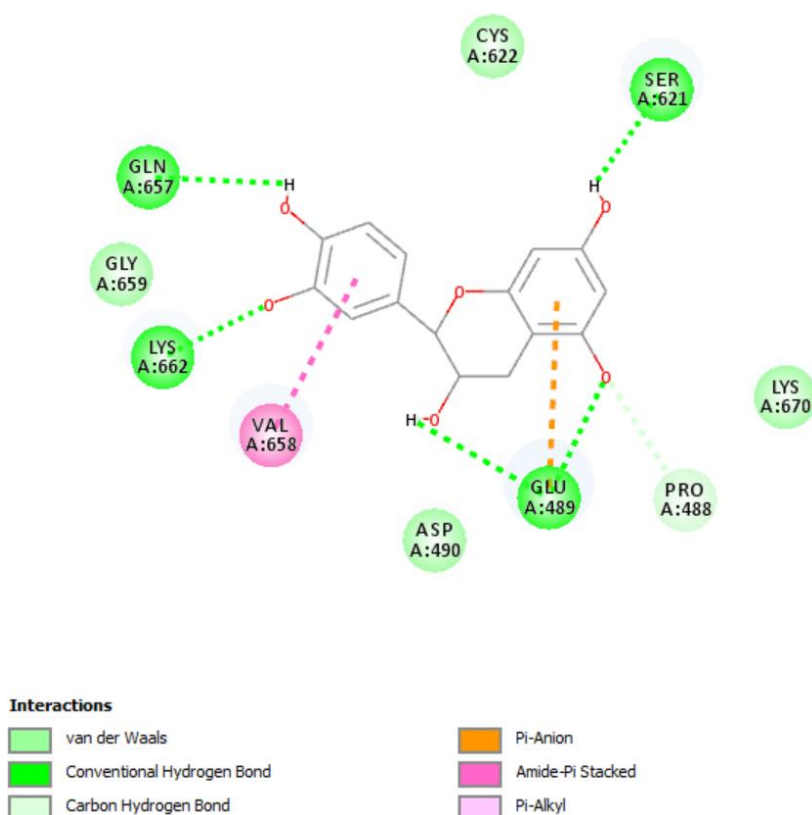


Fig no:5.40. Interaction of Epicatechin with target

Epicatechin has 5 conventional hydrogen bonds with GLN A:657, SER A:621, GLU A:489 and LYS A:662. It has carbon hydrogen bond with PRO A:488 and van der Waals interaction with GLY A:659, LYS A:670, ASP A:490. It has a Pi-anion bond with GLU A:489. It also has a Pi-alkyl bond and an Amide-Pi stacked with VAL A:658.

5.6.4 Hesperetin

Hesperetin is found in citrus fruits, mainly lemons and sweet oranges. It has neuroprotective and antidiabetic properties as well anti-inflammatory and cytoprotective actions in models of acute liver toxicity and is used for the treatment of inflammatory liver diseases accompanied by extensive hepatocyte injury, such as fulminant hepatitis.

