CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Identification of Anti-Viral Metabolites of *Nigella sativa*

by

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A thesis submitted in partial fulfillment for the degree of Master of Science

in the

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I dedicate this thesis to my parents and teachers

CERTIFICATE OF APPROVAL

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Abstract

It is estimated that by the end of 2025, 226 million people worldwide will be affected with viral infections. Although many treatments for viral infections are available, but some new herbal medicines need to be discovered that will have fewer side effects instead of synthetic ones which can cure the disease and also show less side effect. The motive of the present research was to discover potential antiviral components from *Nigella sativa*. 29 bio compounds representatives of classes namely cycloleucalenol, thymol, strychinine, tirucallol, dithymoquinone, lophenol, isoquinoline, taraxerol, betaamyrin, butyrospermol, cycloartenol, alphahederin and gramisterol were selected. Virtual screening of these ligands was carried out against drug targets by CB-dock. Physicochemical and Pharmacokinetics properties determine the final destiny of compounds as drug or non-drug compounds. *Thymol* belongs to class of organic compounds known as aromatic monoterpenoids containing at least one aromatic ring. It has been verified from this research that the drug. *Thymol* is better than the already available drug Peramivir. The Docking Score comparison in between the above drugs shows the reliability; (Peramivir -4.4 & Strchinine -4.2) knowingly with a fact that higher the docked interface complex score – the better the complex is. Further it has been approved in the research that the effectiveness of thymol is greater than the synthetic peramivir (ADMET properties).

Keywords: Antiviral, *Nigella sativa*, molecular docking, thymol, peramivir, lophenol, isoquinoline and beta pinene

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Abbreviations

BBB	Blood Brain Barrier
CNS	Central Nervous System
FDA	Food and Drug Administration
Fu	Fraction Unbound
Ns	*Nigella sativa*
PDB	Protein Data Bank
TQ	Thymoquinone
VDss	Volume of distribution

Chapter 1

Introduction

Nigella sativa is popularly called kalonji which is an important member of the Ranunculaceae family is nowadays world-widely recognized as herbal medicine because it has phenomenal roles in the cure of different diseases. This dicotyledonous plant is mostly cultivated as herbal medicine and has an important role in the cure of diabetes, viral and bacterial infections, asthma, diarrhea. Prophet (S.A.W) mentioned that black seeds can cure every disease except death. Great Muslim scholars also mentioned the importance and uses of *Nigella sativa* in their books [1].

For example, Ibn e Sina who was a great philosopher and renowned physician has written a book under the title of Canon of Medicine which is well recognized in the history and used as a guideline in Europe; in this book he advised to use *Nigella sativa* in fever, headache, common cold, wounds, fungal infections and against bites of harmful animals and worms. Because of their black color, these seeds are nomenclatures with many names like Habba Al Barakah meaning that these seeds are a blessing, Habba tul Alsawada. Black cumin, black caraway, shuniz are also referred to frequently used names of *Nigella sativa* in the world [2].

Nigella sativa is commonly cultured in the regions of the Middle East, North Africa, Europe, and Asia. It is a flowering shrub that may attain a height of 90 centimeters approximately. Flowers are produced annually on the plant. Flowers

of this important plant are very delicate, consisting of 5-10 petals and available in different colors including pink, purple, white, blue, and yellow color. Leaves are green in color, which contain threadlike narrow dividing segments. Moreover, the fruit is a flattened large capsule that contains, small dark-colored seeds; ovulate in shape known as black seed commonly as they turn black when getting exposure with the air [3].

Many active components have been isolated and reported from the oil-containing seed which includes thymoquinone, thymohydroquinone, thymol, p-cymene, terpineol, longifolene, alkaloids including nigellidine and nigellicine, saponins,carvacol, alpha hederin. Some components are present in trace amounts also in the black seeds. The seeds of *Nigella sativa* contain an essential oil that possesses properties against hypertension, diarrhea, cough, fever, viral infections, inflammation, bacteria, parasites, asthma, and cancer mostly [4].

Mostly biological activity is due to the presence of thymoquinone in the seeds of the black cumin because of its importance in both fixed and essential oil. A report of any toxic reaction from thymoquinone is not present. Approximately 30-50% quinine in *Nigella sativa* is present in the form of thymoquinone, which is an important bioactive constituent. Whereas it also contains other active components like carvacol, p-cymene, thymol, thymohydroquinone and dihydrothymoquinone [5].

These seeds are of vital importance as they are used as alternative and traditional medicines for the cure of several disorders and diseases since a long time ago. Numerous studies have been conducted on laboratory animals and as well as on the human beings to investigate the uses and benefits of this miracle preserved in them by nature [5].

Most old traditional cultures preferably in Asia treatment of various allergies were done by the use of *Nigella sativa*. Immunity is also stimulated and blood sugar level can be maintained by the use of black seeds. Liver functioning, immune system, respiratory and digestive system are treated by the use of black seeds in ancient times. For the loss of appetite, worm, and skin wounds, and many other

issues were treated by the tincture of the black seed. On the other hand, oil is applied as antiseptic and anesthetic effect on the external surface of the body. Roasted *Nigella sativa*'s seeds are used to stop frequent vomiting [6].

Nigella sativa's seeds can be added as food preservatives and flavoring agents in the preparation of tasty food dishes. In the Subcontinent, *Nigella sativa* is used to enhance the taste and aroma of the food. In the case of developed countries, herbal medicines are used as magic against gastrointestinal disorders and skin related illnesses mostly [7]. Researchers are using nowadays herbal plants in their modern scientific techniques to verify this miracle of God to cure a vast range of diseases and illnesses. Many such pharmacological activities were investigated. A few of them are elaborated below:

Interestingly, Nigella seed oil can be used in the cosmetics as it has shown the properties against sun protective factor (SPF). This is due to the presence of aroma components in the black seeds. So, Black cumin can also be used to treat skin issues as researchers were successful in obtaining better results when it comes to decreasing the size of lesions. It can spread melanin in the skin layers. The underlying mechanism may be that it increases the sensitivity of receptors inside the skin. Hence, thymoquinine can be used to cure external skin problems like pigmentation [2, 8].

As microbial agents are becoming resistant to normal medicines because of their excessive use so it is need of time to explore herbal products against different bacteria and viruses so in the case of *Nigella sativa*, Thymoquinone an important component in the black seed essential oil which has proved to be shown antibacterial activity. Mostly gram-positive cocci bacteria are not resistant to this important and active component of *Nigella sativa* so they can be killed [8, 9].

The Paper disc diffusion method was used and black seeds give efficient results against bacteria specially *H. pylori*. It is also effective against such bacteria which in clinical trials are multi-drug resistant. Growth of bacteria is stunted as by the use of Black cumin seeds containing melanin and most importantly thymoquinone is present. So, these findings suggest further research in this field to get more

effective and better results of an herbal product without any or minimal side effects [9].

Antiviral drugs show a narrow spectrum, less available and rate of efficiency is low so it is need of the hour to move such herbal medicines which are more efficient and effective and treat ailments on large scale with minor side effects so to do so researchers have tested different products including *Nigella sativa*. *Nigella sativa* stimulate and enhance T cells and induce proliferation of cells and allow to help in antiviral activity so that virus can be eliminated.

This also indicates the role of cytotoxic T cells in decreasing virus load as studied earlier by the researchers. It kills harmful microorganisms and thus plays a role against viruses and bacteria.

Moreover, *Nigella sativa*'s has anti analgesic role which prevents pain in different parts of the body. Black seeds are also effective against immunodeficiency virus protease. Several experiments on laboratory animals especially mice very tested to know the role of *Nigella sativa* against the viral activity [10].

1.1 Problem Statement

Probably it is estimated that by the end of 2025, 226 million people worldwide will be affected with viral infections. Although many treatments for viral infections are available, but some new herbal medicine need to be discovered that will have fewer side effects instead of synthetic ones that will cure the disease and also show less side effect. Plant extracts have been used in ethno- medical treatments that have fewer side effects as compared with synthetic treatment.

1.2 Aims of the Study

The aim of the current study was to identify natural anti-viral compounds from *Nigella sativa* effective against nucleoprotein of virus.

1.3 Objectives of the Study

To achieve the goal we have following objectives:

1. To identify various bioactive compounds of *Nigella sativa* as potential inhibitors of nucleoproteins.
2. To analyze the binding conformation between targeted proteins and other inhibitors as standard anti-viral agents.
3. To identify the lead compound as antiviral drug candidate.

1.4 Scope

In low-income countries, people prefer traditional medicines over modern medicine thanks to low cost and lesser side effects. Major issues with these traditional medicines are limited bioavailability, quantity and validity. This research is an attempt to determine novel antiviral compounds which would be stronger drug targets of the near future for these diseases like inflammation, cancer, microbial, hypertension, and neural diseases.

Chapter 2

Literature Review

2.1 Importance of Medicinal Plants

Nature is very kind and it shows the qualities of the plants and animals existence at a same place and area. They both contain many natural products which are specifically used for the treatment of various human illness and disorders [23]. Currently these special herbal plants are in demand and society is accepting them slowly. They have healing properties and having useful medicinal effects on the human or animal body and these plants also called medicinal herbs [24]. These plants include a wide range of different plants which have different medicinal properties. These plants contain a large number of such important compounds which can be used to prepare organic and healthy drugs [25].

Commonly, medicinal plants parts are involved for drugs production such as seeds, root, flower etc. Mostly medicinal plants contain bioactive compounds which have directly or indirectly which can heal and cure wide range of diseases has role in different medicinal agents. So these plants are globally used as complementary or alternative medicines [26].

Generally, medicinal plants have been used since earliest times. It can be said that before the history of medicinal plants, the ancient people used these plants for many purposes [27]. In ancient time, less information is present that how

to cure diseases by plants for healing of various illness and how to use them for medicinal purposes [28]. Figure 1 shows different uses of whole medicinal plant.

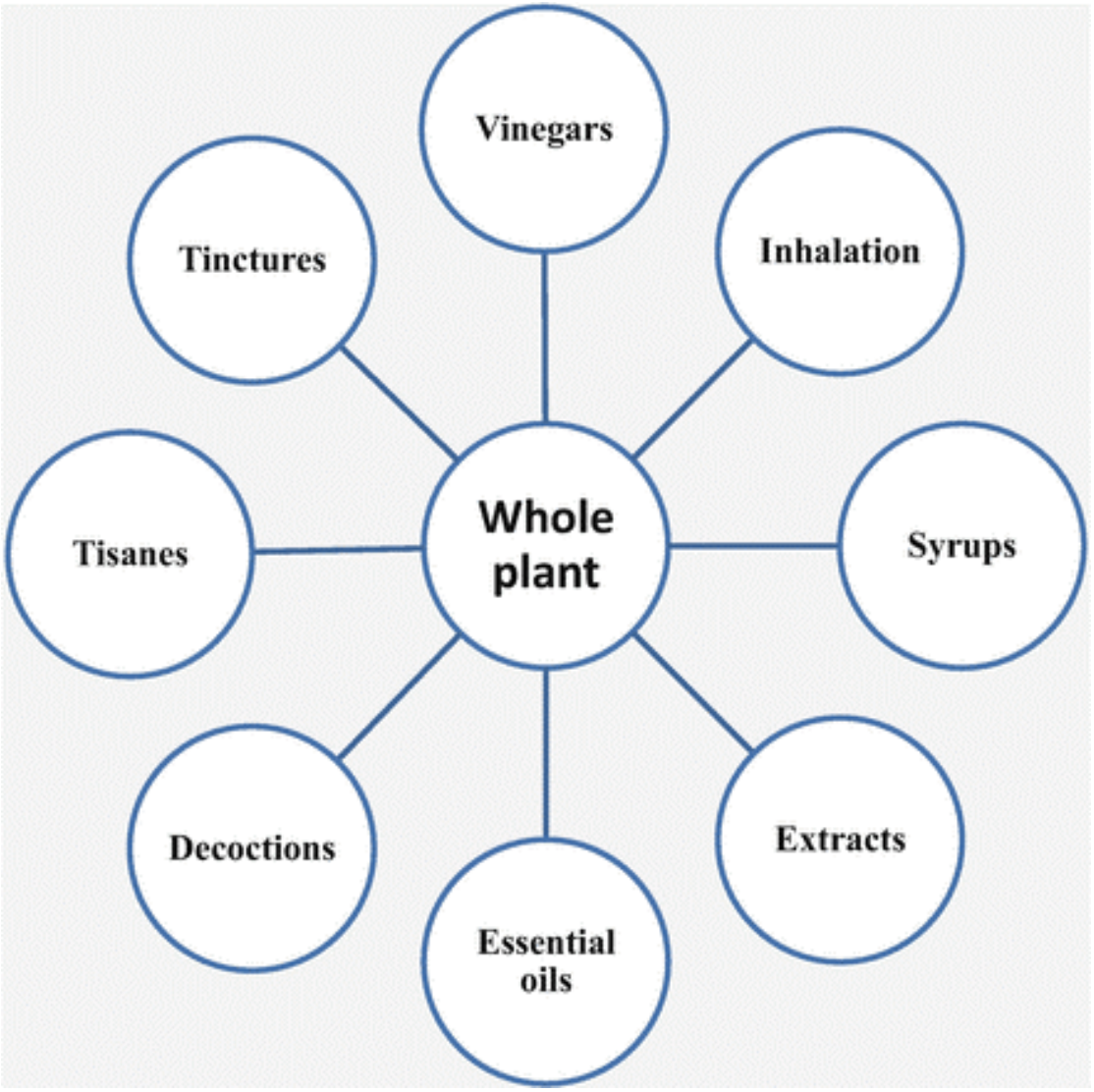


FIGURE 2.1: Uses of Medicinal Plants [28].

But now a day's people generally use medicinal plants and spices because these plants contain a lot of bio-active compounds and essential oils which is beneficial for human health. So these plants and spices contain special compounds which prevent various diseases. [1, 29] .Toxic and negative harmful side effects caused by different products of pharmaceutical industry is a significant factor sudden increase its demand in population and it also decreases the use of chemicals so a person can be healed more easily [30]. On the other hand, the presence of such precious herbal plants worldwide is different and they are mostly obtained from

wild populations [31]. Therefore, in recent decades, for herbal products demand from wild resources has reached to 15% yearly from in Europe, North America and Asia [32]. Medicinal plants have a promising future as long as they exist. Nearly half a million plants worldwide are still need to be explore for medical purpose, So upcoming studies for medicinal plants maybe effective for the treatment of diseases [33]. Drug discovery process can be understood by Figure 2.2.

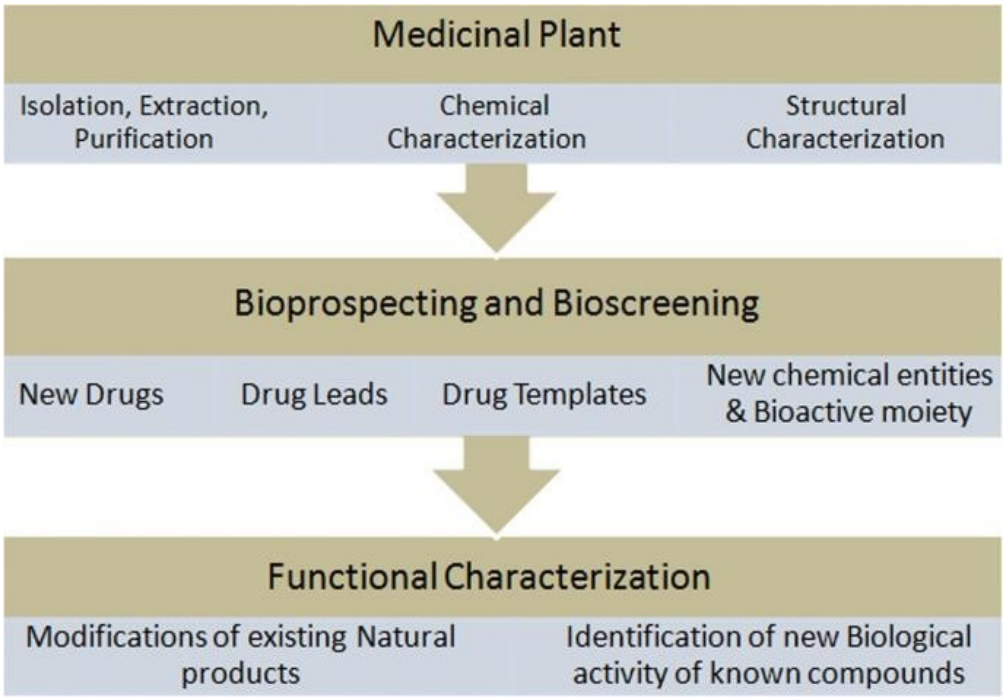


FIGURE 2.2: Uses of Medicinal Plants in Drug Discovery Process [33].

Marchese et.al illustrated that extracts of plants, include secondary metabolites which are important source of antimicrobial substances whereas monoterpines are the most important constituents of essential oils which are produced by the process of liquid extraction and steam distillation done for such plants which are used for herbal purpose and can also be eaten [34].

2.2 *Nigella sativa*

Zaher et.al noticed that for the improvement of production rate and health of animals that are raised in farms, that helps to improve the whole body specially

immunity system and thus prevent the body from further diseases and infections. So, in search of herbal products, *Nigella sativa* is key product and helps to increase the average gain of animals on daily basis, enhance the capacity of digestion of their feed and also to gain maximum nutrition from daily taken diet and feed.

It also has quality to stimulate immunity system, enhance reproductive performance and also function of thyroid in farmed animals as shown in the Figure 2.3 [35].

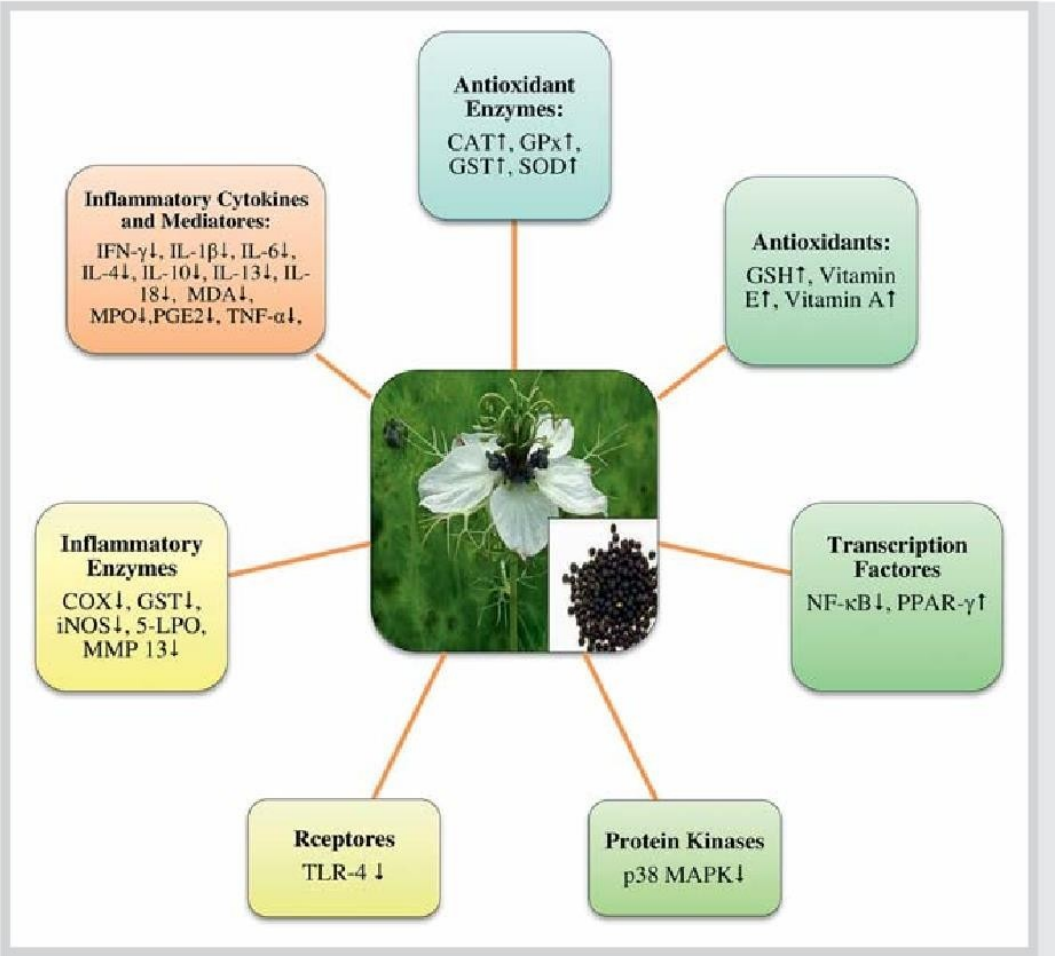


FIGURE 2.3: Different Properties of *Nigella sativa* [35].