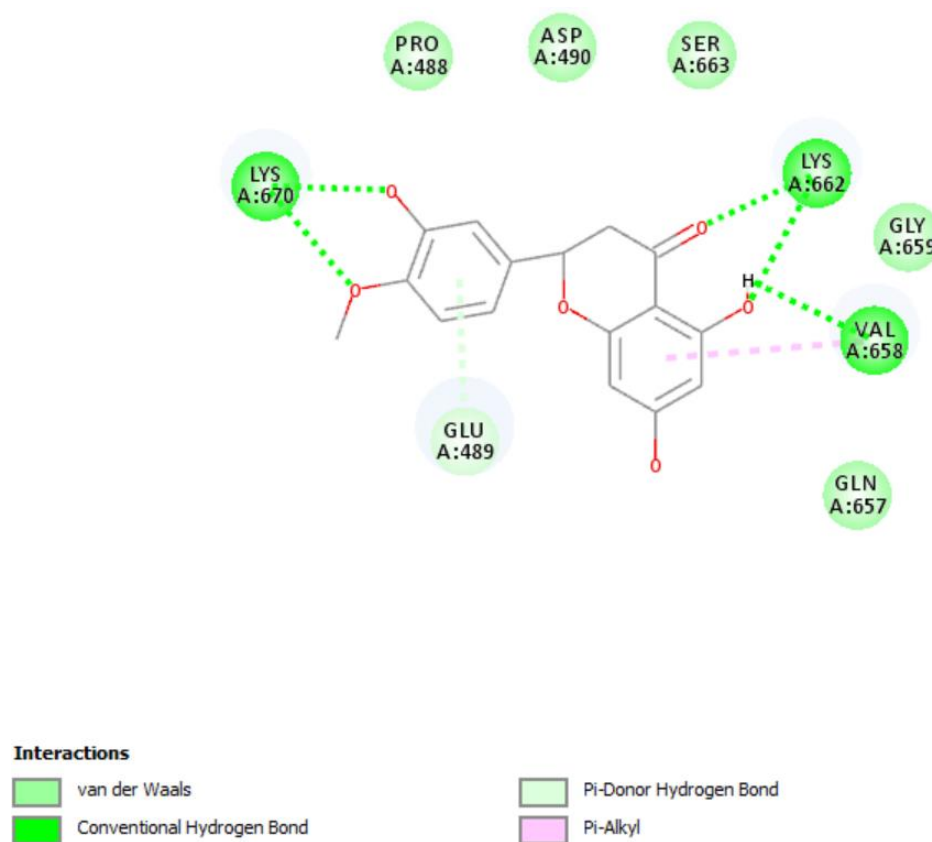


Fig no:5.41. Interaction of Hesperetin with target

Hesperetin has 5 conventional hydrogen bonds with LYS A:670, LYS A:662, VAL A:658. It has 1 Pi-donor hydrogen bond with GLU A:489. It has van der Waals interaction with PRO A:488, GLN A:657, ASP A:490, SER A:663, GLY A:659 and 1 Pi-alkyl interaction with VAL A:658.

5.6.5 Maslinic Acid

Maslinic Acid is found in olive, red dates, eucalyptus, crape myrtle, sage, plantain and apples.

It is used in skin care products and dietary supplements. It also has a wide range of biological benefits including antitumor, antidiabetic, anti-obesity, antioxidant, anti-inflammatory, cardioprotective, neuroprotective and antimicrobial properties

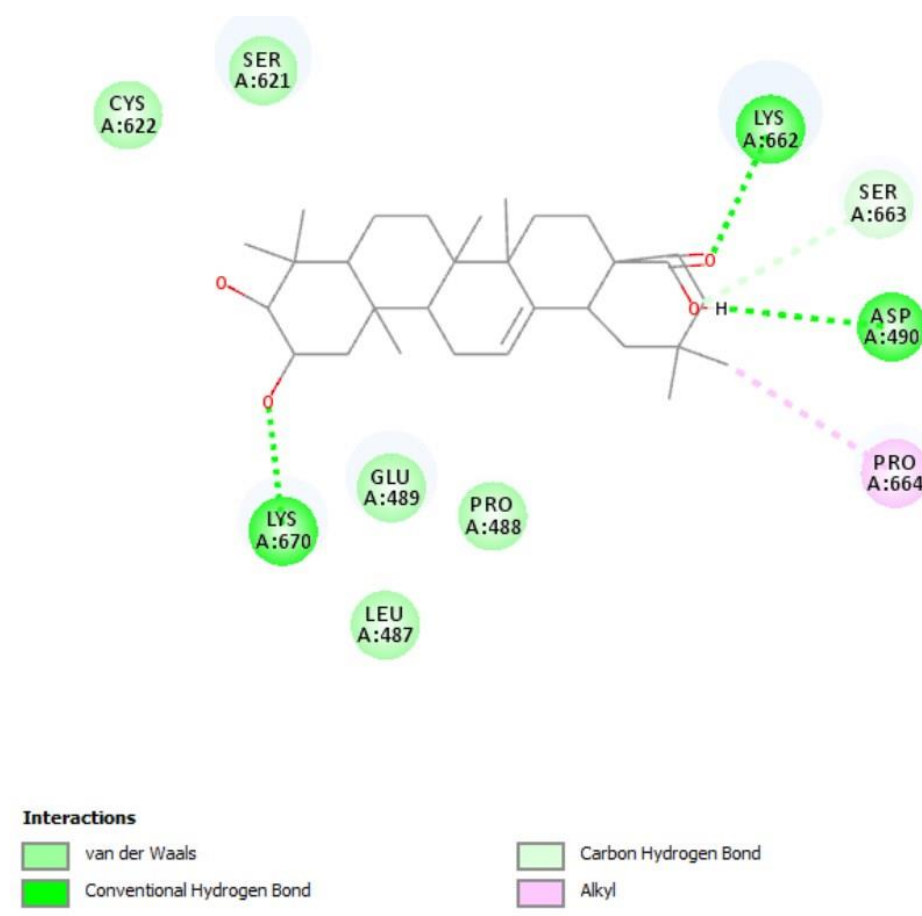


Fig no:5.42. Interaction of Maslinic Acid with target

Maslinic Acid has 3 conventional hydrogen bonds with LYS A:670, LYS A:662, ASP A:490. It has carbon hydrogen bond with SER A:663. It has van der Waals interaction with SER A:621, CYS A:622, GLU A:489, LEU A:487, PRO A:488 and an alkyl bond with PRO A:664.

5.6.6 Hydroxytyrosol

Hydroxytyrosol is found in olives, virgin olive oil and wine. Its regular consumption has several beneficial effects such as antioxidant, anti-inflammatory, anticancer, and as a protector of skin and eyes.

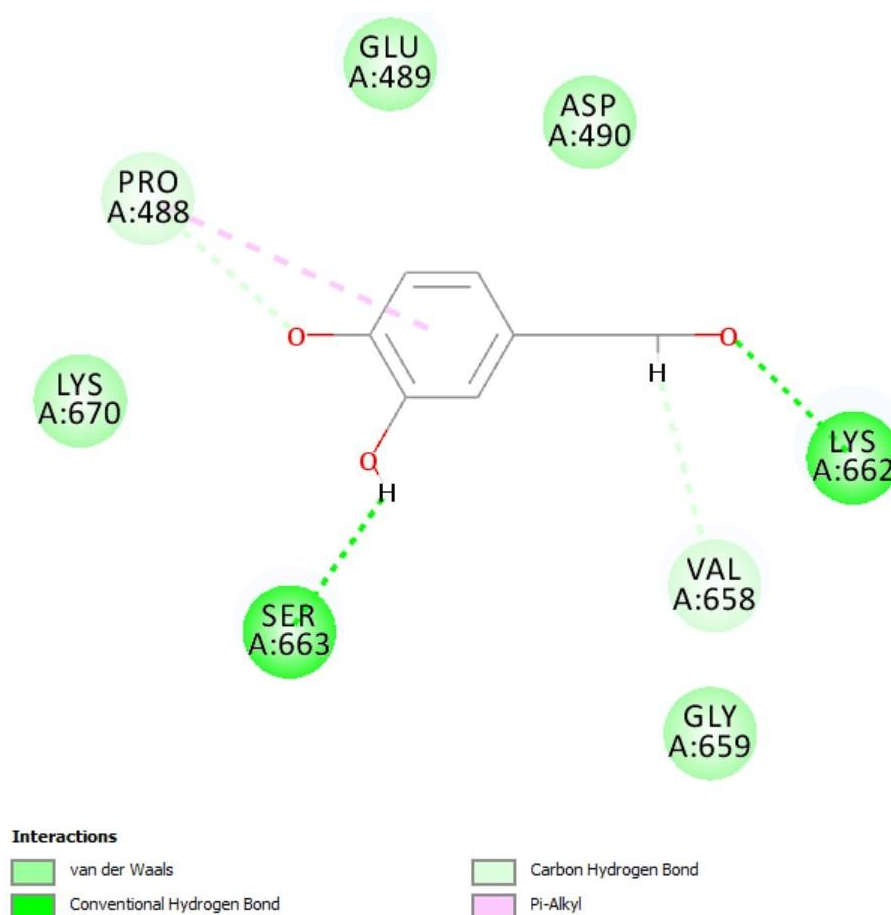


Fig no:5.43 Interaction of Hydroxytyrosol with target

Hydroxytyrosol has 2 conventional hydrogen bonds with SER A:663, LYS A:662 and carbon hydrogen bonds with VAL A:658, PRO A:488. It has van der Waals interaction with LYS A:670, GLU A:489, ASP A:490, GLY A:659 and Pi-alkyl interaction with PRO A:488.

5.6.7 Lariciresinol

Lariciresinol is found in carrots and sesame seeds. It has anti-inflammatory and anti-diabetic effects. It is also used as an antifungal agent