Nigella sativa can also be used when murine cytomegalo viral infection rises in cattle. It is basically used to strengthen the immune system and this is quite successful medicine. *Nigella sativa* along with other plants herbal extract also cure cancer and many such other diseases. Scientifically this treatment is still not proved so that efficiency of this method cannot be evaluated [36].

FIGURE 2.4: The given figure represents *Nigella sativa* flowering plant, seeds and oil [36].

2.3 Morphology of *Nigella sativa*

According to Forouzanfar, et.al *Nigella sativa* is a plant which produce flowers annually and it is approximately 20-30 cm tall in height and consist of leaves which are linear and their head is like lance. The flowers contain 5-10 delicate petals. The flowers are of mostly and usually of white, pink, yellow, pale blue or pale purple colour. Fruit bearded by *Nigella sativa* is large in size and has inflated capsule which is composed of 3-7 follicles which are united and contain large number of seeds in respective follicle. The seeds colour is black which are funnel like shaped, sometimes angular and oblong, may be flattened. The length of these seeds ranges from 0.2 cm and 0.1 cm in width [16]. Shamim Molla et.al depicted that *Nigella sativa*, which belongs to Ranunculaceae family and is dicotyledonous, cultivated and harvested for its special properties which ranges from being a spice in the food ;it also act as preservative for food. Figure 2.4 shows flowers ,black seeds and oil of *Nigella sativa*. It can also be used as a natural remedy for various diseases and disorders.it is also recommended by our Holy Prophet (S.A.W) for healing

purposes and commonly known as black seed, which is also mentioned in holy bible as black cumin [37].

2.4 Medicinal Uses of *Nigella sativa*

Sallehuddin et.al demonstrated that *Nigella sativa* and thymoquinone play important role in medical field as it is revealed *in vivo* studies that it can be used to cure inflammation, cancer, bacterial and viral infections, protect neurons and nephrons, also act as anti-oxidant. Because of *Nigella sativa* the presence of angiogenesis induction in wound healing of skin, proliferation of fibroblasts and frequent synthesis of collagen occurs at a very positive rate.

It is also concluded that *Nigella sativa* also reduce damage to the tissues and decrease and maintain the amount of white blood cells, moreover it also help to kick out the amount of bacteria ;hence also reduce bacterial infections. Thymoquinone is the most active and prominent component of black seeds. All these health promoting activities are shown in Figure 2.5 [38].

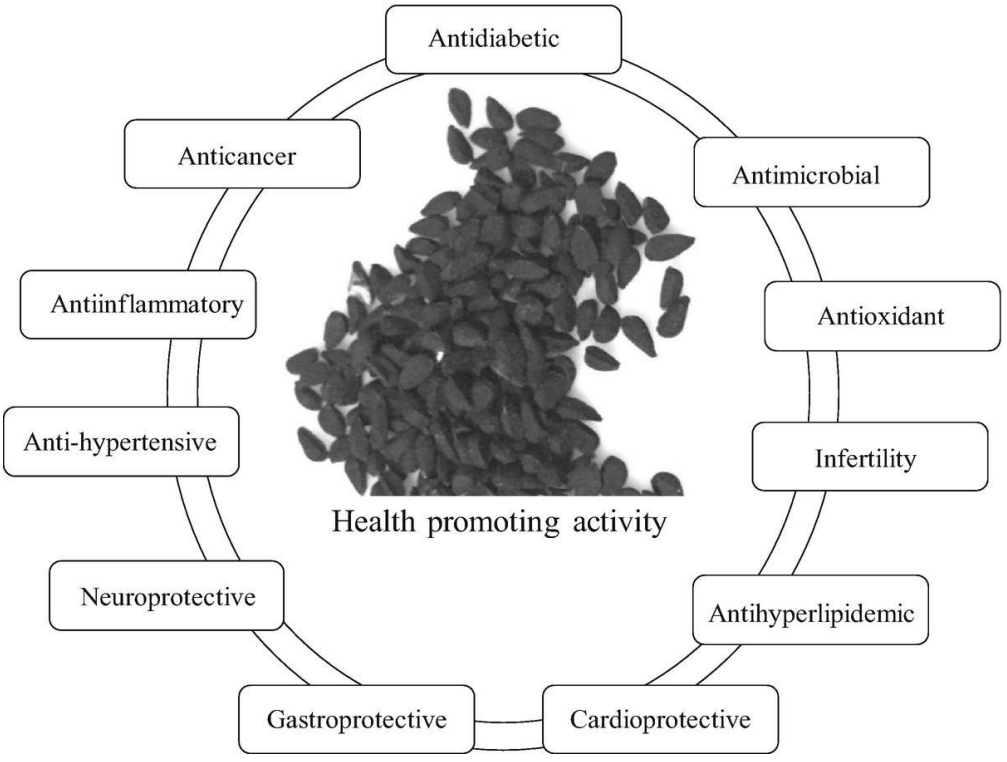
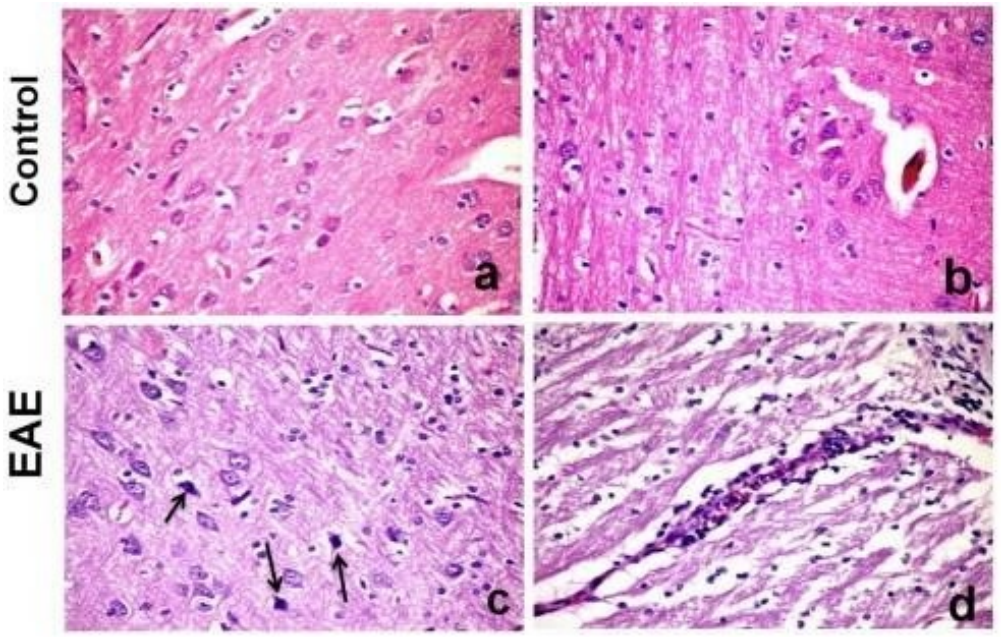


FIGURE 2.5: Health Promoting Activities of *Nigella sativa* [38].

After more than 400 empirical evidances, four properties namely anticancer, antioxidant, anti- inflammatory and hepatoprotective are indicating properties of thymohydroquinione are evaluated by Khader et al. Moreover, some more properties are also predicted by Darakhshan and his coworkers which are against asthma, microbes and protects gastric system, neurons and nephrons [39, 40].

2.4.1 Anti inflammatory Activity

Khader et.al found that there are many reports on the anti-inflammatory activity of TQ and stated that the anti-inflammatory effect of TQ is caused by the upregulated expression of heme-oxygenase 1 (HO-1) in human keratinocytes (HaCaT) by activating nuclear factor (NF)-erythroid2-(E2)-related factor-2 (Nrf2) via reactive oxygen species (ROS)-mediated phosphorylation of protein kinase B (PKB/Akt) and cyclic AMP-activated protein kinase-alpha (AMPKalpha) [40, 45]. According to Bai et al, TQ attenuated thioacetamide (TAA)-induced liver fibrosis accompanied by reduced protein and mRNA expression of of α-smooth muscle actin (α-SMA), collagen-I and tissue inhibitor of toll-like receptor 4 (TLR4) and decreased pro-inflammatory cytokine levels. It also inhibited phosphatidylinositol 3-kinase phosphorylation and enhanced the phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) and liver kinase B (LKB) as shown below [41].



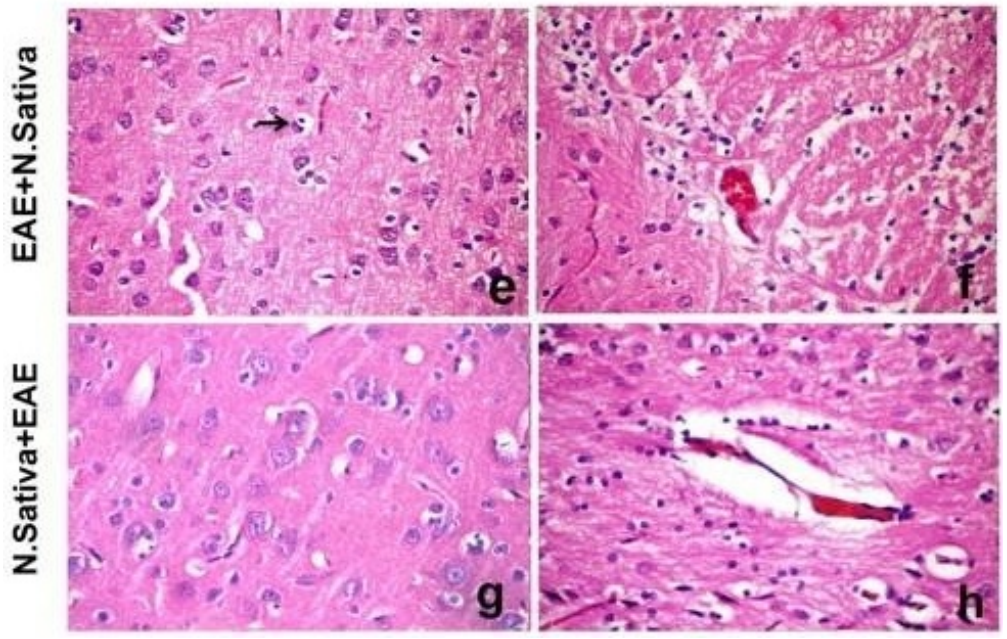


FIGURE 2.6: Role of *Nigella sativa* as Anti-Inflammatory Agent [41].

2.4.2 Antimicrobial activity

Nigella sativa exhibited strong antimicrobial activity against Salmonella typhi, Pseudomonas aeruginosa and others. Marchese et.al illustrated that Plant extracts and their secondary metabolites are rich sources of antimicrobial substances, including coumarins and psoralens, acetylenes, flavonoid and non-flavonoid polyphenols, and terpenes. Monoterpenes (i.e., eucalyptol, borneol, camphor, bornylacetate, carvacrol, menthol, γ-terpinene, α-pinene, β-pinene, and p-cymene) are the most important constituents of essential oils produced through liquid extraction and steam distillation of edible and medicinal plants. The essential oil has been shown to have activity against Gram-positive and Gram-negative bacteria. However, sensitivity against Grampositive bacteria such as Staphylococcus aureus and Vibrio cholerae was found to be stronger. Bacteria like Staphylococcus aureus, S. pyogenes and S. viridans are more susceptible to *Nigella sativa*. In an in-vitro study, volatile oil showed activity comparable to ampicillin. The activity of the volatile oil also extended to drug resistant strains of Shigella spp, Vibrio cholerae and Escherichia coli and was found to have a synergistic action with streptomycin and gentamycin [42].

2.4.3 Hepatoprotective activity

In *Nigella sativa*, thymoquinone is the most active ingredient which is known for its hepatoprotective action. Alanine transaminase and aspartic transaminase shows decreased leakage while trypan blue has decreased uptake activity when it is demonstrated in vitro.

This in vitro study concluded that protective effect is present against tert-butyl hydroperoxide and for hepatocytes, it induced oxidative damage [43].

2.5 Classification of *Nigella sativa*

Nigella sativa can be classified as following:

Table 2.1: Taxonomic Hierarchy of *Nigella sativa* [44].

Kingdom	Plantae
Division	Magoliophyta
Order	Ranunculales
Family	Ranunculaceae
Genus	*Nigella*
Specie	*sativa*

Nigella sativa belongs to kingdom plantae ,and division is magnoliophyta,wheras order is ranunculales and belongs to family ranunculaceae, genus is Nigella and sativa is the species name as shown in the Table 2.1.

2.6 Composition of *Nigella sativa*

Seeds contain numerous esters of structurally unusual unsaturated fatty acids with terpene alcohols (7%); furthermore, traces of alkaloids are found which belong

to two different types: isochinoline alkaloids are represented by nigellimin and nigellimin-N-oxide, and pyrazol alkaloids include nigellidin and nigellicin [45]. Dinagaran et.al investigated that *Nigella sativa* seed contains fixed oil that ranges between 28 to 36% and chiefly composed of unsaturated fatty acids that are arachidonic, eicosadienoic, linoleic and linolenic and saturated fatty acids that includes palmitic, stearic and myristic [46].

The seed oil contains compounds such as cholesterol, campesterol, stigmasterol, β- sitosterol, α-spinasterol, citronellol, limonene, p-cymene, citronellyl acetate, carvone, nigellone, arachidic, linolenic, linoleic, myristic, oleic, palmitic, palmitoleic and stearic acids [47]. Seed oil contains fixed oils like linoleic acid (55.6%), oleic acid (23.4%) and palmitic acid (12.5%) and volatile oils like trans-anethole (38.3%), p-cymene (14.8%), limonene (4.3%), and carvone (4.0%) [48].The seeds also contain a fatty oil rich in unsaturated fatty acids, mainly linoleic acid (50 60%), oleic acid (20%), eicodadienoic acid (3%) and dihomolinoleic acid (10%).

Saturated fatty acids (palmitic, stearic acid) amount to about 30% or less [49]. Also contain parts of the essential oil, mostly thymoquinone, by which it acquires an aromatic flavor. The seeds give on steam-distillation a yellowish brown volatile oil with an unpleasant odor. The oil contains carvone, d –limonene, and a carbonyl compound, nigellone. This composition of oil can be seen in Figure 2.7 [50].

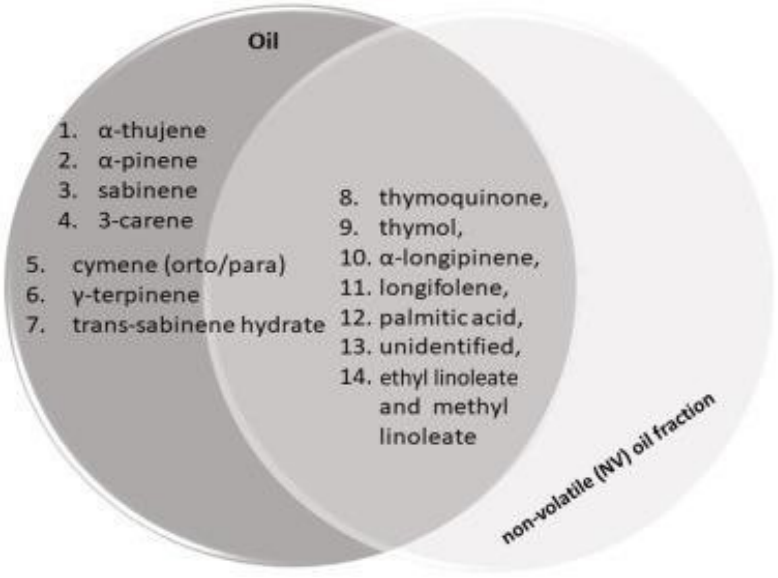


FIGURE 2.7: Non-Volatile Components of *Nigella sativa*'s Oil [50].

2.7 Signs and symptoms of Viral Infections

Symptoms of viral diseases vary depending on the specific type of virus causing infection, the area of the body that is infected, the age and health history of the patient, and other factors.

The symptoms of viral diseases can affect almost any area of the body or body system. Symptoms of viral diseases can include flu like symptoms which may include fatigue, fever, headache, cough, aches and pain mostly diarrhea, vomiting, stuffy nose congestion, runny nose, or postnasal drip, swollen lymph nodes, swollen tonsils, unexplained weight loss [51]. In infants, signs of a viral disease can also include bulging of the soft spot on the top of the head, difficulty with feeding, excessive crying or fussiness and excessive sleepiness [52].

2.8 Comparison of Viral Infections

Viral infections are basically of two types: acute and chronic viral infections. Acute viral infections is a process in which non equilibrium is maintained and both virus and host both continually change up till then the process of viral infection is resolved, then the host is killed or in either case the infection leads to chronic viral infection. In a virus or immune system, several genes are present which are activated during acute viral infections only. And if these genes become nonfunctional or over effective then this may result in severe and disgusting results and consequences. Whereas chronic infection is process of dynamic and meta-stable equilibrium. In this type, both virus and host balance each other so in this process sometimes viruses manage themselves and they persist the immune system and thus sometimes no disease can be caused and this interesting phenomenon is still like a mystery which needs to be solved in the field of immunity and microbiology. This difference can be observed in Figure 2.8 [53].

Approximately 6.75 billion human population is affected from different chronic viruses which is a large number as so many humans are carrying this severe chronic

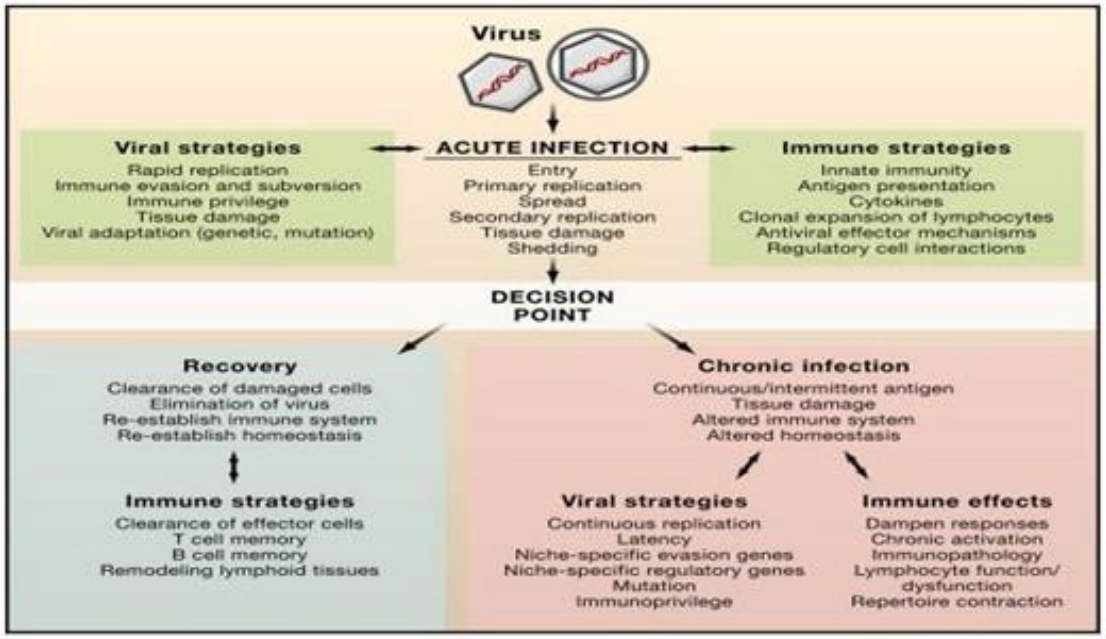


FIGURE 2.8: Difference Between Acute and Chronic Viral Infections [53].

viral infections. In some cases this affected percentage is low, for example in case of adenovirus, this prevalence percentage of disease sticks to 1 percent only [54-55].

The Figure 2.9 shown below give us the idea of different viruses and their prevalence. Following viruses' prevalence is included in the graph: xenotrophic murine leukemia virus related virus, human T cell leukemia virus and polyomaviruses. These are the estimated amount of viruses as they relate to human population globally [55].

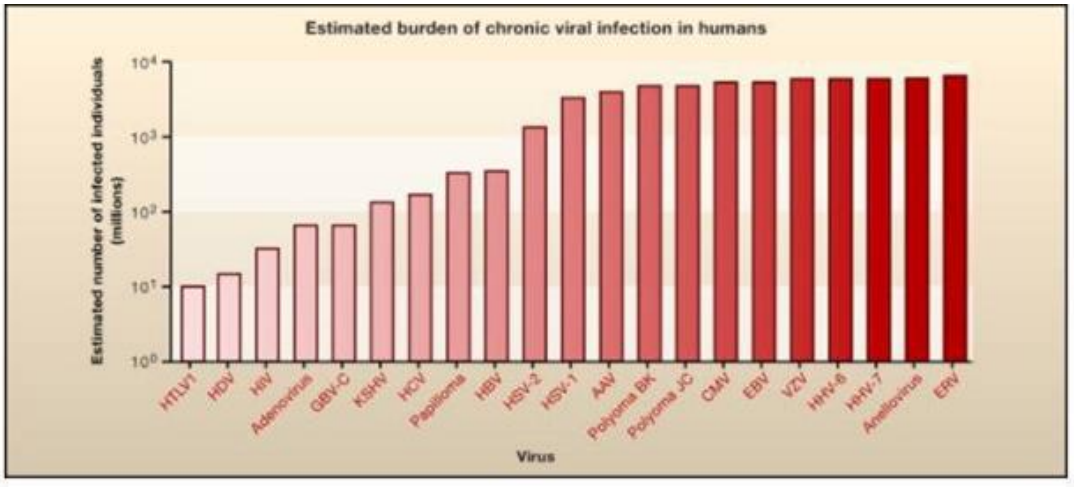


FIGURE 2.9: Estimated Burden of Viral Infections in Humans [55].