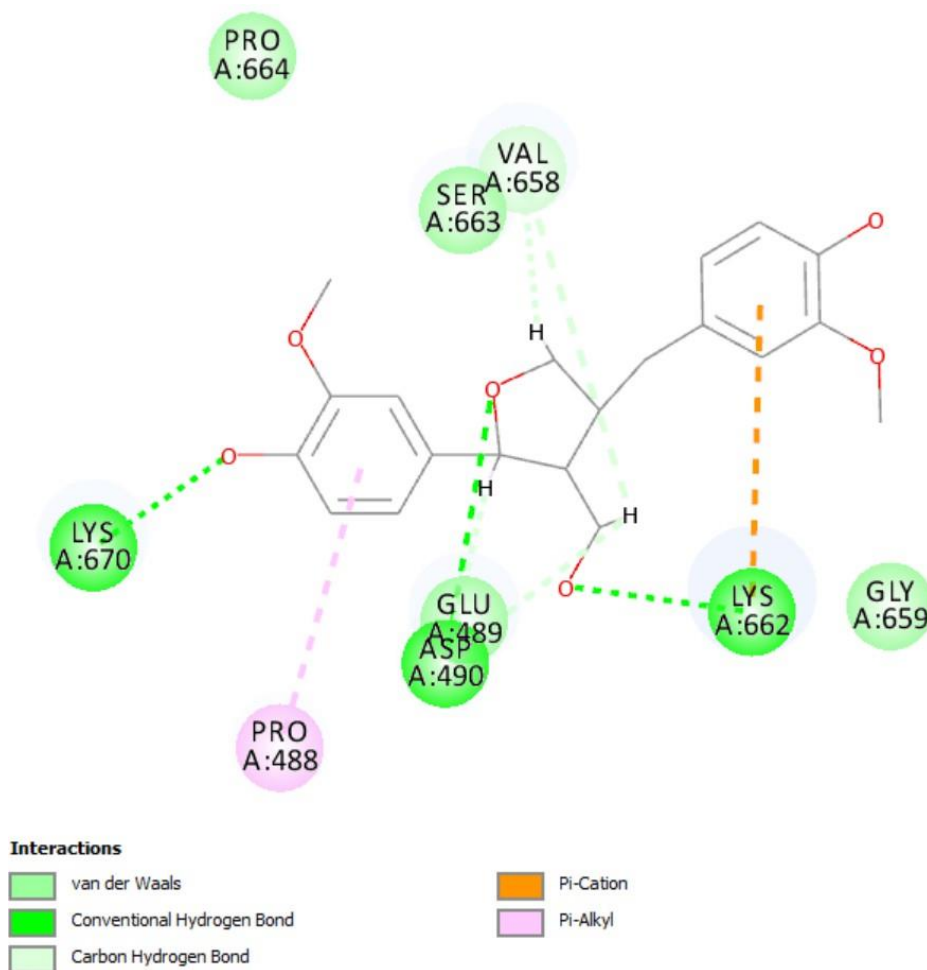


Fig no:5.44. Interaction of Lariciresinol with target

Lariciresinol has 3 conventional hydrogen bonds with LYS A:670, ASP A:490, LYS A:662 and 3 carbon hydrogen bonds with VAL A:658, GLU A:489. It has van der Waals interaction with PRO A:664, SER A:663, GLY A:659. It also has Pi-cation interaction with LYS A:662 and Pi-alkyl interaction with PRO A:488

5.6.8 Ferulic acid

Ferulic acid is found in fennel, rice, wheat, oats, beans, coffee seeds, artichoke, peanuts, bamboo, sweetcorn, garlic, grapefruit, orange and pineapples. It possesses many physiological functions like anti-inflammatory, antioxidant, antimicrobial activity, anticancer, and antidiabetic effect. It is vastly used in skin care treatments.

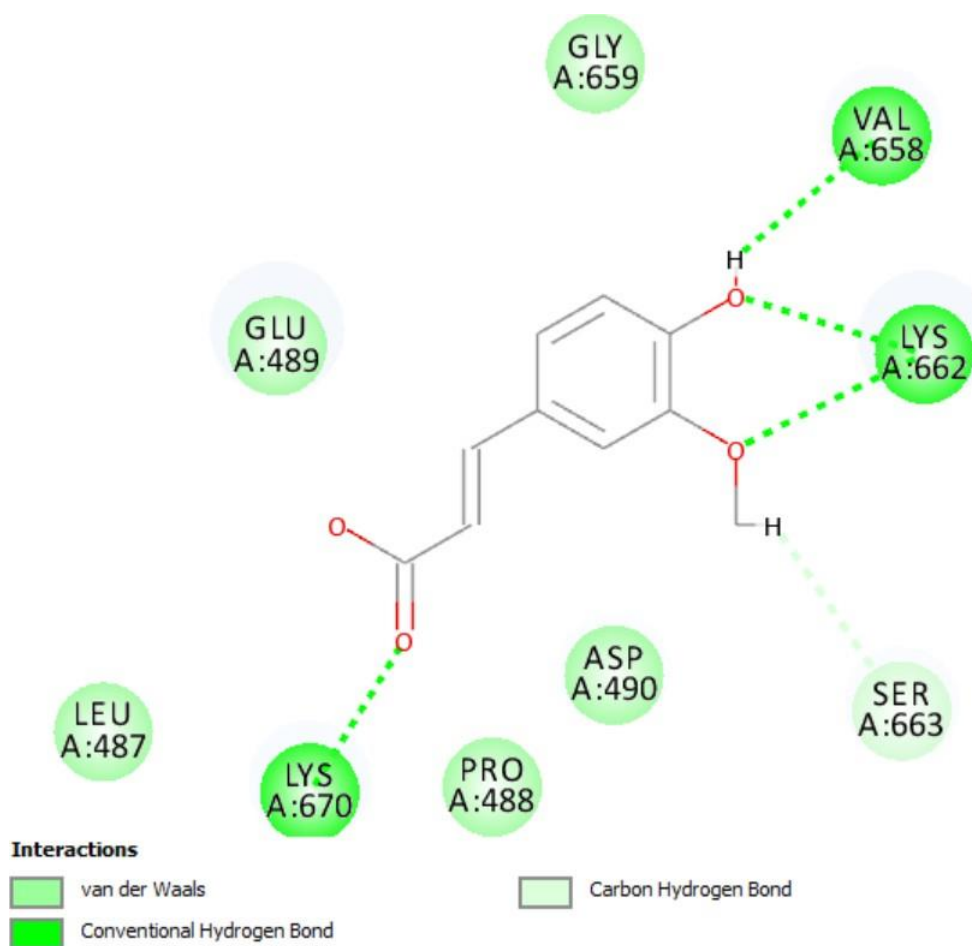


Fig no:5.45. Interaction of Ferulic acid with target

Ferulic acid has 4 conventional hydrogen bonds with LYS A:670, VAL A:658, LYS A:662. It has 1 carbon hydrogen bond with SER A:663 and van der Waals interaction with GLY A:659, ASP A:490, PRO A:488, LEU A:487 and GLU A:489

5.6.9 Sinapic acid

Sinapic acid is found in orange, grapefruits, cranberries, mustard seed and canola. It has antioxidant, anti-inflammatory, anticancer, antimutagenic, antiglycemic, neuroprotective and antibacterial properties.

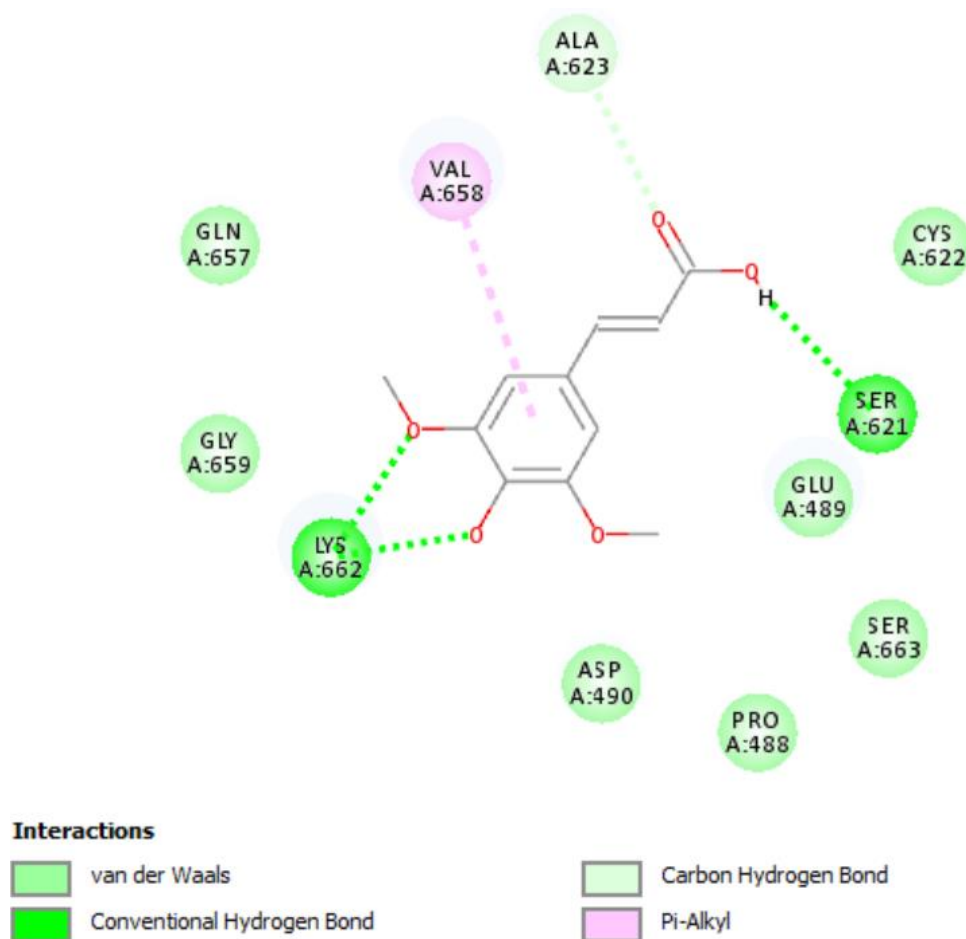


Fig no:5.46. Interaction of Sinapic acid with target

Sinapic acid has 3 conventional hydrogen bonds with LYS A:662 and SER A:621. It has 1 carbon hydrogen bond with ALA A:623 and a Pi-alkyl interaction with VAL A:658. It also has van der Waals interaction with GLN A:657, CYS A:622, GLU A:489, SER A:663, PRO A:488, ASP A:490, GLY A:659, and GLN A:657.

5.6.10 Caffeic Acid

Caffeic Acid is found in carrot, coffee, tea, red wine, berries, apples, olives, artichokes, and pears. It has anti-inflammatory, anticancer, and antiviral abilities. It may help boost the performance of athletes.