2.9 Molecular Docking

By performing molecular docking it is now easy and possible to identify such competent able inhibitors which can act against interested target molecules. This technique is computer based and after simulation process, complex of ligand and receptor is predicted. Each and every process of docking is composed of specific algorithms which aid in prediction and conformation of binary complex [56, 57].

It is used to know the strength bond between a ligand and a target protein through a special scoring function. The 3D structure of the target proteins and the ligands is taken as the input for docking. It represents a frequently used approach in structure-based drug design since it requires a 3D structure of a target protein. It can be used to determine the correct structure of the ligand

within the target binding site, and to estimate the strength of the binding between the ligand and the target proteins through a specific scoring function. The process is elaborated in Figure 2.10 [58, 59].

It also helps in the recognition of new small molecular compounds, revealing the essential properties, such as high interaction between binding with target protein [60].So the docking process includes compounds which are discussed below.

1. The docking process requires a 3D structure of protein which is downloaded from Protein data bank (PDB).
2. To minimum size of molecules or compounds or virtual compounds that contain a database required.
3. A computational framework is also needed to perform the docking and find the scoring process.

Protein and ligand docking is one of the key areas of molecular docking, which has obtained high popularity and appreciation due to its role in structure-based drug designing [61]

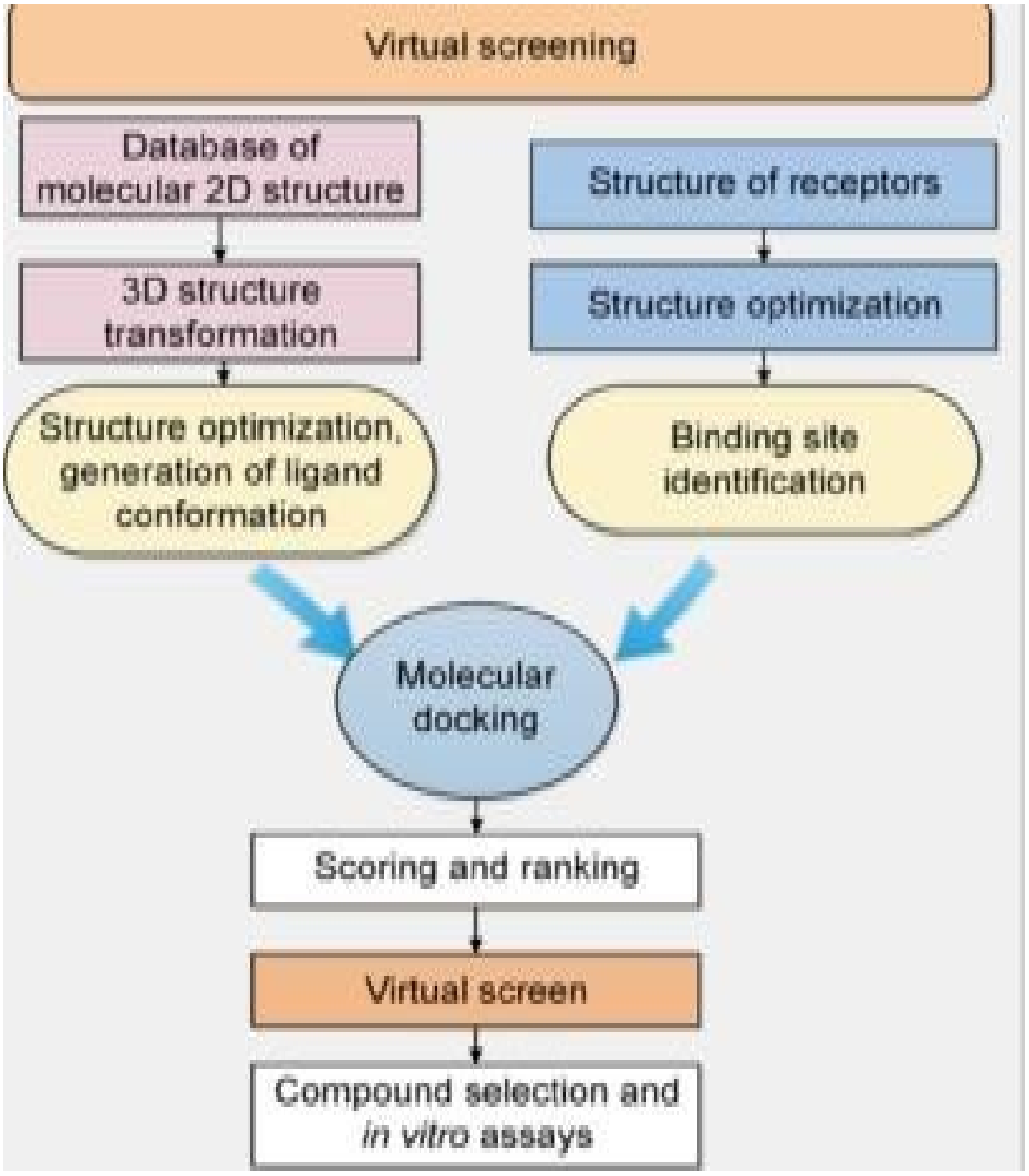


FIGURE 2.10: The Process of Molecular Docking [58].

Molecular dynamics, distance geometry method and genetics algorithm etc are most widely used algorithm in molecular docking and the most frequent software used for molecular docking are Auto Dock vina, Auto Dock, CB Dock and ICM. most frequent software used for molecular docking. Qamar et. all described the docking site by searching from already reported such residues of different proteins by different computational tools including MOE site finder and electrostatic surface map in order to find that particular molecular docking site. NS1, NS3, NS5 Proteins are docked by using MOE dock tool and ligand phytochemicals database is also used. Algorithm applied was triangular matcher which was taken as and

used for ligand replacement method in order to get 10000 best docked molecules. This process of reverse docking is illustrated in figure 2.11 [62].

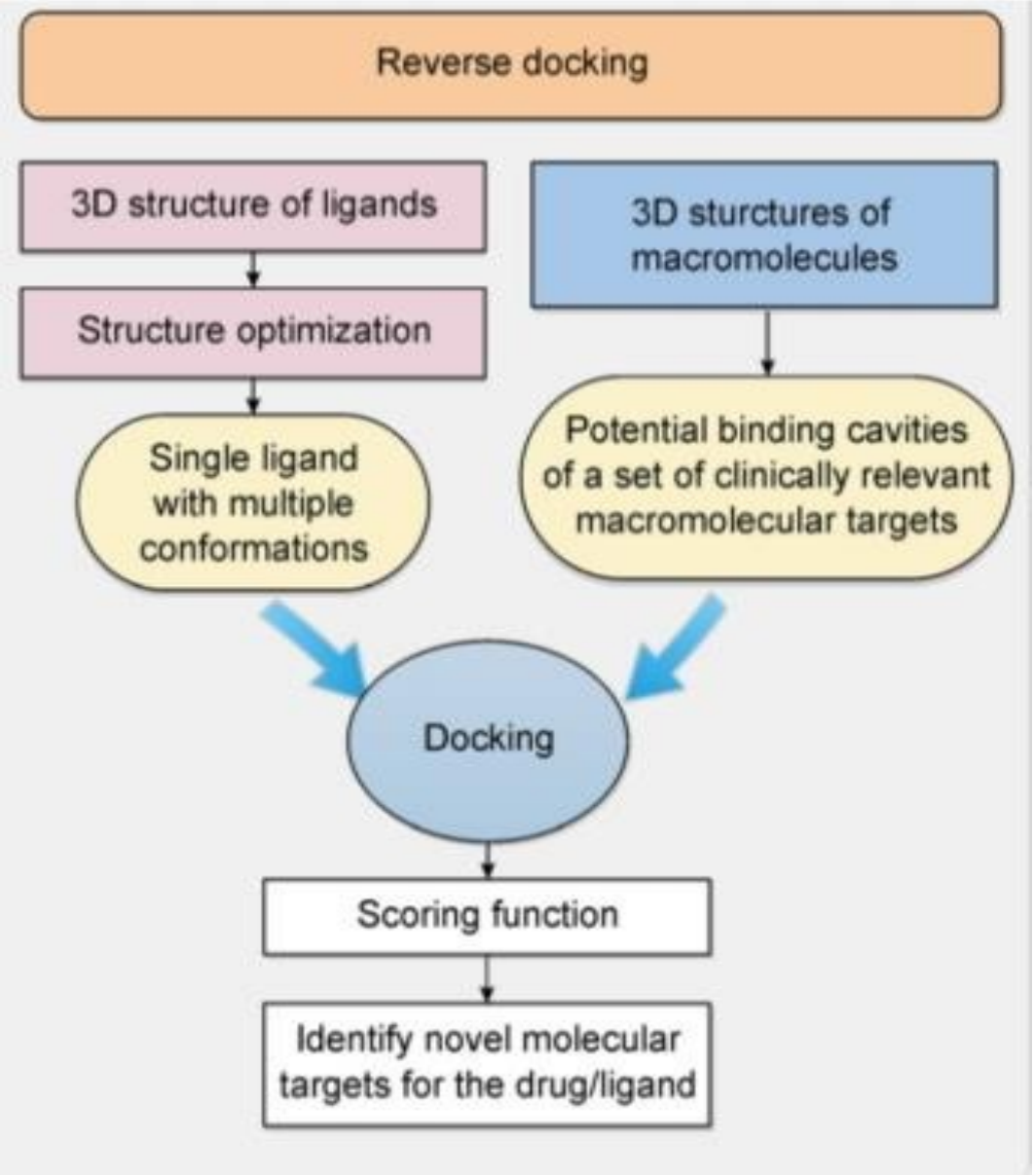


FIGURE 2.11: Reverse Docking Technique [62].

London Dg scoring function is used for rescoring of simulated poses and number of molecules generated by this method were 10 which were minimized by use of algorithm of force field refinement so that the final binding energy can be found out by the use of solvation of generalized born by making the receptors residues rigid and firm. The obtained compounds were ranked on the base of affinity of binding score and lastly upon root mean square deviation value [63]. Now the next step is

to choose such compounds only for further analysis and active the proteins with docked molecules score. So such inhibitors are again proceeded through docking process by the ligand with different proteins namely NS,NS3 and NS5. This was done by use of molecular docking process which helps in further binding process in order to validate the phytochemical properties of different pockets of catalyst [64].

Bouchentouf et.. al performed different experiments and concluded that 6LU7 is the energy complex with the lowest value and it gives best docking results when score of nigellidine is compared with other docked compounds, Chloroquine is the compound which has score near to nigellidine. It also shows better docking score than favipiravir and hydroxychloroquine [65, 66, 67].

Chapter 3

Materials and Methods

The methodology include step by step analysis of components of *Nigella sativa*.

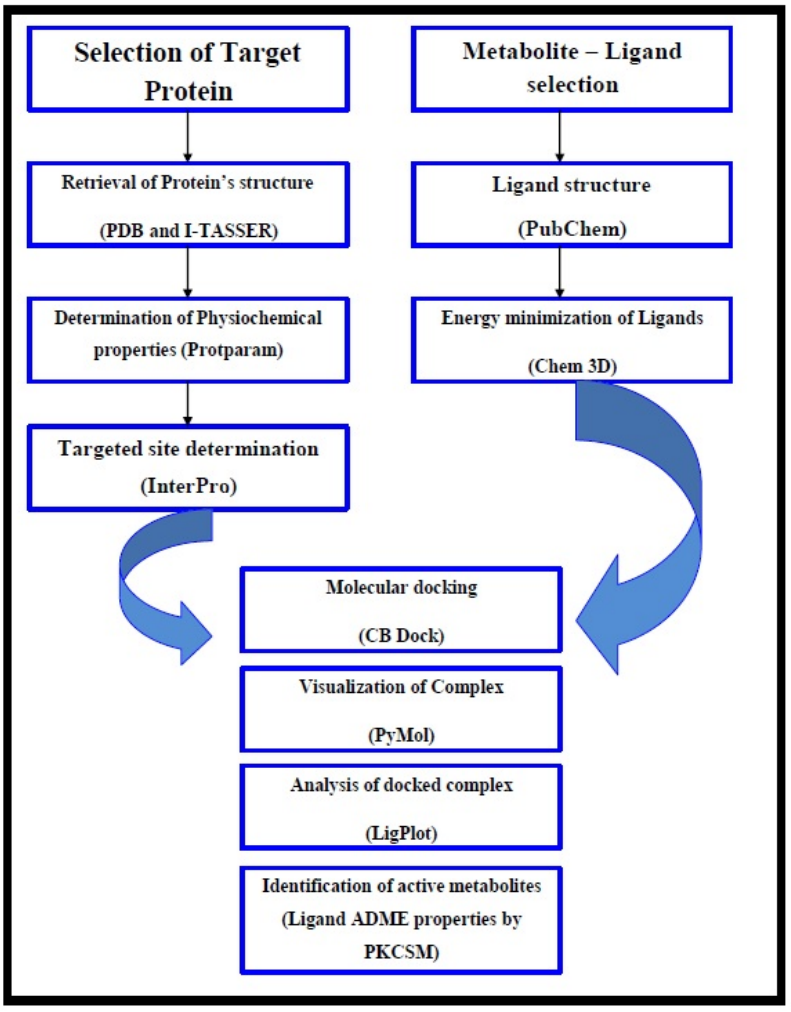


FIGURE 3.1: Methodology used for the drug-targeting and epitope-based study

3.1 Selection of Problem

When virus proliferate inside the human body specially then it will lead to cause the infection inside the body commonly known as viral infection. After the entrance of the virus, it become active and start the process of replication at a very high rate and many replicate copies are formed in very short time so that the burden will shift on the host body and as a result it will burst and bodies of the virus will be free and spread in the host cell. So these newly formed viral particles will spread to the whole body of the virus and also affect other healthy cells. On the other hand, viral particles can kill the host cell and before doing so, they will cutoff themselves from the host. After the spread of the virus and due to damage and destruction occurred to the cell and tissues and relevant immune system and immune responses, symptoms of illness caused by the virus start arising. In today's world, covid-19 which causes severe issue of the respiratory track and thought to be spreaded from the birds and then also then affect human beings on such a large scale is the example of such transmission on a large scale so it is need of the hour to study such problem which is affecting the world on such broad spectrum [68].

3.2 Target Protein Selection

It is possible to manage viral infections, the key factor involved, several active metabolites in *Nigella sativa*. So these active metabolites are involved in the viral pathways which play a vital role to inhibit viral infections. Selection of primary sequence (FASTA format) of target proteins (nucleoprotein) from PDB (Protein Data Bank) is done [69].

3.3 3D Structure Prediction of Protein

I-TASSER (https://zhanglab.ccmb.med.umich.edu/I-TASSER/) stands for Iterative Threading Assembly Refinement. It is an online server used for the prediction

of the structure and function of the protein in 3 dimensions. This online server firstly identifies the structural model of the PDB through various strategies which include the atomic models of full length and they are built by using simulations of the different threading fragments. This server also analysis and predicts different and multiple regions of secondary structure from the sequence of the protein structures and include and involve alpha helixes, beta sheets and coils from the sequence of the amino acids. The I-TASSER server also predict the 3D structure of proteins and these server gives us five 3D structure of proteins so on the basis of C-score we can select the best 3D structure of the protein [70].

3.4 Structure Analysis By Use of PyMOL

PyMOL is a cross platform molecular graphics tool, that has been used world widely for the three dimensional analysis and visualization of many proteins, small molecules including nucleic acids, densities of different electrons and varying surfaces, and also the trajectories. It is also used for editing the molecules, tracing the ray and also to make animations and movies. This is software that is based on python, and also contain many plugin tools in order to enhance its use and also facilitate the drug targeting and designing by the use of PyMOL software [71]. After downloading the protein structure, the extra constituents attached to the protein need to be removed which was done by the use of an open-source system PyMol. The linear chain of the protein consisted of a range of 1-306 amino acids and was referred to as the A chain and remaining all the constituents of the protein was eliminated so that further process is done effectively [72].

3.5 Active Site Identification

The ligand shows maximum or highest interaction with the protein where the target protein has their active site. Amino acids are highly involved in the formation of complex of ligand to protein. Protein binding pockets were identified by

CASTp.

3.6 Retrieval of Chemical Structure of Ligands

Those ligands were selected that had previously shown some antiviral properties. These include the terpenes, monoterpenes, sesquiterpenes, phenolic compounds, flavonoids, Coumarins, and sterols. By using the database PubChem, we downloaded the 3-dimensional structure of the above selected ligands.

PubChem is under the National Center of Biotechnology Information (NCBI) and is a database that contains information regarding the chemical molecules. The information stored is related to the chemical names, molecular formulas.

3 dimensional or simple structures, their isomers, canonical similes, and information regarding the activities of the molecules against the biological assays [42]. The structure of the ligands which were obtained from PubChem were all downloaded and then the ligands MM2 energy was minimized by using Chem3D ultra.

If in case the selected ligand structure was not available, then our next attempt would be to download the canonical similes from PubChem and then insert them in the software ChemDraw and after obtaining the 3D structure repeat the energy minimization step using Chem3D ultra. In the end, the SDF format was selected to save energy minimized the structure of the ligand [73].

3.7 Bioactivity Analysis of Ligands and Toxicity Measurement

Chemical compounds that were used as ligand were selected from PubChem database. Selected compounds follow the Lipinski rule of five and those are likely to be used as active drug in humans [74]. The potential success of a compound depends on its ADMET properties. PkCSM (https://omictools.com/pkcsm-tool) is an online

tool that helps to find the ADMET properties of the compounds. The rules are as follow:

1. The logP value of most "drug-like" molecules should be limited to 5.
2. Molecular weight should be under 500.
3. Maximum number of H-bond acceptor should be 10.
4. Maximum number of H-bond donor should be 5 [75].

3.8 Molecular Docking of Targeted Proteins

The purpose of molecular docking is to find the best conformational interaction between target proteins and compounds. The two essential requirements for docking are the target protein and the candidate ligand [76]. Active metabolites of *Nigella sativa* found after the review of literature were used as the ligands and target protein was nucleoprotein. CB dock is an online docking server which automatically identifies binding sites and is used to perform docking. It can simplify docking procedures and improve accuracy by predicting target protein binding sites [77].

3.9 Process of Molecular Docking

The first step in performing the docking process is to create a ligand and target protein files. First, the target protein file was compiled following a few steps. PDB file of target proteins was given to CB dock as input file one by one. After these amendments target protein file was saved in pdbqt format. After compilation of protein file the ligands file were prepared by following the same procedure and saved in PDB format in same directory. Then Setting up Grid box around protein ligand structure was performed [78]. For this purpose macromolecules option was selected from Grid and pdbqt target protein file was opened then from set map type option ligand structure was opened. After performing these steps from grid, grid

box option was selected to design grid around protein- ligand complex. Grid box with all parameters appeared and file was saved as Grid Parameters File (GPF) in same directory after selecting parameters. Utilizing docking related commands docking was performed.

These commands help to get the directory path where the docking readable prepared protein ligand files have been saved along grid parameters file. Docking files were created for chosen data set after completion of this step and results were saved in pdbqt format [79].The interaction of the active pockets of the ligand and the protein were calculated for the interpretation of docking results. Three types of interactions are studied; ionic bonding, hydrogen bonding and hydrophobic bonding. Using Ligplot plus (version v.1.4.5) the protein ligand interaction were studied. Then, protein ligand interactions in the form of diagrammatic representation, results were given in the file format of PDB [80]

Chapter 4

Results and Discussion

4.1 Primary Structure Retrieval

The structure of nucleoprotein was obtained through protein data bank(PDB) with ID 2NB2 in Fasta sequence with sequence length of 38 residues (https://www. rcsb .org/).

Nucleoprotein is selected as target protein because anti-viral activity of *Nigella sativa* is targeted. To study such activity, nucleoprotein is targeted as it is playing role in replication of virus. If nucleoprotein is inhibited then process of replication can be inhibited easily.