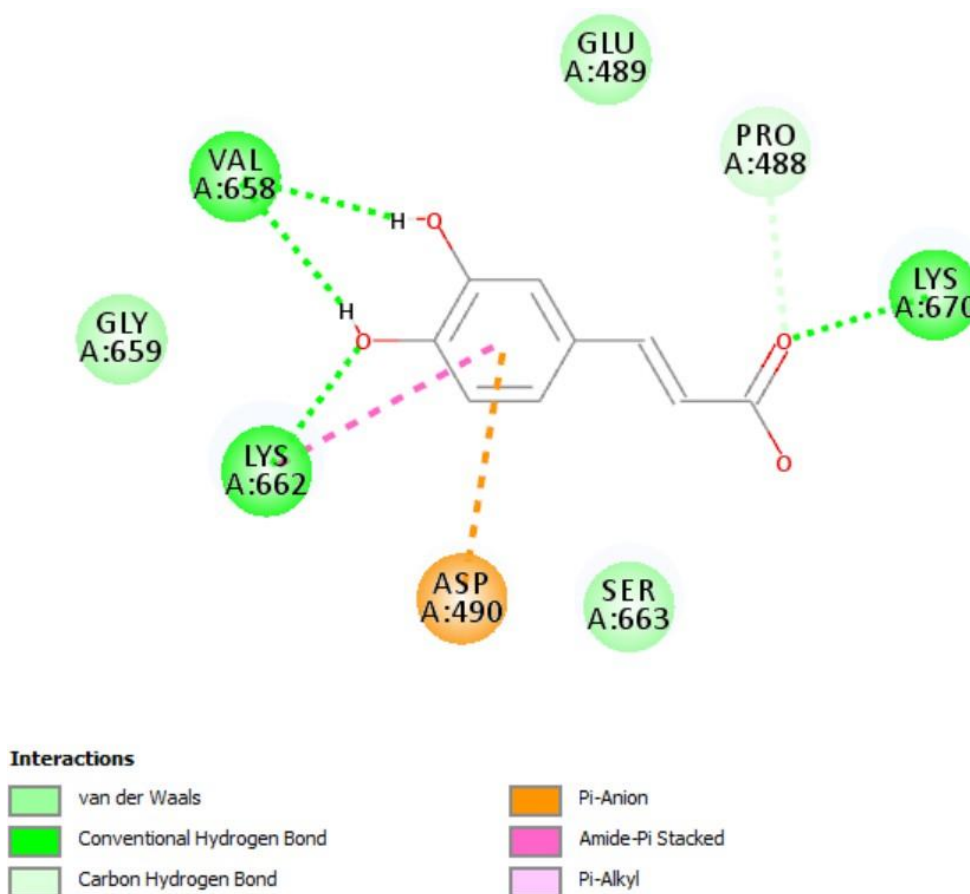


Fig no:5.47. Interaction of Caffeic Acid with target

Caffeic Acid has 4 conventional hydrogen bonds with LYS A:662, VAL A:658, LYS A:670. It has carbon hydrogen bond with PRO A:488 and van der Waals interaction with GLU A:489, SER A:663, GLY A:659. It has Pi-alkyl interaction and Amide-Pi stacked with LYS A:662. It also has Pi-anion interaction with ASP A:490.

5.6.11 Quercetin

Quercetin is found in orange, onions, grapes, berries, cherries, broccoli, garlic and citrus fruits

It is a versatile antioxidant known to possess protective abilities against tissue injury induced by various drug toxicities. It provides beneficial effects on health, including protection against various diseases such as osteoporosis, lung cancer, and cardiovascular disease.

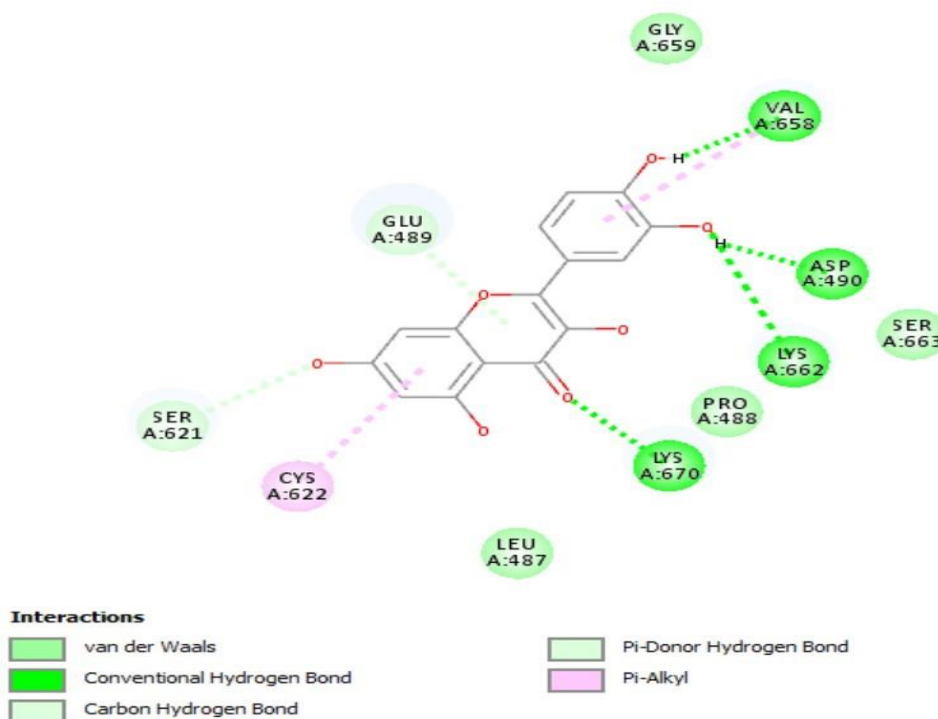


Fig no:5.48. Interaction of Quercetin with target

Quercetin has 4 conventional hydrogen bonds with VAL A:658, ASP A:490, LYS A:662 and LYS A:670. It has 1 carbon hydrogen bond with SER A:621 and van der Waals interaction with GLY A:659, SER A:663, PRO A:488, LEU A:487. It also has a Pi-donor hydrogen bond with GLU A:489 and Pi-alkyl bonds with CYS A:622, VAL A:658

5.6.12 Apigenin

Apigenin is found in parsley, chamomile, celery, vine-spinach, artichokes, onions, oranges, tea, grapefruit, garlic and oregano

It is used to induce muscle relaxation and sedation. It is also active as an antioxidant, anti-inflammatory, anti-amyloidogenic, neuroprotective, and it is also under study as a cognition-enhancing substance with interesting potential in the treatment/prevention of Alzheimer's disease.

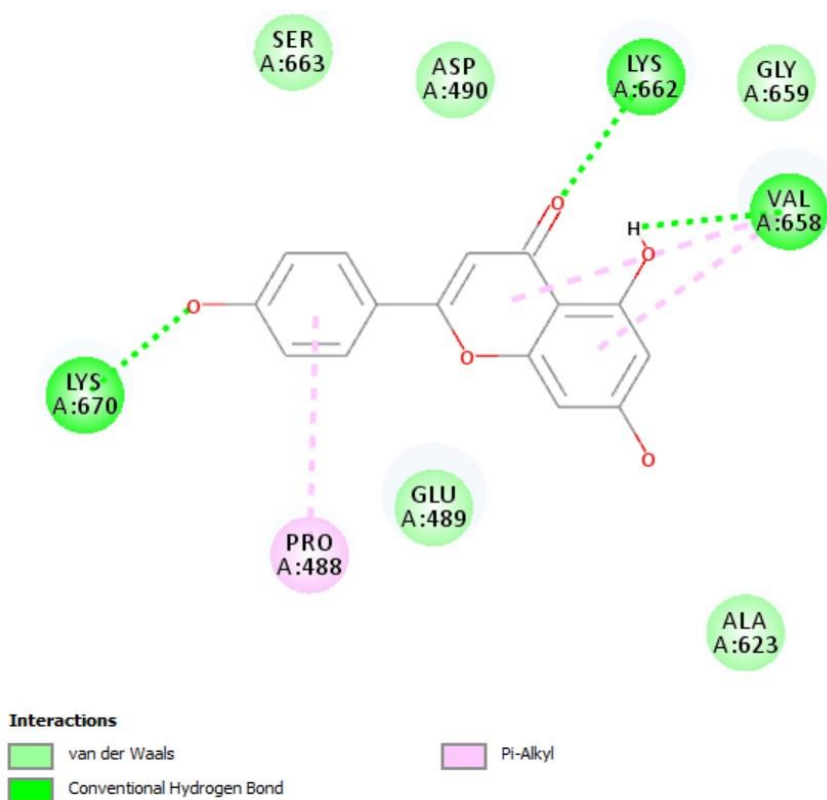


Fig no:5.49. Interaction of Apigenin with target

Apigenin has 3 conventional hydrogen bonds with LYS A:662, LYS A:670 and VAL A:658. It has Pi-alkyl bonds with VAL A:658, PRO A:488 and van der Waals interaction with SER A:663, ASP A:490, GLY A:659, ALA A:623 and GLU A:489.

5.6.13 Octanoic acid

Octanoic acid is found in olive, flax seeds, red wine and vegetable oils. It has antioxidant properties and anti-inflammatory effects