The protein sequence of the obtained protein is as under:

>2QVJ_1|Chains A, B, C, D, E|Nucleocapsid protein|Vesicular stomatitis Indiana virus (11277)SVTVKRIIDNTVIVPKLPANEDPVEYPADYFRKSKEIPLYINTTKSLSDLRG YVYQGLKSGNVSIIHVNSYLYGALKDIRGKLDKDWSSFGINIGKAGDTIGIFDLVS LKALDGVLPDGVSDASRTSADDKWLPLYLLGLYRVGRTQMPEYRKKLMDGLT NQCKMINEQFEPLVPEGRDIFDVWGNDSNYTKIVAAVDMFFHMFKKHECASFRY GTIVSRFKDCAALATFGHLCKITGMSTEDVTTWILNREVADEMVQMMLPGQEID KADSYMPYLIDFGLSSKSPYWSVKNPAFHFWGQLTALLLRSTRARNARQPDDIE YTSLTTAGLLYAYAVGSSADLAQQFCVGDNKYTPDDSTGGLTTNAPPQGRDVV EWLGWFEDQNRKPTPDMMQYAKRAVMSLQGLREKTIGKYAKSEFDK

4.1.1 Physiochemical Characterization of *Nigella sativa*

ProtParam is a tool of Expasy which is used online for the prediction of different parameters including both physical and chemical properties of stored protein. These several parameters calculate and estimate the following: molecular weight (MW) ,composition of amino acid , theoretical value of Protein index ,atomic composition of protein ,extinction coefficient ,estimated half-life of protein instability and aliphatic index, grand average of hydropathicity which was abbreviated as GRAVY.

The calculated PI greater than 7 represents the basic nature of the protein while less than 7 shows acidic nature of protein. Extinction coefficient represents light absorption. Instability index if less than 40 show stability of the protein while greater than 40 indicates the instability of protein [82]. The physiochemical properties of nucleoprotein are shown in Table 4.1

Table 4.1: Showing Physiochemical Properties of Nucleoprotein

MW	**PI**	**NR**	**PR**	**Ext. Co 1**	**Ext. Co 2**	**Instability Index**	**Aliphatic Index**	**GRAVY**
4737 7.01	6. 26	52	50	755 40	752 90	30.56	80.14	-0.338

The aliphatic index represents the aliphatic content of a protein. The high value of the aliphatic index indicates the thermo stability of the protein. Molecular weight contains both positive and negative charged residues of protein. At 280nm the ranging extinction coefficient of 73980, 67965, 20105, and 112270 indicates Tyr and Trp high concentration. Low GRAVY shows better interaction with water molecules. All these parameters which were selected for this research work were taken according to previous research work [120]. MW stands for molecular weight, pl for theoretical isoelectric point (pH at which protein is neutral, without any charge), NR for total number of negatively charged residues (Asp + Glu),PR for total number of positively charged residues (Arg +Lys), Ext.Co1 for extinction

coefficients when assuming all pairs of Cys residues form cystines , Ext. Co2 for extinction coefficients when assuming all Cys residues are reduced, and GRAVY for grand average of hydropathicity [83].

There is only 1 amino acid chain (chain A) including 38 residues comprises of THR-36, SER-37, LEU-35, ARG-38, CYS-34, ALA-33, PRO-32, ALA-31, CYS-30, LEU-29, GLY-28, ILE-27, CYS-26, ALA-25, ARG-24, MET-23, GLY-22, ALA-21, TYR-20, ASP-19, PRG-18, ILE-17, TYR-16, THR-15, CYS-14, ARG-13, SER-12, ASN-11, CYS-10, GLU-9, SER-8, LEU-7, CYS- 6, ASP-5, GLN-4, TYR-3, ARG-2 AND ASP-1.

Further it comprises of 3 regions where the polar contact has been made within the bond range of (hydrogen bond in between 1.9 – 2.3).

4.2 3D Structure Prediction of Protein

3D Structures of targeted proteins were downloaded from RCSB PDB in PDB format. Protein Data Bank may be a three-dimensional database of complex molecules of living organisms, like proteins and nucleic acids. I-TASSER (Iterative threading ASSEmbly Renement) may be a special procedure in order to predict the structure of the protein, also structure and function of the Nigelline Protein.

This online server firstly identifies the templates of the given structure retrieved by the PDB obtained by applying different approaches including the multiple threading approach LOMETS, by involving atomic models of full length which are built by the simulations assembled by fragments that are template based.

This server has been widely used for protein structure and performance predictions in biological and biomedical investigations. I-TASSER predicts regions of secondary protein structure which may include like alpha helix, beta sheet and coils obtained by the sequence of the organic compound [84].

I-TASSER server team mails complete results of job id with five models and on the base of C-score best 3D structural model are often easily selected.

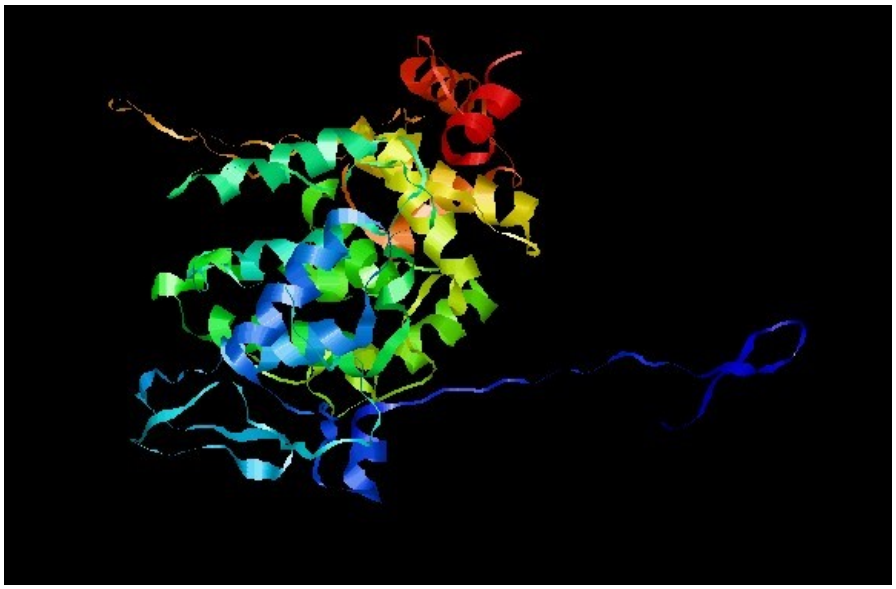


FIGURE 4.1: Structure of Nucleoprotein Obtained by I-Tasser [84].

4.2.1 Functional Domain Identification of Protein

Data base Interpro was used to identify the domains and functional sites of 2QVJ. Interpro is a resource for functional analysis of protein sequences. Conserved domains are involved in sequence/structure/relationship. Proteins can have more than one functional domain that perform different functions. Functional domain is the active part of a protein that is involved in interactions of proteins with other substances. Figure 4.2 shows the functional domains of the protein to be targeted. Two promoters A and B combine to form a single polypeptide. It consists of 1-306 residues. Each promoter has 3 domains, Domain I has 8-101 residues whereas Domain II has 102-184 residues. The third domain has got 201-303 residues. The Domain I and II has a cleft which acts as a substrate binding site as shown in Figure 4.2 [85].

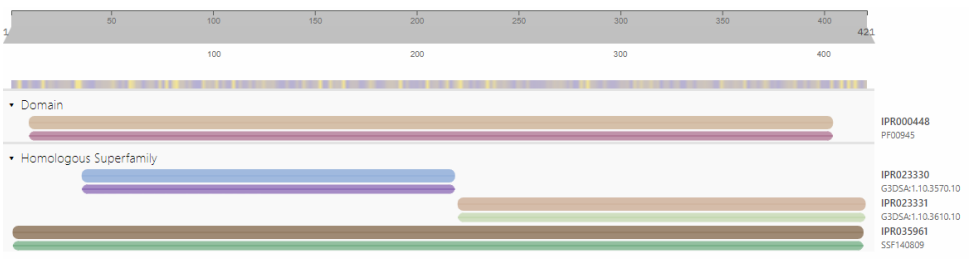


FIGURE 4.2: Functional domains of protein.

4.3 Ligand Selection

Protein data bank contains a large amount of protein ligand complex, especially for the protein target. Therefore, the selection of ligands is based on the best resolution of the structure, the chemical class of the co-crystal ligand bound to the protein structure and the best binding affinity. Conformational selection is a process in which ligand selectively binds to one of these conformers, strengthening it and increasing its population with respect to the total population of the protein, ultimately resulting in the observed complex [86]. Ligands (compounds of the selected plant) were searched out from the chemical information database: PubChem, from here the information can be accessed freely around the globe. Their 3-D structures were downloaded from PubChem in SDF format and they are present in Table 1 Appendix A. After selection of ligands was done which was carried out by chem pro software (chem 3D v 12.0.2). This was a mandatory step in the preparation of ligands for docking because unstable ligands will show unreliable vina scores in docking results. Bioactive antiviral compounds of *Nigella sativa* were selected as ligands for the present study.

4.4 Active Site Identification

To identify active sites of protein, caspT software is used which predicts available pockets for binding and also tells about surface area and volume of pockets [87]. Figure 4.3 shows available pockets for ligands whereas Table 4 shows area and volume of binding pockets.

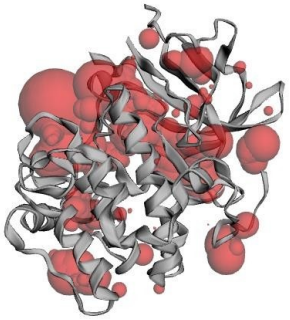


FIGURE 4.3: Structure of Protein Showing Available Pockets for Ligands [87].

The 3D structures and information of selected ligands that are cycloleucalenol, thymol, strychinine, tirucallol, dithymoquinone, lophenol, Isoquinoline, taraxerol, betaamyrin, butyrospermol, cycloartenol, alpha-hederin, alphapinene, alphathujene, betapinene, borylacetate, carvacol, carvone, gammaterpinene, mycrene, longifolene, nigellicine, limonene, nigellidine, pcymene, sabinene, thymohydroquinone, thymoquinone and gramisterol are downloaded from PubChem. This database (https://pubchem.ncbi.nlm.nih.gov) is used as information centre for public in which knowledge about chemical substances alongwith their biological activities and functions is present [87].

TABLE 4.2: Area and Volume of Binding Pockets Obtained by caspT [87].

Pocket ID	**Area (SA)**	**Volume (SA)**
1	827.806	671.098
2	147.392	100.729
3	109.722	75.081
4	86.810	54.825
5	155.182	41.793
6	41.662	34.001
7	29.150	20.769
8	39.489	9.374
9	15.664	7.045
10	14.533	3.487
11	8.285	2.934
12	15.669	1.576
13	5.460	0.992
14	1.669	0.986
15	3.127	0.280
16	2.251	0.161
17	1.234	0.146
18	1.743	0.139
19	2.008	0.137

20	1.985	0.109
21	2.120	0.075
22	0.726	0.054
23	0.707	0.034
24	0.955	0.028
25	0.687	0.020

4.4 Virtual Screening and Toxicity Prediction through Lipinski Rule of Five For compounds to be separated as drug-like and non-drug-like Lipinski rule of five and ADME properties are followed. The Lipinski rule deals with certain parameters like Molecular weight which should be ≤ 500, log P ≤ 5, H-bond donors ≤ 5, H-bond acceptors ≤ 10. These rules are to be followed by orally active compounds. The drug-like is dependent on the mode of administration. A compound is considered a drug when it follows 3 or more rules and if a compound violates two or more rules it is considered poorly absorbed [88]. Table 4.4 gives the value of Lipinski Rule for the selected Ligands.

4.5 Molecular Docking

Molecular Docking is technique used to estimate the strength of a bond between a ligand and a target protein through a special scoring function and to determine the correct structure of the ligand within the target binding site. The 3D structure of the target proteins and the ligands is taken as the input for docking. After preparing proteins and ligands ready for docking, docking were performed by CB dock which is a well trusted online blind auto docking tool [86]. The results and time required for docking depends upon structures of receptors, ligands, requirements, and net speed. It may take several hours for a single result so patience was shown while doing docking. CB dock gave us possible possess and receptor models and among these possess best one was selected by observing certain properties like vena score and size of cavity etc [87].

Molecular docking without having information of binding sites is performed by using a user friendly blind docking web server called as CB Dock, which predicts and estimate a binding site for a given protein and calculate centers and sizes with a novel rotation cavity detection method and perform docking with the popular docking program known as Auto dock Vina. So, the obtained data is given in the table.

TABLE 4.3: Results of CB dock [87]

S. No	**Name**	**Binding Score**	**Cavity Size**	**Grid Map**	**Min-energy (Kcl/ mol)**	**Max-energy (Kcl/ mol)**
1	Cycloleucalenol	-5.8	126	23	0	1.60E+00
2	Thymol	-4.2	126	17	0	1.60E+00
3	Strychinine	-6.2	126	19	0	1.60E+00
4	Tirucallol	-6.3	4	24	0	1.60E+00
5	Dithymoquinone	-5.8	126	24	0	1.60E+00
6	Lophenol	-5.8	126	23	0	1.60E+00
7	Isoquinoline	-4.1	126	16	0	1.60E+00
8	Taraxerol	-6.2	126	23	0	1.60E+00
9	Beta Amyrin	-6.4	126	23	0	1.60E+00
10	Butyrospermol	-6.2	4	24	0	1.60E+00
11	Cycloartenol	-6.6	30	24	0	1.60E+00
12	Alphahederin	-6.8	30	27	0	1.60E+00
13	Alphapinene	-3.7	126	16	0	1.60E+00
14	Alphathujene	-3.8	126	17	0	1.60E+00
15	Betapinene	-3.8	126	17	0	1.60E+00
16	Borylacetate	-4.3	126	17	0	1.60E+00
17	Carvacol	-4.2	126	17	0	1.60E+00
18	Carvone	-4.2	126	17	0	1.60E+00
19	Gammaterpinene	-3.8	126	17	0	1.60E+00

20	Mycrene	-3.5	126	18	0	1.60E+00
21	Longifolene	-4.7	126	17	0	1.60E+00
22	Nigellicine	-5.4	126	19	0	1.60E+00
23	Limonene	-5.4	126	19	0	1.60E+00
24	Nigellidine	-6.1	126	20	0	1.60E+00
25	P-Cymene	-4	126	17	0	1.60E+00
26	Sabinene	-3.7	126	17	0	1.60E+00
27	Thymohydroquinone	-4.2	126	17	0	1.60E+00
28	Thymoquinone	-4.2	126	17	0	1.60E+00
29	Gramisterol	-5.7	30	23	0	1.60E+00

4.6 Interaction of Ligands and Target Protein

The docking analysis is performed by using LigPlot+(version v.1.4.5) and PyMol Edu (v1.7.4.5). Interactions of ligands and target proteins are predicted by using Ligplot plus (version v.1.4.5). The graphical system of LigPlot + automatically generates several 2 dimensional diagrams which are derived from 3 dimensional coordinates. These 2D diagrams shows the interactions of the hydrogen bonds. Moreover it also predicts the hydrophobic interactions present between the ligands with the other elements of the target proteins [88].

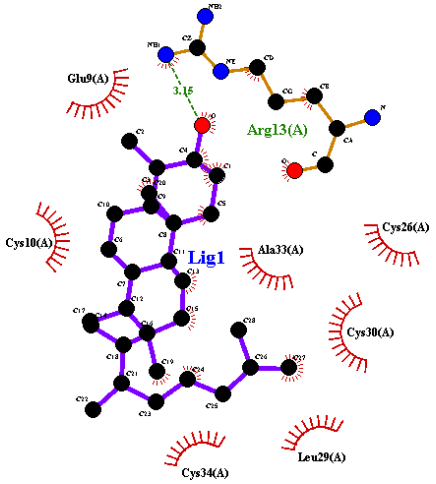


FIGURE 4.4: Interactions of cycloeucalenol By Ligplot [88].

Figure 4.4 shows the interaction of cycloleucalenol with receptor protein. It shows that cycloleucalenol has formed seven hydrophobic interactions and one hydrogen bond.

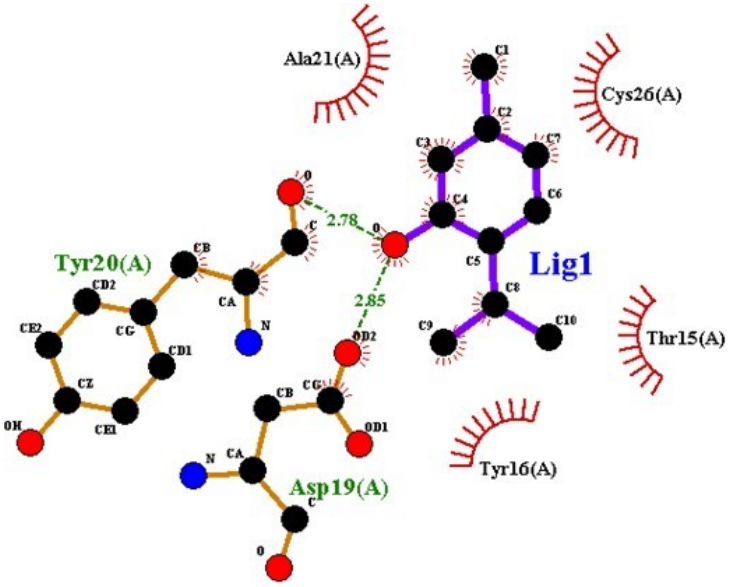


FIGURE 4.5: Interactions of thymol By Ligplot [88].

Figure 4.5 shows the interaction of thymol with receptor protein. It shows that thymol has formed four hydrophobic interactions and two hydrogen bonds.

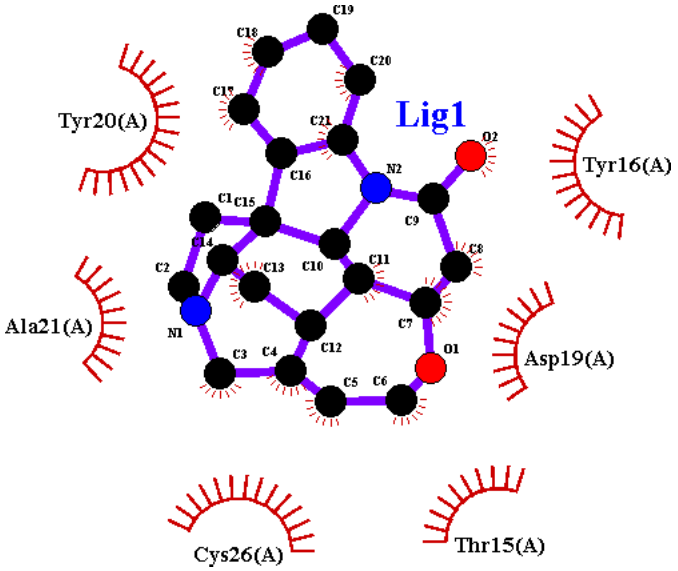


FIGURE 4.6: Interactions of strychinine By Ligplot [88].

Figure 4.7 shows the interaction of strychinine with receptor protein. It shows that strychinine has formed six hydrophobic interactions only.

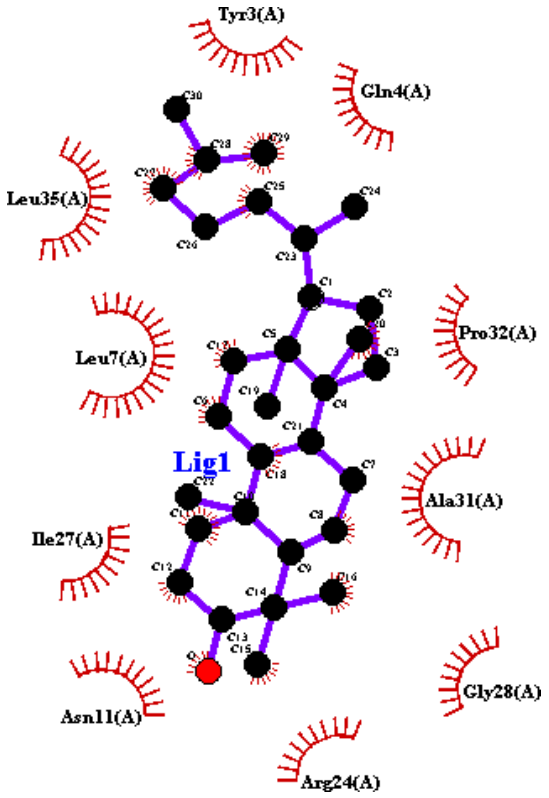


FIGURE 4.7: Interactions of triucallol By Ligplot [88].

Figure 4.7 shows the interaction of triucallol with receptor protein. It shows that triucallol has formed ten hydrophobic interactions only.

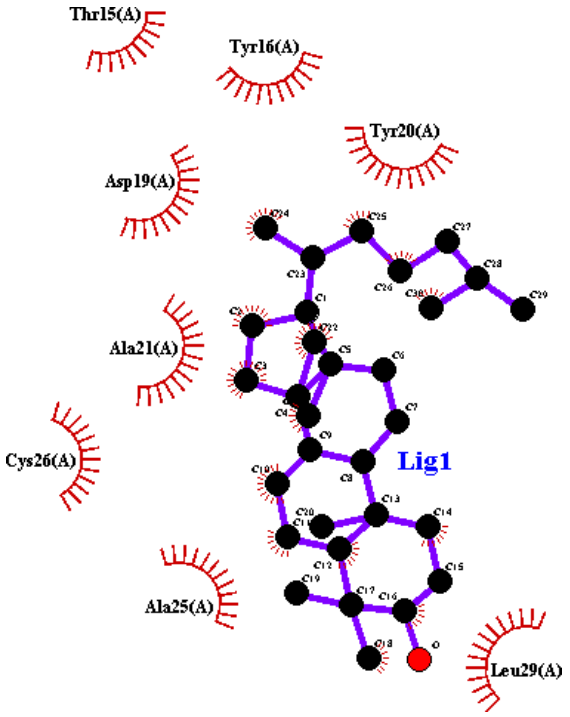


FIGURE 4.8: Interactions of dithymoquinone By Ligplot [88].

Figure 4.8 shows the interaction of dithymoquinone with receptor protein. It shows that dithymoquinone has formed seven hydrophobic interactions only.

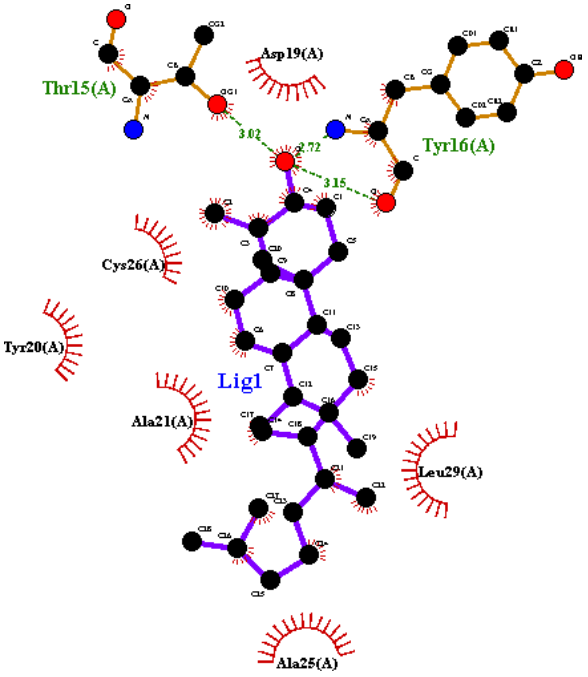


FIGURE 4.9: Interactions of lophenol By Ligplot [88].

Figure 4.9 shows the interaction of lophenol with receptor protein. It shows that lophenol has formed six hydrophobic interactions and two hydrogen bonds.

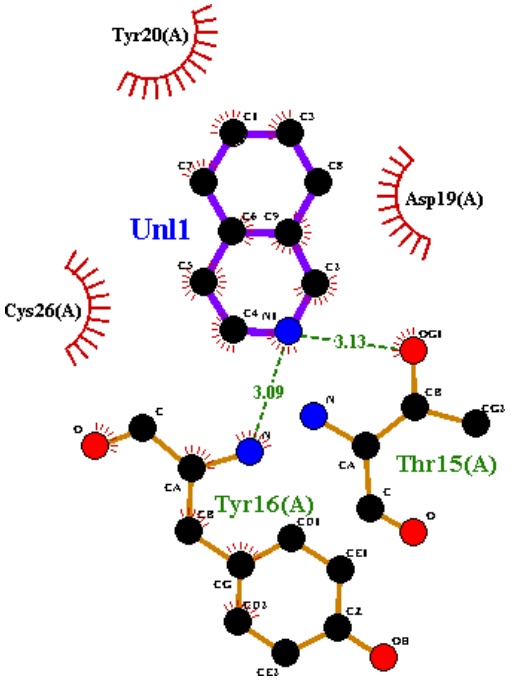


FIGURE 4.10: Interactions of isoquinoline By Ligplot [88].

Figure 4.10 shows the interaction of isoquinoline with receptor protein. It shows that isoquinoline has formed three hydrophobic interactions and two hydrogen bonds.

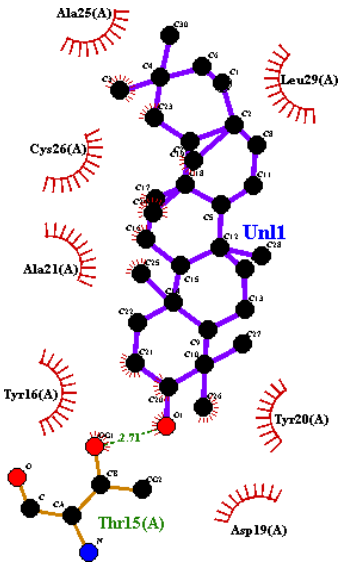


FIGURE 4.11: Interactions of taraxerol By Ligplot [88].

Figure 4.11 shows the interaction of taraxerol with receptor protein. It shows that taraxerol has formed seven hydrophobic interactions and one hydrogen bond.

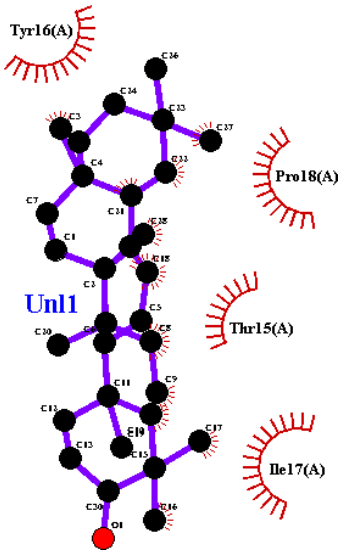


FIGURE 4.12: Interactions of betaamyrin By Ligplot [88].

Figure 4.12 shows the interaction of betaamyrin with receptor protein. It shows betaamyrin that has formed four hydrophobic interactions only.

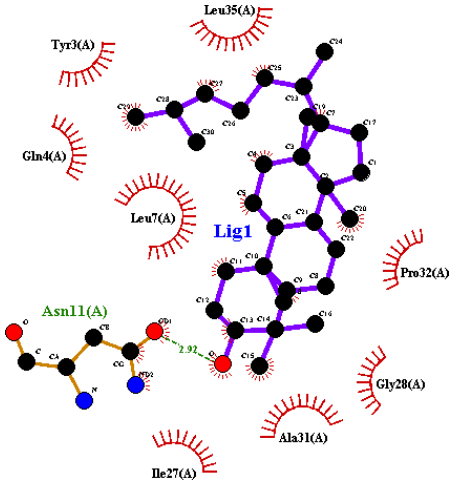


FIGURE 4.13: Interactions of butyrospermol By Ligplot [88].

Figure 4.13 shows the interaction of butyrospermol with receptor protein. It shows that butyrospermol has formed eight hydrophobic interactions and one hydrogen bond only.

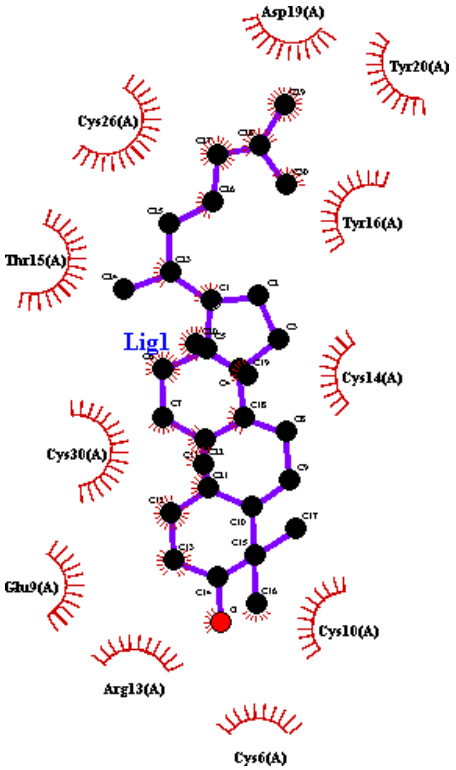


FIGURE 4.14: Interactions of cycloatrtenol By Ligplot [88].

Figure 4.14 shows the interaction of cycloartenol with receptor protein. It shows cycloartenol that has formed ten hydrophobic interactions only.

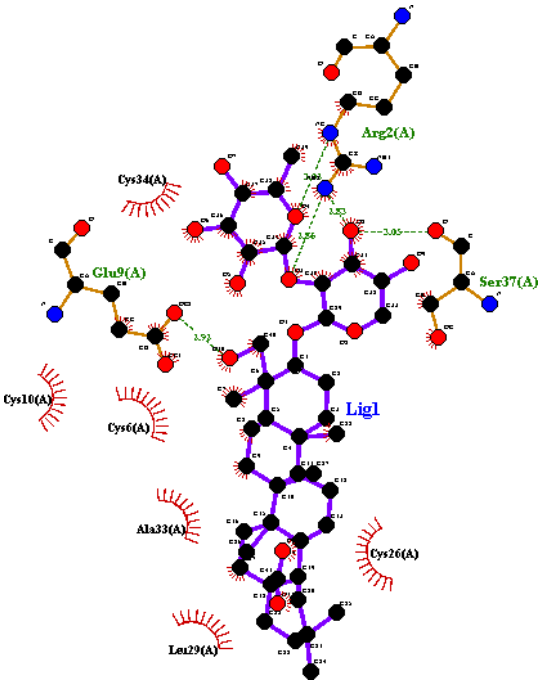


FIGURE 4.15: Interactions of alphahederin By Ligplot [88].

Figure 4.15 shows the interaction of alphahederin with receptor protein. It shows alphahederin that has formed six hydrophobic interactions and five hydrogen bonds.

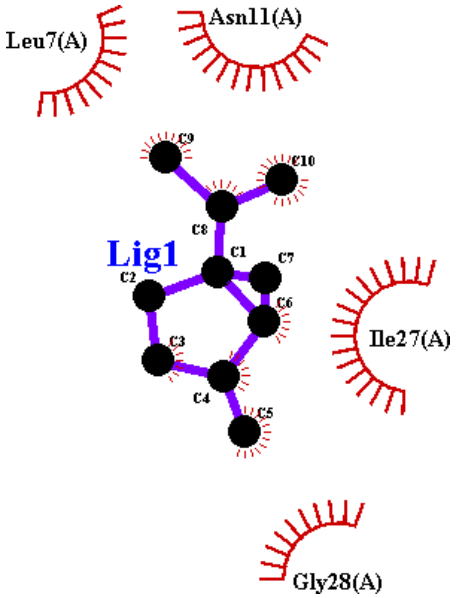


FIGURE 4.16: Interactions of alphapinene By Ligplot [88].

Figure 4.16 shows the interaction of sabinene with receptor protein 2NB2. It shows sabinene that has formed five hydrophobic interactions only.

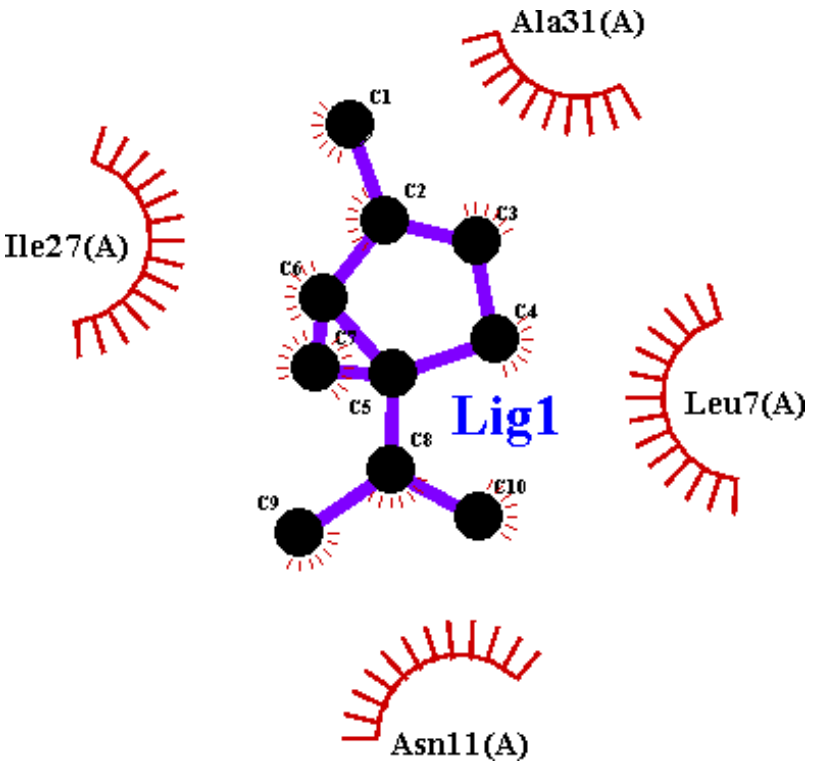


FIGURE 4.17: Interactions of alphathujene By Ligplot [88].

Figure 4.17 shows the interaction of alphathujene with receptor protein. It shows alphathujene that has formed four hydrophobic interactions only.

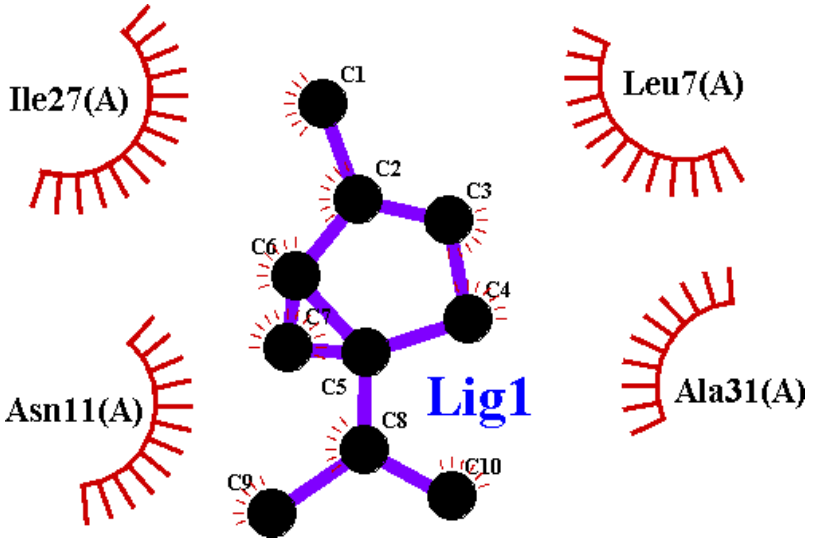


FIGURE 4.18: Interactions of betapinene By Ligplot [88].

Figure 4.18 shows the interaction of betapinene with receptor protein. It shows that betapinene has formed four hydrophobic interactions only.

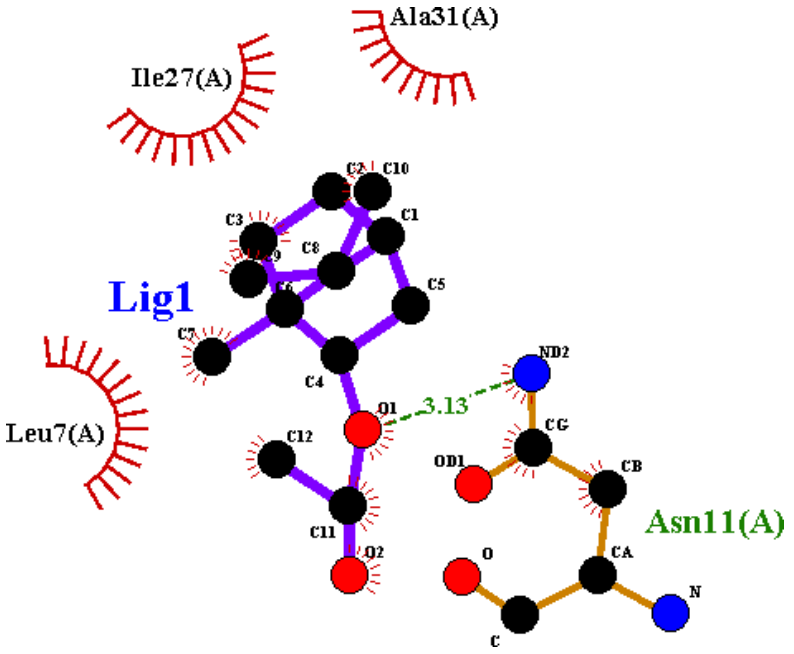


FIGURE 4.19: Interactions of bornylacetate By Ligplot [88].

Figure 4.19 shows the interaction of bornylacetate with receptor protein. It shows that bornylacetate has formed four hydrophobic interactions only.

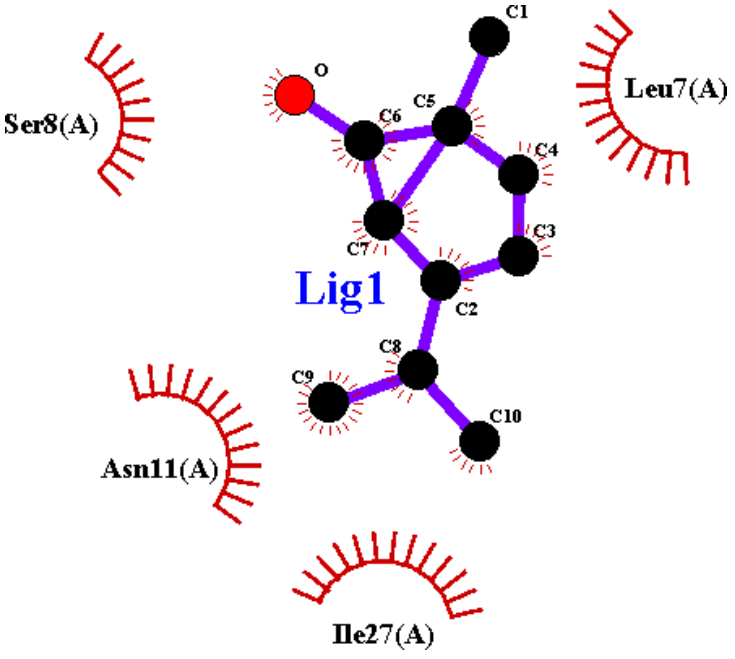


FIGURE 4.20: Interactions of carvacol By Ligplot [88].

Figure 4.20 shows the interaction of carvacol with receptor protein. It shows that carvacol has formed four hydrophobic interactions only.

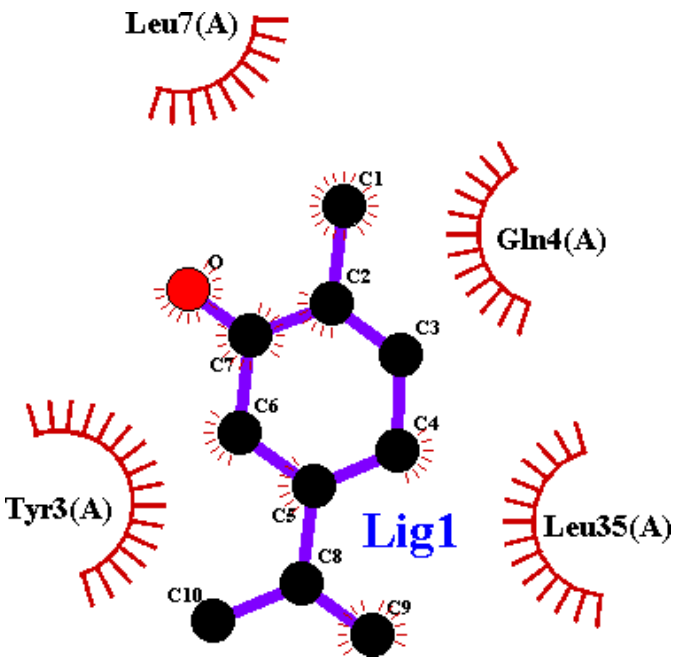


FIGURE 4.21: Interactions of carvone By Ligplot [88].

Figure 4.21 shows the interaction ofcarvone with receptor protein. It shows that carvone has formed four hydrophobic interactions only.

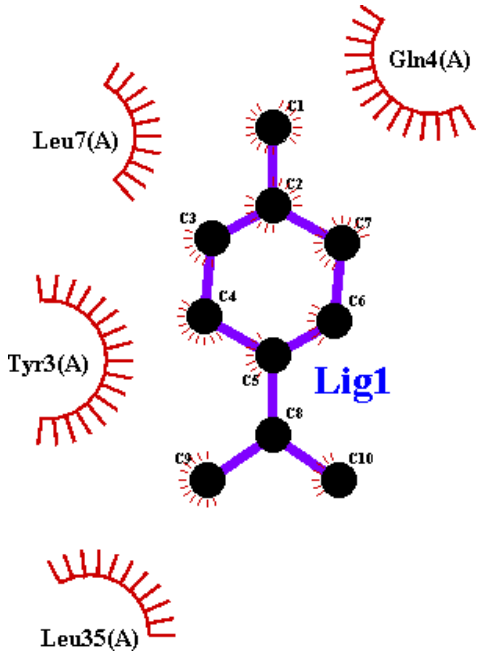


FIGURE 4.22: Interactions of gammaterpinene By Ligplot [88].

Figure 4.22 shows the interaction of gammaterpinene with receptor protein. It shows that gammaterpinene has formed four hydrophobic interactions only.