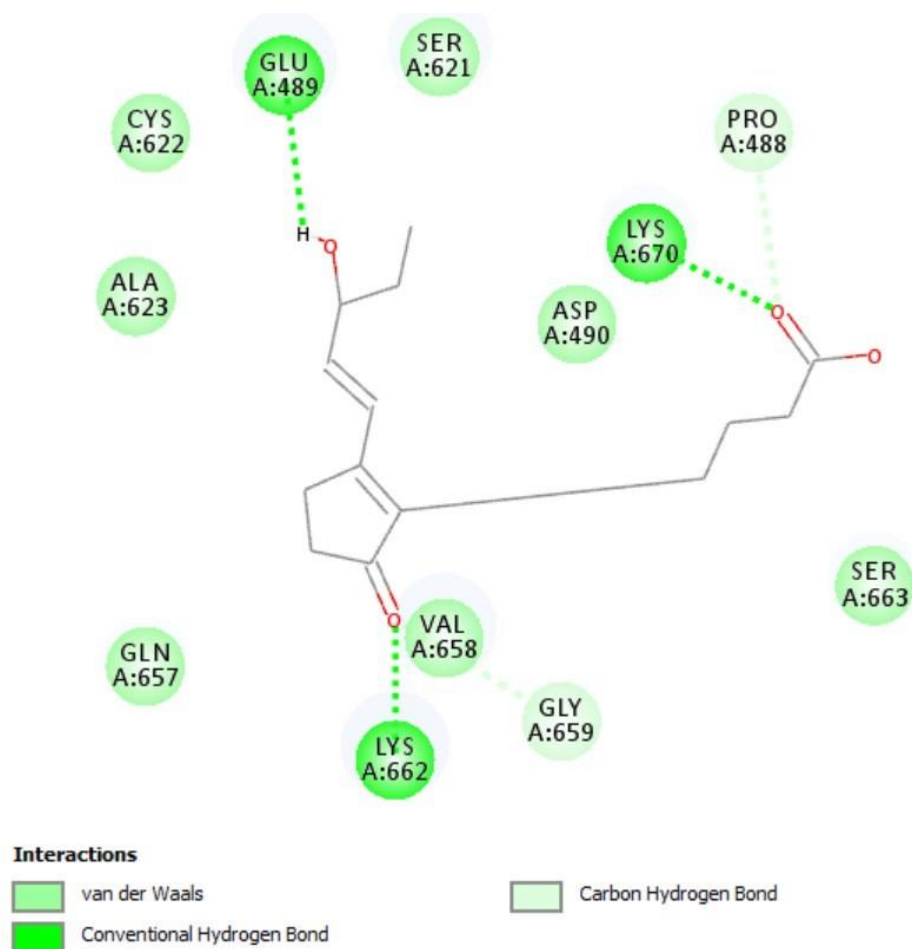


Fig no:5.50. Interaction of Octanoic acid with target

Octanoic acid has 3 conventional hydrogen bonds with GLU A:489, LYS A:670 and LYS A:662. It has carbon hydrogen bonds with PRO A:488, GLY A:656 AND van der Waals interaction with CYS A:622, SER A:621, ASP A:490, SER A:663, VAL A:658, GLN A:657 and ALA A:623

CHAPTER 6

SUMMARY AND CONCLUSIONS

Plants encompass numerous phytochemical constituents, many of which are known to be biologically active compounds and are responsible for displaying diverse pharmacological activities. Some of the secondary metabolites of plants are important sources of natural antioxidants that are favored over synthetic ones because of safety concerns. The bio active secondary metabolites have been shown to reduce the risk and progression of diseases such as Cancer, Cardiovascular, neuro- degenerative diseases, etc. by scavenging free radicals through various biological mechanisms.

The resulting compounds-Catechin, Secoisolariciresinol, Epicatechin, Hesperetin, Maslinic Acid, Hydroxytyrosol, Lariciresinol, Ferulic acid, Sinapic acid, Caffeic Acid, Quercetin, Apigenin and Octanoic acid could be deservedly isolated or synthetically derivatized for further evaluation. They also deserve more exploration for application in the field of complementary medicines against various diseases. Their additive/synergistic activities could be rationally tapped as an opportunity for effective combination therapy in prostate oncology. Individually, they display enhanced potentials for replacing the currently available anti prostate drugs with various shortfalls.

The study requires the incorporation of more sophisticated computing algorithms and experimental research to accurately assess the 13 phytochemicals- Secoisolariciresinol, Epicatechin, Hesperetin, Maslinic Acid, Hydroxytyrosol, Lariciresinol, Ferulic acid, Sinapic acid, Caffeic Acid, Quercetin, Apigenin and Octanoic acid as bona fide agents. More so, basic and clinical experimental evaluations are necessarily required *in vitro* and *in vivo* for the affirmation of the interesting pharmacology and biosafety. However, it represents a promising model for future therapeutic designs for overcoming the incessant resistance, accessibility, and safety challenges associated with the currently available orthodox anti prostate drugs. There is abundant proof of the role of plant extracts as bio-reductive agents against various diseases. Also, considerable studies have been carried out on

herbal drugs elucidating their role as promising Sjogren's syndrome (SS).

In this study, a commercial drug and herbal compounds selected for comparison in In-silico studies against gene responsible for Sjogren's syndrome. In this study, small molecules from natural sources were evaluated of which Catechin, Secoisolariciresinol, Epicatechin, Hesperetin, Maslinic Acid, Hydroxytyrosol, Lariciresinol, Ferulic acid, Sinapic acid, Caffeic Acid, Quercetin, Apigenin and Octanoic acid confirmed to be in with good binding activity against NF-kB-inducing Kinase (NIK) showed potential in becoming a drug that can be used in the treatment of Sjogren's syndrome.

Furthermore, studies like clinical trials are required to confirm these compounds for commercialization in order to treat Sjogren's syndrome (SS).

CHAPTER 7

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