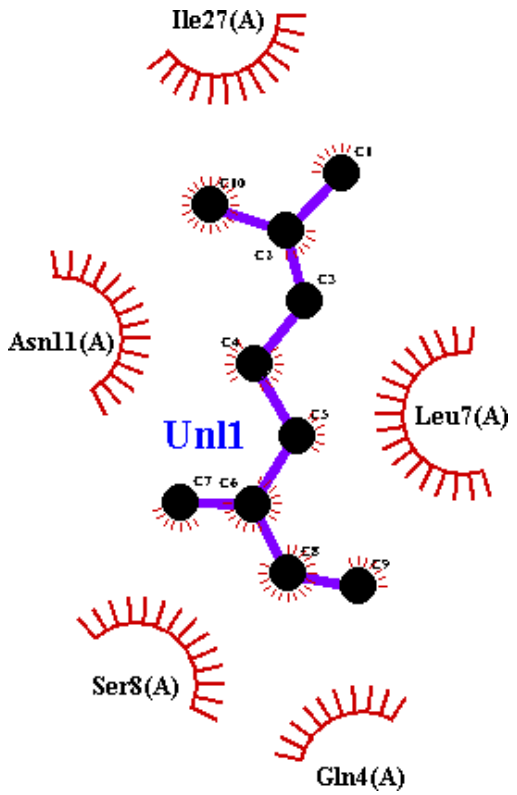


FIGURE 4.23: Interactions of myrcene By Ligplot [88].

Figure 4.23 shows the interaction of mycrene with receptor protein. It shows that mycrene has formed five hydrophobic interactions only.

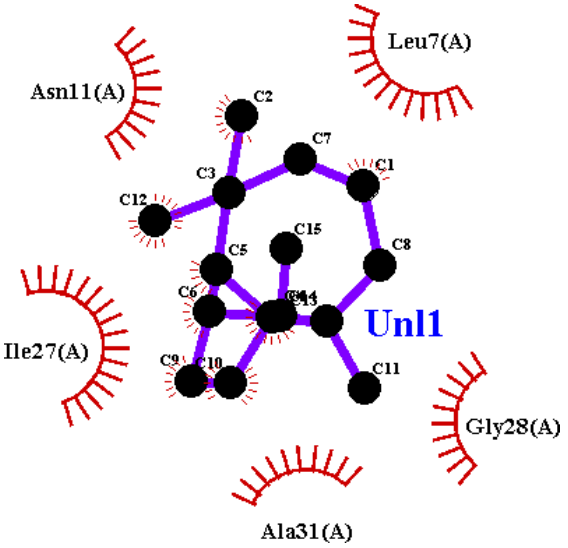


FIGURE 4.24: Interactions of longifolene By Ligplot [88].

Figure 4.24 shows the interaction of longifolene with receptor protein. It shows that longifolene has formed five hydrophobic interactions only.

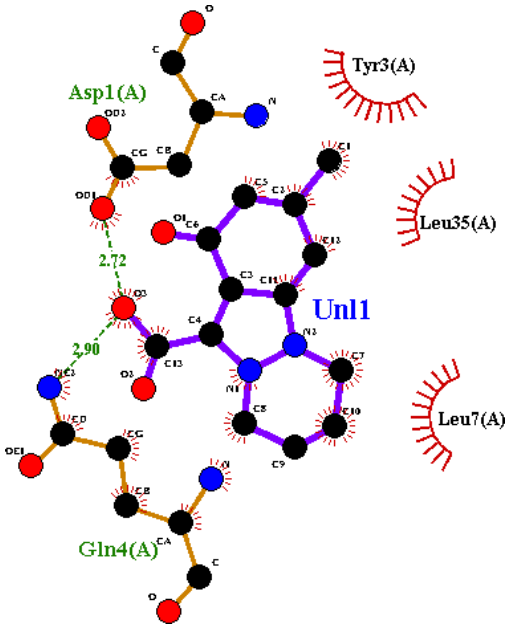


FIGURE 4.25: Interactions of nigellicine By Ligplot [88].

Figure 4.26 shows the interaction of nigellicine with receptor protein. It shows that nigellicine has formed three hydrophobic interactions and two hydrogen bonds only.

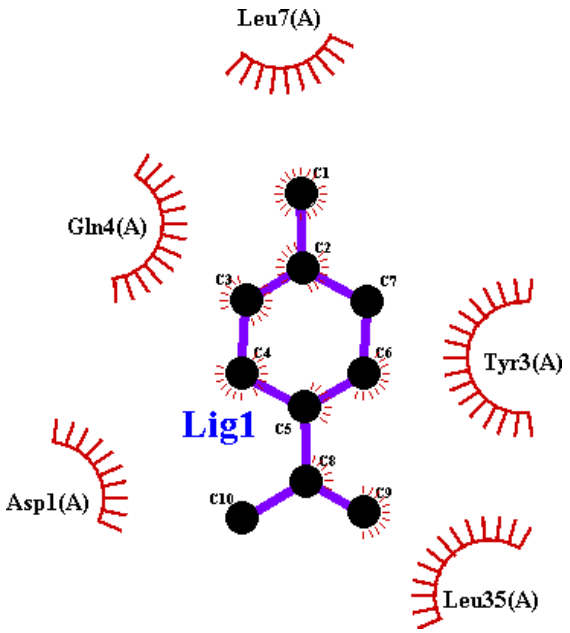


FIGURE 4.26: Interactions of limonene By Ligplot [88].

Figure 4.26 shows the interaction of limonene with receptor protein. It shows that limonene has formed five hydrophobic interactions.

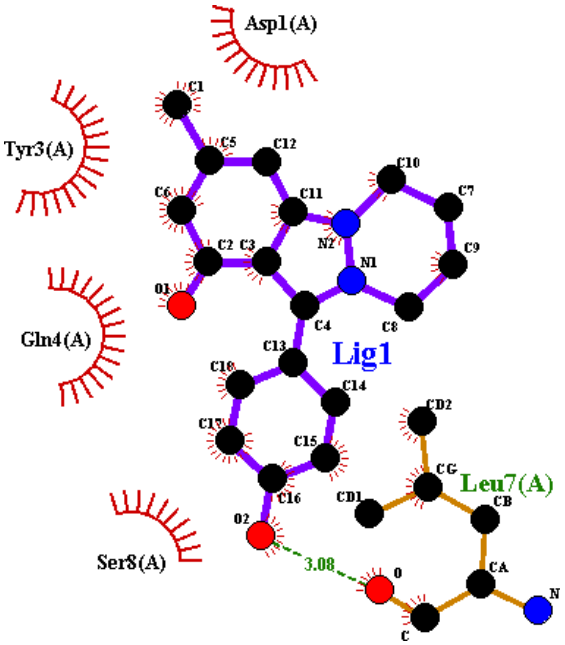


FIGURE 4.27: Interactions of nigellidine By Ligplot [88].

Figure 4.27 shows the interaction ofnigellidine with receptor protein. It shows that nigellidine has formed four hydrophobic interactions and one hydrogen bond only.

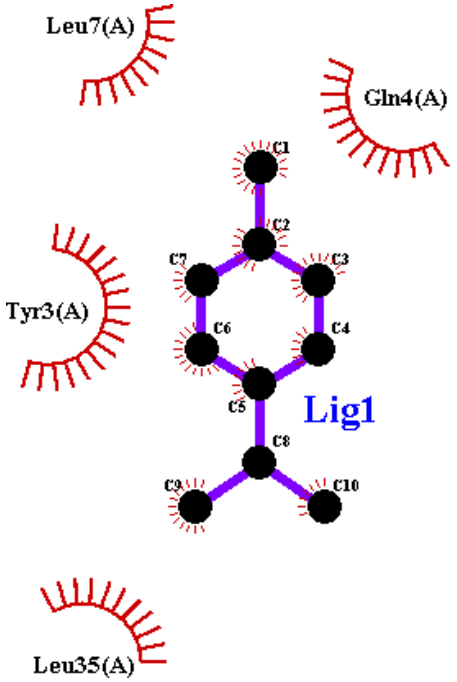


FIGURE 4.28: Interactions of p-cymene By Ligplot [88].

Figure 4.28 shows the interaction of pcymene with receptor protein. It shows pcymene that has formed four hydrophobic interactions only.

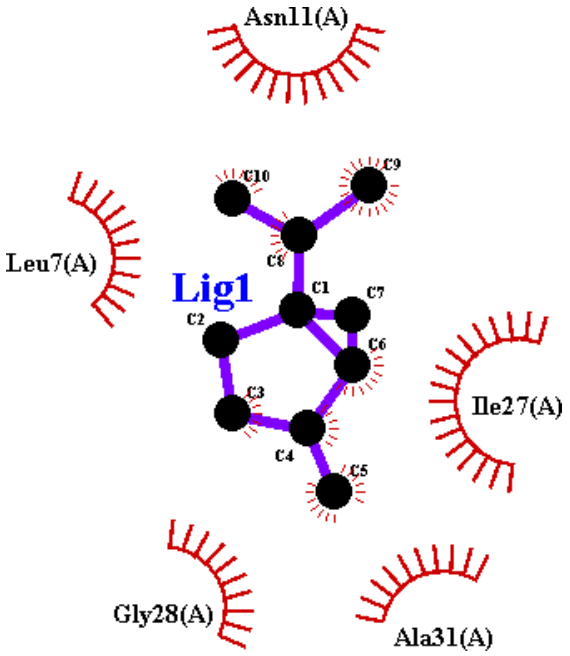


FIGURE 4.29: Interactions of sabinene By Ligplot [88].

Figure 4.29 shows the interaction of sabinene with receptor protein 2NB2. It shows sabinene that has formed five hydrophobic interactions only.

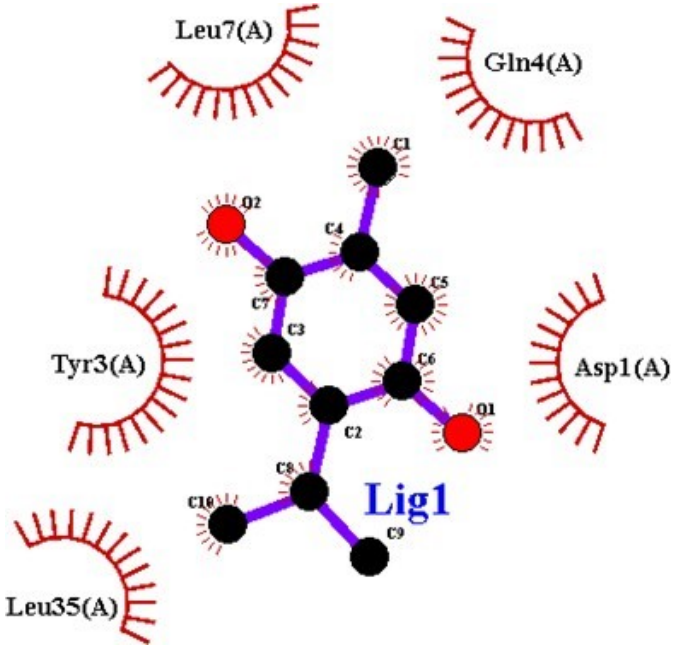


FIGURE 4.30: Interactions of thymohydroquinone By Ligplot [88].

Figure 4.30 shows the interaction of thymohydroquinone with receptor protein. It shows thymohydroquinone that has formed five hydrophobic interactions only.

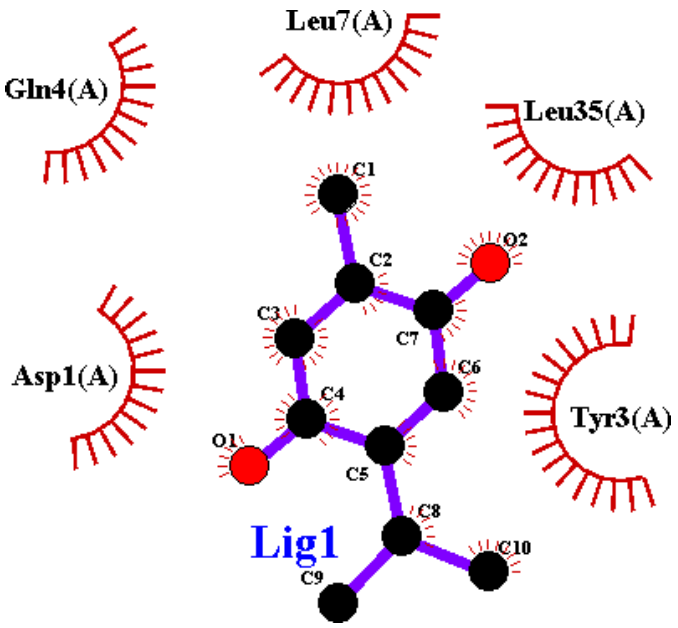


FIGURE 4.31: Interactions of thymoquinone By Ligplot [88].

Figure 4.31 shows the interaction of thymoquinone with receptor protein 2NB2. It shows thymoquinone that has formed five hydrophobic interactions only

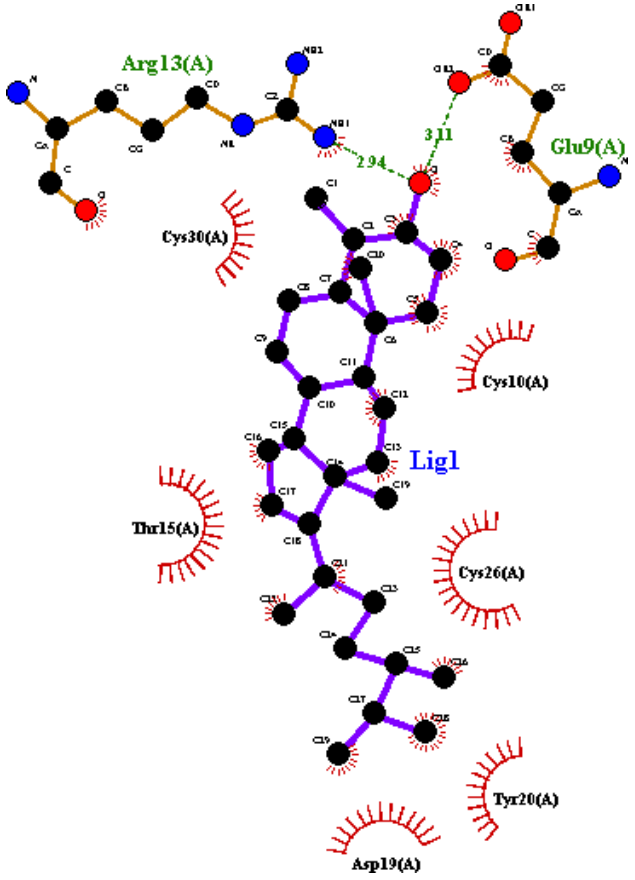


FIGURE 4.32: Interactions of gramisterol By Ligplot [88].

Figure 4.32 shows the interaction of gramisterol with receptor protein. It shows that gramisterol has formed six hydrophobic interactions and two hydrogen bonds.

The Table 2 (refer to Appendix B) shows properties of compounds obtained by ligplus.these properties include hydrogen bonds,their distance,aminoacids and hydrophobic bonding.

4.7 ADMET Properties of Ligands

Lipinski's five-drug law used as a first step in assessing verbal bioavailability and artificial availability. A second study was performed by calculating the ADMET properties of ligands as a measure of pharmacokinetics using the online tool pkCSM [89].

In pharmacology there two broad terms the one is pharmacodynamics and the other is pharmacokinetics.

4.7.1 Pharmacodynamics

Pharmacodynamics is a branch of pharmacology in which we study the effect of drugs on the body [89].

4.7.2 Pharmacokinetics

In pharmacokinetics we study the absorption of drugs, distribution of drugs, metabolism of the drug and excretion of the drugs [89].

4.7.3 Absorption

In pharmacology (specifically pharmacokinetics), the transfer of a drug from the bloodstream into the tissues is called absorption. So the chemical composition of

a drug, as well as the environment into which a drug is placed, work together to determine the rate and extent of drug absorption.

Absorption is one of ADMET properties which predict absorption of orally administered drugs and includes Water solubility, CaCO2 permeability, Intestinal absorption, Skin permeability, P-glycoprotein substrate, and P- glycoprotein I & II inhibitors [90]. Water solubility (log S) of a compound predicts its solubility in water at 25 Degree Centigrade. It is predicted as a molar concentration logarithm (log mol / L). Lipid soluble drugs are less soluble in water than water-soluble drugs. The CaCO2 permeability model predicts the logarithm of the apparent permeability coefficient (log Papp; logcm/s). A compound has a high CaCO2 absorbency if it has a Papp > 8 Ö 10-6cm /s. Intestinal absorption predicts the percentage that will enter a person's small intestine. A compound with less than 30% absorption is considered to be less absorbent. The skin permeability model predicts the absorbency in log Kp and this model has a special interest in the formation of transdermal drugs [91].

The element with the log Kp > -2.5 means it has low skin penetration. The P-glycoprotein substrate acts as a natural barrier and removes toxins and xenobiotics from the cells. This model predicts whether the given compound may be P-glycoprotein (Pgp) substratum or not. This means if a compound is a Pgpsubstrate (categorically yes), it may be show low oral absorption. P-gp substrates can be easily pumped out of the cells to reduce their absorption. P- glycoprotein I/II inhibitor model predicts that the compound is likely to be a P-gb I/II inhibitor or not. P-gp inhibitors reduce the pumping activity of P-gp and may have high absorption [92].

4.7.3.1 Absorption Properties of Ligands

Cycloleucalenol shows low water solubility, normal CaCO2 permeability, approximately 97% intestinal absorption in humans, shows low skin permeability and negative value for P- glucoprotein substrate, P-glucoprotein I inhibitor and P-glucoprotein II inhibitor.

Water solubility value for all other ligands is low, normal CaCO2 value for all ligands except alphahederin who has negative value, intestinal absorption for humans is more than 90% for all ligands.

Only dithymoquinone and nigellicine shows 100% absorption, thymol, isoquinoline, alphahederin, alphapinene, alphathujene, betapinene, carvacol,carvone, gammaterpinene, mycrenelongifolene, nigellicine,p- cymene,sabinene,thymoquinoline shows negative value for P- glucoprotein substrate, P-glucoprotein I inhibitor and P-glucoprotein II inhibitor while other ligands shows positive value for one, two or all three factors. If a compound is positive for Pgp substrate then its means that it can be easily pumped out of the cells to reduce its absorption [93].

TABLE 4.4: Absorption Properties of Ligands [93].

S. No.	**Ligands Name**	**Water Solubility**	**Caco2 Permeability**	**Intestinal Absorption (human)**	**Skin Permeability**	**P-gluco protein Substrate**	**P-glucoprotein I Inhibitor**	**P-glucoprotein II Inhibitor**
1	Cycloleucalenol	-5.455	1.211	96.659	-2.732	No	No	Yes
2	Thymol	-2.789	1.606	91	-1.62	No	No	No
3	Strychinine	-4.418	1.08	94.524	-2.75	Yes	Yes	Yes
4	Tirucallol	-7.498	1.203	93.119	-2.926	No	Yes	Yes
5	Dithymoquinone	-3.654	1.367	100	-3.189	No	Yes	No
6	Lophenol	-6.538	1.206	95.178	-2.769	No	No	Yes

7	Isoquinoline	-1.721	1.549	97.359	-1.824	No	No	No
8	Taraxerol	-6.31	1.212	94.036	-2.788	No	Yes	Yes
9	Beta Amyrin	-6.531	1.226	93.733	-2.811	No	Yes	Yes
10	Butyrospermol	-7.081	1.205	93.83	-2.831	No	Yes	Yes
11	Cycloartenol	-5.762	1.194	95.248	-2.737	No	Yes	Yes
12	Alphahederin	-3.012	-3.012	38.881	-2.735	Yes	No	No
13	Alphapinene	-3.733	1.38	96.041	-1.827	No	No	No
14	Alphathujene	-4.294	1.386	95.256	-1.371	No	No	No
15	Betapinene	-4.191	1.385	95.525	-1.653	No	No	No
16	Borylacetate	-4.99	1.924	95.811	-2.424	No	Yes	No
17	Carvacol	-2.789	1.606	90.843	-1.62	No	No	No
18	Carvone	-2.324	1.413	97.702	-2.145	No	No	No
19	Gammaterpinene	-3.941	1.414	96.219	-1.489	No	No	No
20	Mycrene	-4.497	1.4	94.696	-1.043	No	No	No
21	Longifolene	-5.668	1.404	95.767	-1.74	No	No	No

22	Nigellicine	-2.148	0.453	100	-2.73	No	No	No
23	Limonene	-3.568	1.401	95.898	-1.721	Yes	No	No
24	Nigellidine	-3.651	1.304	95.368	-2.916	No	Yes	No
25	P-Cymene	-4.081	1.527	93.544	-1.192	No	No	No
26	Sabinene	-4.629	1.404	95.356	-1.342	No	No	No
27	Thymohydroquinone	-1.839	1.549	90.669	-2.729	Yes	No	No
28	Thymoquinone	-1.613	1.271	99.382	-2.461	No	No	No
29	Gramisterol	-6.658	1.216	95.686	-2.779	No	Yes	Yes

4.7.4 Distribution Properties of Ligands

Distribution in pharmacology is a branch of pharmacokinetics which deals with the movement of drug within the body from one location to another location. Distribution as one of ADMET property includes four models namely as Volume of distribution in human (VDss expressed as log L/kg). Fraction unbound in humans(Fu), Blood brain barrier (BBB) permeability expressed as log BB.

Central nervous system permeability (CNS permeability) expressed as log PS Model-1 explains the theoretical volume that the total amount of drug that will be needed to be evenly distributed to provide the same concentration as in blood plasma [94]. VDss is considered low if it is less than 0.71 L / kg (log VDss < 0.15) and higher if it is above 2.81L / kg (log VDss> 0.45). If VDss is high, it means that more of the drug is still distributed to the tissues than to plasma.If a compound shows more Fu value, its mean it is more effective.

BBB protects the brain from exogenous compounds so BBB permeability is an important parameter. If predicted value of log BB >0.3 then it mean given substance can crossBBB and if value <-1 then no harm to brain.

Log PS is the product of bloodbrain permeability and surface area, and its value >-2 considered to penetrate the Central Nervous System (CNS), and <-3 considered as safe[95].

Cycloleucalenol, thymol, strychinine ,tirucallol ,lophenol ,isoquinoline ,taraxerol ,beta amyrin ,butyrospermol ,cycloartenol ,alphapinene ,alphathujene ,betapinene ,borylacetate ,carvacol ,carvone ,gammaterpinene ,mycrene ,longifolene , p-cymene ,sabinene , thymoquinone and gramisterol has logBB>0.3 whereas dithymoquinone, dithymohydroquinone, nigecilline, nigedilline and alphahydrenine shows log value less 0.3 [96].

Table 4.5: Distribution Properties of Ligands [96].

S. No.	**Name**	**VDss (human)**	**Fraction Unbound (human)**	**BBB Permeability (human)**	**CNS Permeability**
1	Cycloleucalenol	-0.207	0	0.857	-1.428
2	Thymol	0.512	0.203	0.407	-1.664
3	Strychinine	1.163	0	0.318	-1.036
4	Tirucallol	0.66	0	0.683	-2.254
5	Dithymoquinone	-0.026	0.188	-0.118	-2.719

6	Lophenol	0.14	0	0.781	-1.675
7	Isoquinoline	0.024	0.338	0.316	-1.91
8	Taraxerol	0.206	0	0.678	-1.891
9	Beta Amyrin	0.268	0	0.667	-1.773
10	Butyrospermol	0.364	0	0.716	-1.984
11	Cycloartenol	-0.075	0	0.794	-1.714
12	Alphahederin	-1.129	0.303	-1.077	-3.25
13	Alphapinene	0.667	0.425	0.791	-2.201
14	Alphathujene	0.575	0.356	0.81	-1.793
15	Betapinene	0.685	0.35	0.818	-1.857
16	Borylacetate	-2.424	0	0.364	-1.399
17	Carvacol	0.512	0.203	0.407	-1.664
18	Carvone	0.179	0.53	0.588	-2.478
19	Gammaterpinene	0.412	0.42	0.754	-2.049
20	Mycrene	0.363	0.39	0.781	-1.902
21	Longifolene	0.781	0.189	0.808	-1.949
22	Nigellicine	-0.801	0.489	-0.144	-2.944
23	Limonene	0.396	0.48	0.732	-2.37
24	Nigellidine	0.508	0.123	-0.104	-2.16
25	P-Cymene	0.697	0.159	0.478	-1.397
26	Sabinene	0.566	0.295	0.836	-1.463
27	Thymohydroquinone	0.315	0.335	0.117	-1.738
28	Thymoquinone	-0.026	0.527	0.326	-2.269
29	Gramisterol	0.173	0	0.795	-1.721

4.7.5 Metabolism

CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 models of the various isoforms of Cytochrome P450 which is an important cleansing enzyme found in the liver. This enzyme reacts to xenobiotics to facilitate their release. Some drugs

are triggered by this enzyme while most drugs are neutralized by it [97]. Metabolic properties of ligands are given below.

TABLE 4.6: Metabolism of Different Ligands [97].

S. No.	**Ligand Name**	**CYP-2D6 Substrate**	**CYP-3A4 Substrate**	**CYP-2D6 Inhibitor**	**CYP-2C19 Inhibitor**	**CYP-2C9 Inhibitor**	**CYP-2D6 Inhibitor**	**CYP-3A4 Inhibitor**
1	Cycloleucalenol	No	Yes	No	No	No	No	No
2	Thymol	No	No	Yes	No	No	No	No
3	Strychinine	Yes	Yes	No	No	No	Yes	Yes
4	Tirucallol	No	Yes	No	No	No	No	No
5	Dithymoquinone	No	Yes	No	No	No	No	No
6	Lophenol	No	Yes	No	No	No	No	No
7	Isoquinoline	No	No	Yes	No	No	No	No
8	Taraxerol	No	Yes	No	No	No	No	No
9	Beta Amyrin	No	Yes	No	No	No	No	No
10	Butyrospermol	No	Yes	No	No	No	No	No
11	Cycloartenol	No	Yes	No	No	No	No	No
12	Alphahederin	No	Yes	No	No	No	No	No
13	Alphapinene	No	No	No	No	No	No	No
14	Alphathujene	No	No	No	No	No	No	No

15	Beta-pinene	No	No	No	No	No	No	No
16	Boryl-acetate	No	Yes	Yes	Yes	Yes	No	No
17	Carvacol	No	Yes	No	No	No	No	No
18	Carvone	No	No	No	No	No	No	No
19	Gamma-terpinene	No	No	No	No	No	No	No
20	Mycrene	No	No	No	No	No	No	No
21	Longi-folene	No	Yes	No	No	No	No	No
22	Nigel-licine	No	No	No	No	No	No	No
23	Limo-nene	No	No	No	No	No	No	No
24	Nigel-lidine	No	Yes	No	Yes	No	No	No
25	P-Cymene	No	No	Yes	No	No	No	No
26	Sabinene	No	No	No	No	No	No	No
27	Thymohy droquinone	No	No	Yes	No	No	No	No
28	Thymo-quinone	No	No	No	No	No	No	No
29	Gram-isterol	No	Yes	No	No	No	No	No

4.7.6 Excretion

The organs involved in drug excretion are the kidneys, which play important role in excretion (renal excretion) and the liver (biliary excretion). Other organs may

also be involved in excretion, such as the lungs for volatile or gaseous agents. Drugs can also be excreted in sweat, saliva and tears.

Models of Excretion property are Total Clearance (CLtot) expressed as log (CL tot) in ml/min/kg and second one is Renal OCT2 substrate which predicts results as Yes /No. OCT2 (organic cation transporter 2) is a renal uptake transporter that plays role in disposition and renal clearance of drugs. All ligands showed negative result for model Renal OCT2 substrate except dithymoquinone and nigellidine. Most of the compounds exhibit fair total clearance, except for dithymoquinine, Taraxerol, Beta Amyrin and alphahederin [98]. Excretory properties are listed below.

TABLE 4.7: Excretion Properties of Ligands [98].

S. No.	**Ligand Name**	**Total Clearance**	**Renal OCT2 Substrate**
1	Cycloleucalenol	0.388	No
2	Thymol	0.211	No
3	Strychinine	1.139	No
4	Tirucallol	0.403	No
5	Dithymoquinone	-0.016	Yes
6	Lophenol	0.555	No
7	Isoquinoline	0.286	No
8	Taraxerol	-0.081	No
9	Beta Amyrin	-0.044	No
10	Butyrospermol	0.396	No
11	Cycloartenol	0.262	No
12	Alphahederin	-0.031	No
13	Alphapinene	0.043	No
14	Alphathujene	0.077	No
15	Betapinene	0.03	No
16	Borylacetate	0.207	No
17	Carvacol	0.207	No

18	Carvone	0.225	No
19	Gammaterpinene	0.217	No
20	Mycrene	0.438	No
21	Longifolene	0.901	No
22	Nigellicine	0.55	No
23	Limonene	0.213	No
24	Nigellidine	0.511	Yes
25	P-Cymene	0.239	No
26	Sabinene	0.071	No
27	Thymohydroquinone	0.24	No
28	Thymoquinone	0.225	No
29	Gramisterol	0.24	No

4.7.7 Toxicity Prediction

PkCSM is an online tool used to find the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of bioactive compounds and drugs. The maximum tolerated dose (MRTD) provides a measure of toxic chemical on individuals.

This will help in directing the first recommended dose of the treatment regimen in phase 1 clinical trials. MRTD is expressed in the form of logarithms (log mg / kg / day). In a given compound MRTD less than or equal to 0.477 log (mg / kg / day) is considered to be lower and higher if it is higher than 0.477 log (mg / kg / day).

The maximum tolerated dose (MRTD) provides a measure of toxic chemical on individuals. This will help in directing the first recommended dose of the treatment regimen in phase 1 clinical trials. MRTD is expressed in the form of logarithms. In a given compound MRTD less than or equal to 0.477 log is considered to be lower and higher if it is higher than 0.477 log [99]. Table 4.8 shows toxicity properties.

TABLE 4.8: Toxicity Properties of Ligands

Ligand	**Max. Tolerated Dose (Human) (Mg/Kg)**	**Herg I Inhibitor**	**Herg II Inhibitor**	**Oral Rat Acute Toxicity (Mol/Kg)**	**Oral Rat Chronic Toxicity (Mol/Kg)**	**Hepatoxicity**	**Skin Sensitisation**	**T. Pyriformis Toxicity (Log Ug/L)**	**Minnow Toxicity (Log Mm)**
Cycloleucalenol	-0.351	No	Yes	3.002	0.855	No	No	0.294	-1.714
Thymol	1.007	No	No	2.074	2.212	Yes	Yes	0.387	1.213
Strychinine	-0.197	No	Yes	2.894	1.811	No	No	0.289	-0.763
Tirucallol	-0.568	No	Yes	1.906	0.788	No	No	0.736	-1.757
Dithymoquinone	0.534	No	No	1.649	1.53	No	No	0.445	1.323
Lophenol	-0.695	No	Yes	2.411	0.892	No	No	0.42	-1.856
Isoquinoline	0.694	No	No	2.126	2.189	No	Yes	0.148	0.972
Taraxerol	-0.623	No	Yes	2.386	0.808	No	No	0.359	-1.511
Beta Amyrin	-0.56	No	Yes	2.478	0.873	No	No	0.383	-1.345

Butyrrospermol	-0.668	No	Yes	2.239	0.796	No	No	0.523	-1.824
Cycloartenol	-0.46	No	Yes	2.627	0.806	No	No	0.305	-1.928
Alphahederin	-0.105	No	No	2.963	1.766	No	No	0.285	1.925
Alphapinene	0.48	No	No	1.77	2.262	No	No	0.45	1.159
Alphathujene	0.353	No	No	1.589	2.243	No	No	0.597	0.995
Betapinene	0.371	No	No	1.673	2.28	No	No	0.628	1.012
Bornylacetate	0.841	No	No	2.596	1.196	No	No	1.335	-0.214
Carvacol	1.007	No	No	2.074	2.212	Yes	Yes	0.387	1.213
Carvone	0.775	No	No	1.86	1.972	No	Yes	0.41	1.445
Gamma Terpinene	0.756	No	No	1.766	2.394	No	No	0.627	0.906
Myrcrene	0.617	No	No	1.643	2.406	No	No	0.894	0.736
Longifolene	0.072	No	No	1.58	1.353	No	No	1.367	0.371
Nigellicine	0.283	No	No	2.265	1.17	Yes	No	0.237	1.776
Limonene	0.777	No	No	1.88	2.336	No	Yes	0.579	1.203

Nigellidine	-0.425	No	No	2.423	1.081	Yes	No	1.437	1.17
P-Cymene	0.903	No	No	1.827	2.328	No	Yes	0.462	0.869
Sabinene	0.369	No	No	1.549	2.309	No	No	0.788	0.726
Thymohydroquinone	0.441	No	No	2.367	1.96	Yes	Yes	0.839	1.252
Thymoquinone	0.89	No	No	1.743	2.378	Yes	Yes	0.138	1.758
Gramisterol	-0.6	No	Yes	2.395	0.818	No	No	0.426	-1.973

The hERG I & II inhibitors model is said to cause the inhibition of potassium channels induced by the h ERG (human ether-a-go-go gene) are the main causes of the development of chronic QT syndrome leading to fatal ventricular arrhythmia. The inhibition of h ERG channels has led to the withdrawal of many items from the pharmaceutical market. LD50 is the quantity of a compound that causes the deaths of 50% of experimental animals (mice) [99]. The LD50 (mol / kg) predicts toxicity of a probable compound where as LOAEL aims to identify the lowest dosage of a compound with a significant adverse effect. Exposure to low to moderate chemical doses for a long time is very important in medicine and is expressed in a log (mg / kg- bw / day). Hepatotoxicity reveals drug-induced liver damage and is a major safety concern for drug development. Skin sensitivity is a potential adverse effect of skin care & applied products. T. pyriformis is a protozoans bacteria, whose toxin is often used as a toxic endpoint (IGC50) and inhibits 50% growth. p IGC50 (negative concentration logarithm required to prevent 50% growth) in log ug / L predicted value > - 0.5 log ug / L is considered

toxic. The lethal concentrations (LC50) represent the concentration of molecules needed to cause the death of 50% of Flathead Minnows (small bait fishes). In Minnow toxicity LC50 values below 0.5 m M (log LC 50 <-0.3) are regarded as high acute toxicity [100]. Toxicity predicted values of selected ligands were listed in table. 4.8. Thymol, dithymoquinone, isoquinoline, alphapinene, bronylacetate, carvacol, carvone, gammaterpinene, liminone, myrcrene, p-cymene, thymoquinone shows values higher than 0.477 which are above the max tolerated dose whereas other ligands, strychinine, tirucallol, lophenol, taraxerol, betaamyrin, butyrospermol, cycloartenol, alphahederin, alphathujene, betapinene, longifolene, nigellicine, nigellidine, sabinene and gramisterol have low values than 0.477 [101].

4.8 Lipanski Rule of Five

Lipinski's rule of five are as follow:

1. The logP value of most "drug-like" molecules should be limited to 5.
2. Molecular weight should be under 500.
3. Maximum number of H-bond acceptor should be 10.
4. Maximum number of H-bond donor should be 5 [75].

So, following rules are applied on our compound and hence analysis of different ligands of nigella sativa is checked and results are shown in table 4.10.

Table 4.9: Lipinski;s rule of five [75].

Sr. No	**Ligand**	**LogP**	**Molecular Weight (G/Mol)**	**Hydrogen Bond Acceptor**	**Hydrogen Bond Donar**
1	Cycloleucalenol	8.0248	426.729	1	1
2	Thymol	2.82402	150.221	1	1
3	Strychinine	4.5179	461.005	3	0

4	Tirucallol	8.4791	426.729	1	1
5	Dithymoquinone	2.7134	328.408	4	0
6	Lophenol	7.6347	400.691	1	1
7	Isoquinoline	2.2348	129.162	1	0
8	Taraxerol	8.1689	426.729	1	1
9	Beta Amyrin	8.1689	426.729	1	1
10	Butyrospermol	8.335	426.729	1	1
11	Cycloartenol	8.1689	426.729	1	1
12	Alphahederin	3.5211	750.967	11	7
13	Alphapinene	2.9987	136.238	0	0
14	Alphathujene	2.9987	136.238	0	0
15	Betapinene	2.9987	136.238	0	0
16	Borylacetate	3.2818	322.984	3	0
17	Carvacol	2.82402	150.221	1	1
18	Carvone	2.4879	150.221	1	0
19	Gamma Terpinene	3.3089	136.238	0	0
20	Myrcrene	3.475	136.238	0	0
21	Longifolene	4.415	204.357	0	0
22	Nigellicine	1.55502	246.266	4	1
23	Limonene	3.3089	136.238	0	0
24	Nigellidine	3.22942	294.354	4	1
25	P-Cymene	3.11842	134.222	0	0
26	Sabinene	2.9987	136.238	0	0
27	Thymohydroquinone	2.52962	166.22	2	2
28	Thymoquinone	1.6669	164.204	2	0
29	Gramisterol	7.8008	412.702	1	1

The obtained results shows molecular weight, logP values, hydrogen bond acceptor and donar values of ligands of Nigella sativa. If any compound follow these rules then it can be used as active drug in humans. All the ligands except Alphahederin follows rules. Alphahederin has more hydrogen bond donar as well as hydrogen bond acceptor.

4.9 Lead Compound Identification

Physicochemical and Pharmacokinetics properties determine the final destiny of compounds as drug or non-drug compounds. Physicochemical properties or Lipinski's rule of five works as primary filter and Pharmacokinetics studies as secondary filter in screening of potential compounds. Thymohydroquinone dithymoquinone, dithymohydroquinone, nigecilline, nigedilline and alphahydrenine and alphahederin does not obey Lipinski's rule of five, so they knock out in primary screening while Cycloleucalenol, Tirucallol, Taraxerol,Beta Amyrin, Butyrospermol and Cycloartenol not totally comply with RO5 (log p >5). Pharmacokinetic studies of these compounds screen out cycloleucalenol, thymol, strychinine, tirucallol, lophenol, Isoquinoline, taraxerol, betaamyrin, butyrospermol, cycloartenol, alphapinene, alphathujene, betapinene, borylacetate, carvacol, carvone, gammaterpinene, mycrene, longifolene, p-cymene,sabinene, thymoquinone and gramisterol (log BB > 0.3) Tirucallol, Dithymoquinone, Tirucallol, Alphahederin, Alphapinene, Carvone, Gamma Terpinene,Limonene and Thymoquinone (log BB >0.3 & log PS >-2) Alphahederin, Alphapinene, Carvone, Gamma Terpinene, Nigellicine, Limonene, Nigellidine and Thymoquinone (log PS > - 2) [102].

Table 4.10: Toxicity Prediction of Reference Drug

Ligand	**Max. Tolerated Dose (Human) (Mg/Kg)**	**Herg I Inhibitor**	**Herg II Inhibitor**	**Oral Rat Acute Toxicity (Mol/Kg)**	**Oral Rat Chronic Toxicity (Mol/Kg)**	**Hepatoxicity**	**Skin Sensitisation**	**T. Pyriformis Toxicity (Log Ug/L)**	**Minnow Toxicity (Log Mm)**
Peramivir	0.462	No	No	2.486	2.872	No	No	0.285	2.96

4.10 Peramivir

After docking and physiochemical properties analyses, Peramivir was selected as standard for comparison with lead compound.

It exhibits best binding interactions and minimized score among all selected drugs. Peramivir is widely used in the clinical practice as antiviral drug and proves itself as highly effective.

4.11 Drug ADMET Properties

ADMET properties (Absorption, Distribution, Metabolism, Excretion & Toxicity) of FDA approved antiviral drugs were explored by pkCSM online prediction tool.

4.11.1 Toxicity Prediction of Reference Drug

Table 4.12 shows the Toxicity Properties of Azithromycin. Toxicity parameters value of Peramivir shows that this drug is not toxic towards liver but other parameters are in the range of positive values.

Which indicates that peramivir cannot cause any sensitivity to skin and it also is not a inhibitor of hERG I and hERG II. The dose value of 0.462 is also tolerable. With that a no to AMES toxicity indicates that it is not carcinogenic.

4.11.2 Absorption Properties

As clear from table, Peramivir is less soluble in water and has 26.801% absorption in small intestine of human. Skin permeability is low and shows positive result as Pgp- substrate, and not a PdpI/II inhibitor. Its means standard drug has low oral absorption. Pgp-I/II inhibitor ‘no ’ means Peramivir has increased pumping activity to pump out xenobiotics from cell and have low absorption.

4.11.3 Distribution Properties

Distribution properties consists of four models, among them first one is volume of distribution in human (VDss) expressed as log L/kg.

Peramivir shows low VDss which means less of the drug is distributed in tissue rather than plasma. Fu (fraction unbound) predicts the unbounded friction in plasma, if it is more than drug may be more effective. Our standard drug has 0.381 Fu predicted value.

Third model BBB permeability (blood brain barrier permeability) expressed as log BB shows value of -1.188 is more than -1 and considered as unsafe for brain. Last model named as CNS permeability (central nervous system permeability) expressed as log PS <-3 considered as safe while Peramivir shows logPS = -4.421.

Table 4.11: Distribution Properties of Reference Drug

	Name	**VDss (human)**	**Fraction unbound (human)**	**BBB permeability (human)**	**CNS permeability**
1	Peramivir	-0.028	0.381	-1.188	-4.421

4.11.4 Metabolic Properties

Drugs metabolic properties are given below in Table 4.14. Cytochrome. P450 is a detoxification enzyme present in liver and plays role in excretion of exogenous compounds by oxidizing them.

CYP2D6 & CYP3A4 are two main isoforms of cytochrome P450. First & second model result shows that Peramivir is not metabolized by cytochrome P450. Model no 3-7 shows that drug is not an inhibitor for these isoforms of cytochrome P450.

TABLE 4.12: Metabolic Properties of Reference Drug

	Ligand Name	CYP-2D6 Substrate	CYP-3A4 Substrate	CYP-2D6 Inhibitor	CYP-2C19 Inhibitor	CYP-2C9 Inhibitor	CYP-2D6 Inhibitor	CYP-3A4 Inhibitor
1	Peramivir	No	No	No	No	No	No	No

4.11.5 Excretion Properties

The predicted values of excretion of reference drug are given in Table 4.16. Total clearance expressed as log (CL tot) value is -0.106 ml/min/kg which indicates the hepatic and renal clearance of Peramivir. OCT2 is an organic cation transporter 2 that plays role in disposition and renal clearance of drugs. Peramivir predicts Renal OCT2 substrate 'No' which means it is not interfering in the functioning of OCT2 in the cell.

TABLE 4.13: Excretion Properties of Reference Drug

	Ligand Name	Total Clearance	Renal OCT2 Substrate
1	Peramivir	-0.106	No

4.12 Peramivir Mechanism of Action

Peramivir is a cytoplasmic analogue that binds the influenza virus neuraminidase active site competitively. Peramivir reduces the activity of influenza A and B strains neuraminidase. Neuraminidase influenza virus is the surface glycoprotein that catalyses the splitting of the bond between the sialic acid terminal and the sugar residues close to this terminal. This activity encourages many mechanisms to propagate the virus in the respiratory tract. Viral neuraminidase stimulates virus

release from infected cells; enhances the penetration into respiratory epithelial cells of the virus; suppresses respiratory mucus inactivation; causes cellular apoptosis by triggers beta growth factor transformation and generates cytokines, including interleukin-1 and tumor necrosis factor.

The function of the influenza neuraminidase enzyme is likewise interfered with by Peramivir and subsequently interferes with the disaggregation and release of the virals. Peramivir is little bioavailable by mouth and so is inhaled by mouth. Although the amount of Peramivir inhaled in the respiratory tract relies on patient drivers, for example aspiratory flow, 4 mg Peramivir is administered in vitro under settings approximating to in vivo inhalation. In vitro anti-viral activity depends on the test used and evaluated the virus strain.

4.13 Peramivir Docking

Peramivir as ligand was docked with drug targets by an online automatic docking tool that is CB dock. Best docking score was -4.4. Molecular docking interactions of docked drug with target are listed below in Table 4.16 .it has 7 hydrogen bond donar and 7 hydrogen bond acceptor.

Table 4.14: Docking of Peramivir

Name	**Binding Score**	**Cavity Size**	**Grid Map**	**HBD**	**HBA**	**Molecular Weight g/mol**	**Log P**
Peramivir	-6.3	1385	20	7	7	328.41	-0.3491

4.14 Peramivir and Antiviral Agent Comparison

The standard drug and lead compound was compared for their physiochemical and pharmacokinetic properties to assess their bioavailability, drug likeness, efficacy

and safety. Both of these compounds passed the drug likeness criteria (Lipinski's rule of five).

Peramivir Comparison with Lead Compound. However, thymol has less molecular weight and more log P value than Peramivir and follows lipiski's rule of five.

TABLE 4.15: Compares Both Lead Compounds

S. No.	**Name of compound**	**Log P value**	**Molecular weight**	**H-bond donor**	**H-bond acceptor**
1	Peramivir	-0.3491	328.41	7	7
2	Thymol	2.824	150.2	1	1

4.15 ADMET Properties Comparison

The ADMET properties comparison is done to check the absorption, distribution, metabolic excretion, and toxicity properties of the drug and the lead compound for finding a better drug candidate.

4.15.1 Toxicity Comparison

The toxicity of both the standard drug and lead compound is based upon 9 models. Model 1 of AMES toxicity shows that both the standard and lead compounds are not mutagenic. Model 2 of Maximum tolerated dose gives that if the value is equal or less to 0.477 log mg/kg/day then it is considered low and greater values are considered high.

The table below shows that Peramivir has a low value of tolerated dose. 3rd model is of hERG I and II inhibitors both these compounds are not inhibitors of hERG I but Thymol is not hERG II inhibitor.

4th model of oral rat acute toxicity is used to assess the relative toxicity. Model 5 of oral rat chronic toxicity gives the values of the lowest dose that could result

in an adverse effect. Model 6 of hepatotoxicity shows either the drug can cause damage to the liver. The table shows that both are hepatotoxic.

For the dermal products model, 7 is used for checking the sensitivity towards the skin. Both the standard and lead compounds are not sensitive to skin. Model 8 uses T. Pyriformis and model 9 uses minnows to check the toxicity.

For T. Pyriformis value>-0.5 is considered toxic according to which both are non-toxic and for minnow toxicity values below 0.5mM are considered toxic and here Thymol is toxic.

TABLE 4.16: Comparative Values of Toxicity of Peramivir and Thymol

Ligand	**Max. Tolerated Dose (Human) (Mg/Kg)**	**Her-g I Inhibitor**	**Her-g II Inhibitor**	**Oral Rat Acute Toxicity (Mol/Kg)**	**Oral Rat Chronic Toxicity (Mol/Kg)**	**Hepatoxicity**	**Skin Sensitisation**	**T. Pyriformis Toxicity (Log Ug/L)**	**Minnow Toxicity (Log Mm)**
Peramivir	0.462	No	No	2.486	2.872	No	No	0.285	2.96
Thymol	-0.197	No	Yes	2.894	1.811	No	No	0.289	-0.763

4.15.2 Absorption Properties Comparison

The parameter of absorption is based upon 6 models. The water solubility model gives the value of solubility of the compound in water when at 25℃. The model of CaCO2 solubility is used to predict the drug absorption when given orally. Values greater than 0.90 are considered to have high intestinal absorption, which means Thymol is absorbed more than peramivir. Value of Intestinal absorption model

less than 30% is considered to be poorly absorbed as in the case of peramivir and show that Thymol has high intestinal absorption.

For the transdermal drugs the skin permeability model, value less than log Kp > -2.5 is considered low, according to this both the compounds pass the skin permeability test.

The Pglycoprotein substrate model is very important as P-glycoprotein is an ABC transporter and acts as a biological barrier. Both Thymol and peramivir act as the substrates. The last model of P-glycoprotein inhibitors shows that whether the compound is an inhibitor or not.

The table 4.20 shows that Thymol is an inhibitor of P-glycoprotein I and II whereas peramivir is non inhibitor of P-glycoprotein I and II.

TABLE 4.17: Comparative Values of Absorption Properties of Peramivir and Thymol

	Ligands	**Water Solubility**	**Caco2 Permeability**	**Intestinal Absorption**	**Skin Permeability**	**P-glucoprotein Substrate**	**P-glucoprotein I inhibitor**	**P-glucoprotein II inhibitor**
1	Peramivir	-2.892	-0.488	26.801	-2.735	Yes	No	No
2	Thymol	-4.418	1.08	94.524	-2.75	Yes	Yes	Yes

4.15.3 Metabolic Properties Comparison

Cytochrome P450 is found in the liver mainly and is held responsible for oxidizing the xenobiotic so that they can be excreted easily out from the body hence making cytochrome P450 a detoxification enzyme. Some drugs are activated by it or some are deactivated.

So it is important to assess that whether a compound is a P450 substrate or not, and whether it is an inhibitor to P450 or not.

The table 4.21 shows that Thymol is a CYP2D6 and CYP3A4 substrate and peramivir is not substrate nor any inhibitor. While Thymol is CYP2D6 and CYP3A4 inhibitor.

TABLE 4.18: Comparative Values of Metabolic Properties of Peramivir and Thymol

	Ligand Name	**CYP-2D6 Substrate**	**CYP-3A4 Substrate**	**CYP-2D6 Inhibitor**	**CYP-2C19 Inhibitor**	**CYP-2C9 Inhibitor**	**CYP-2D6 Inhibitor**	**CYP-3A4 Inhibitor**
1	Peramivir	No	No	No	No	No	No	No
2	Thymol	Yes	Yes	No	No	No	Yes	Yes

4.15.4 Distribution Properties Comparison

Table 4.21 shows the comparative distribution properties of Peramivir and Thymol.The distribution parameter is based upon 4 models. The volume of distribution(VDss) is the uniform distribution of the drug in the blood .Both Peramivir and Thymol have a reasonable VDss value. 2nd model is based upon the fraction unbound of the drugs in the plasma as bounded drugs affect the efficiency of the drugs. The given value is the amount of the drug which remains unbounded. For BBB permeability if the value is greater than 0.3 logBB then that drug can easily cross the blood-brain barriers and if the value is less than -1 logBB then the drug is not or poorly distributed to the brain. From these values, it is clear that Peramivir has a low value hence it would be poorly distributed to the brain. Similarly, the model for CNS is based on the values that if the logPS $>$ -2 then that drug can easily penetrate to the CNS while those having value of logPS$<$ -3 will be unable to penetrate the central nervous system. Thymol has a low value hence it will not be able to pass the central nervous system.

TABLE 4.19: Distribution Properties of Peramivir and Thymol

	Name	VDss	Fractioning Bound	BBB Permeability	CNS Permeability
1	Peramivir	-0.028	0.381	-1.188	-4.421
2	Thymol	1.163	0	0.318	-1.036

4.15.5 Excretion Properties Comparison

The value of total clearance is a combination of hepatic and renal clearance and is important so that the dose rates of the drugs can be assessed. Thymol has more total clearance than peramivir. The 2nd model is of the Renal OCT2 (organic cation transporter 2) and this transporter helps in the renal clearance of drugs and other compounds. Being an OCT2 substrate can have an adverse effect in correlation with inhibitors. So both thymol and peramivir are not Renal OCT2 substrates. Table 4.22 shows the values of excretory properties of thymol and peramivir.

TABLE 4.20: Comparative Values of Excretion Properties of Peramivir and Thymol

	Ligand Name	Total Clearance	Renal OCT2 Substrate
1	Peramivir	-0.106	No
2	Thymol	0.211	No

4.16 Physiochemical Properties Comparison

For determining the fundamental properties of the compounds physiochemical properties are studied. Through this screening, it shows that peramivir has 15 carbon atoms, 28 hydrogen atoms, 4 nitrogen atoms, and 4 oxygen atoms whereas

thymol has 10 carbon atoms, 14 hydrogen atoms, and a oxygen atom. This shows that perimivir is a simple bio-compound in relevance to thymol, perimivir can donate 5 hydrogen atoms whereas thymol donate only one hydrogen atom. Although the Log P value of permivir is less than thymol the molecular weight of peramivir is greater than thymol.

TABLE 4.21: Comparison of Physiochemical Properties of Peramivir and Strychnine

	Name of Compound	**Log P Value**	**Molecular Weight**	**Molecular Formula**	**H-bond Donor**	**H-bond Acceptor**
1	Peramivir	-0.3491	328.41	C15H28N4O4	5	4
2	Thymol	2.82402	150.2	C10H14O	1	1

4.17 Docking Score Comparison

Both the standard and the lead compound were docked against the protein and the docking result gives us the best binding score. Table 4.24 shows that the lead compound which is Thymol has a much higher vina score than that of the standard drug which is peramivir. The binding score of peramivir is -4.4and that for Thymol is -4.2 which is higher than that of the standard drug. This result shown in Table 4.24 shows that strychinine can block the or bind with it more efficiently than that of peramivir.

TABLE 4.22: Docking Score Comparison

S. No.	**Name**	**Binding Score**
1	Peramivir	-4.4
2	Thymol	-4.2

Chapter 5

Conclusion and Future Prospects

The study was aimed to determine and identify natural anti-viral compounds from *Nigella sativa*, effective against nucleoprotein of virus.For this purpose, 29 ligands were selected to be docked. The structure of all the 29 ligands was easily available in PubChem and protein structure was also available in PDB. All the ligands were docked against the receptor protein via CB Dock. The results were visualized using PyMol and were analyzed through LigPlot. Out of those 29 ligands, five best scoring phytocompounds namely as thymol, strychinine, lophenol, isoquinoline and beta pinene were identified as hit compounds. Physicochemical and Pharmacokinetics properties determined the final destiny of compounds as drug or non-drug compounds.

Thymol belongs to class of organic compounds known as aromatic monoterpenoids. These are monoterpenoids containing at least one aromatic ring. It was predicted as lead compound by virtual screening results.

Lead compound thymol as per this research results can be explored further as a drug candidate for the treatment of viral infections and related chronic diseases like inflammation, cancer, microbial, hypertension, and neural diseases. All hit and lead compound can also be tested as food preservatives and additives because natural antioxidants prove themselves best preservatives and additives with more efficiency and less or no toxicity than synthetic ones. These potential antiviral

compounds of *Nigella sativa* can be tested for the pharmaceutical and medical industries.

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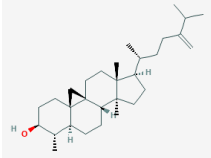
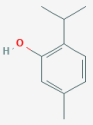
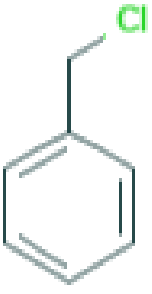
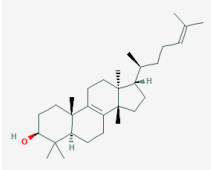
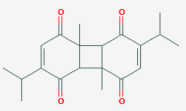
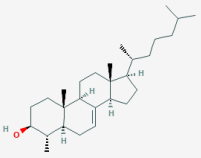
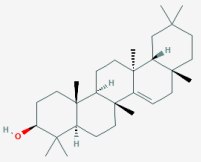
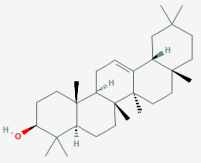
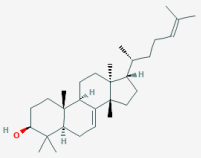
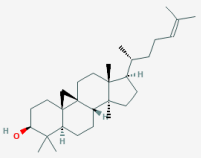
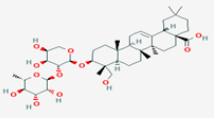
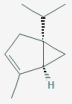
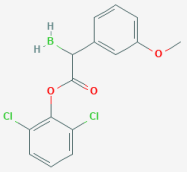
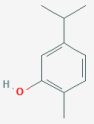
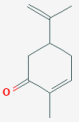
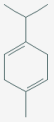
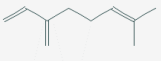
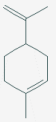
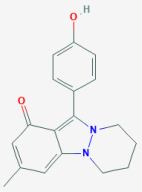
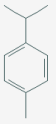
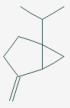
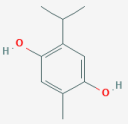
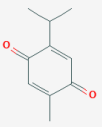
Appendix A

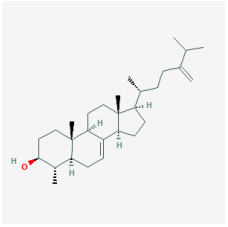
TABLE 1: Selected Ligands with Structural Information

S. No	Name	Molecular Formula	Weight (g/mol)	Molecular Farmula
1	Cycloleucalenol	C30H50O	426.7	
2	Thymol	C10H14O	150.22	
3	Strychinine	C28H29ClN2O2	461.0	
4	Tirucallol	C30H50O	426.7	

5	Dithymoquinone	C20H24O4	328.4	
6	Lophenol	C28H48O	400.7	
7	Isoquinoline	C9H7N	129.16	
8	Taraxerol	C30H50O	426.7	
9	Beta Amyrin	C30H50O	426.7	
10	Butyrospermol	C30H50O	426.7	
11	Cycloartenol	C30H50O	426.7	
12	Alphahederin	C41H66O12	751.0	

13	Alphapinene	C10H16	136.23	
14	Alphathujene	C10H16	136.23	
15	Betapinene	C10H16	136.23	
16	Borylacetate	C15H13BCl2O3	323.0	
17	Carvacol	C10H14O	150.22	
18	Carvone	C10H14O	150.22	
19	Gamma terpinene	C10H16	136.23	
20	Mycrene	C10H16	136.23	

21	Longifolene	C15H24	204.35	
22	Nigellicine	C13H14N2O3	246.26	
23	Limonene	C10H16	136.23	
24	Nigellidine	C18H18N2O2	294.3	
25	P-Cymene	C10H14	134.22	
26	Sabinene	C10H16	136.23	
27	Thymohydroquinone	C10H14O2	166.22	
28	Thymoquinone	C10H12O2	164.20	

29	Gramisterol	C29H48O	412.7	

Appendix B

Table 2: Properties of Compounds Obtained by Ligplus [88].

S. No.	**Ligand Name**	**No of HBs**	**Amino acids**	**Hydrogen bonding distance**	**Hydrophobic bonding**
1	Cycloleu-calenol	1	Arg13	3.15	Cys26
					Cys30
					Cys34
					Cys10
					Glu09
					Leu29
					Ala33
2	Thymol	2	Tyr20	2.78	Tyr16
			Asp19	2.85	Thr15
					Cys26
					Ala21
3	Strychinine	0			Tyr16
					Tyr20
					Ala21
					Cys26
					Thr15
					Asp19
4	Tirucallol	0			Leu35

					Leu07
					Ile27
					Asn11
					Arg24
					Tyr03
					Gln04
					Pro32
					Ala31
					Gly28
5	Dithymoquinone	0			Tyr20
					Tyr16
					Csp26
					Ala25
					Asp19
					Cys26
					Leu29
6	Lophenol	2	Thr15	3.02	Cys26
			Tyr16	2.72	Tyr20
					Ala21
					Ala25
					Leu29
					Asp19
7	Isoquinoline	2	Tyr16	3.09	Asp19
			Thr15	3.13	Cys26
					Tyr20
8	Taraxerol	1	Thr15	2.71	Ala25
					Cys26
					Ala21
					Tyr16
					Asp19
					Tyr20

					Leu29
9	Beta Amyrin	0			Pro18
					Thr15
					Ile17
					Tyr16
10	Butyrospermol	1	Asn11	2.92	Tyr03
					Gln04
					Leu35
					Leu07
					Ala31
					Gly28
					Pro32
					Ile27
11	Cycloartenol	0			Thr15
					Glu09
					Asp19
					Tyr20
					Tyr16
					Csp06
					Csp10
					Csp14
					Csp26
					Csp30
12	Alphahederin	5	Glu09	3.93	Alu33
			Arg02	3.56	Cys 06
			Ser37	3.05	Cys10
					Cys26
					Cys34
					Leu29
13	Alphapinene	0			Asn11
					Ile27

			Leu07
			Gly28
14	Alphathujene	0	Ala31
			Asn11
			Ile27
			Leu07
15	Betapinene	0	Thr15
			Ile17
			Tyr16
			Pro18
16	Borylacetate	0	Leu07
			Ala31
			Ile27
			Asn11
17	Carvacol	0	Leu07
			Ile27
			Asn11
			Ser08
18	Carvone	0	Leu07
			Leu35
			Gln04
			Tyr03
19	Gamma Terpinene	0	Gln04
			Leu07
			Tyr03
			Leu35
20	Myrcrene	0	Leu07
			Gln04
			Ile27
			Asn11

					Ser08
21	Longifolene	0			Leu07
					Gly28
					Ala31
					Ile27
					Asn11
22	Nigellicine	2	Asp01	2.72	Tyr03
			Gln04	2.9	Leu35
					Leu07
23	Limonene	0			Leu07
					Gln04
					Asp01
					Tyr03
					Leu35
24	Nigellidine	1	Leu07	3.08	Asp01
					Tyr03
					Gln04
					Ser08
25	P-Cymene	0			Leu07
					Gln04
					Tyr03
					Leu35
26	Sabinene	0			Leu07
					Ile27
					Asn11
					Ala31
					Gly28
27	Thymohydroquinone	0			Leu07
					Gln04
					Asp01
					Tyr03

						Leu35
28	Thymoquinone	0				Leu07
						Gln04
						Asp01
						Tyr03
						Leu35
29	Gramisterol	2	Arg13	2.94		Cys30
			Glu09	3.11		Thr15
						Asp19
						Tyr20
						Cys26
						Cys10

TABLE 3: Toxicity Properties of Ligands

Ligand	**Max. Tolerated Dose (Human) (Mg/Kg)**	**Herg I Inhibitor**	**Herg II Inhibitor**	**Oral Rat Acute Toxicity (Mol/Kg)**	**Oral Rat Chronic Toxicity (Mol/Kg)**	**Hepatoxicity**	**Skin Sensitisation**	**T. Pyriformis Toxicity (Log Ug/L)**	**Minnow Toxicity (Log Mm)**
Cycloleucalenol	-0.351	No	Yes	3.002	0.855	No	No	0.294	-1.714
Thymol	1.007	No	No	2.074	2.212	Yes	Yes	0.387	1.213
Strychinine	-0.197	No	Yes	2.894	1.811	No	No	0.289	-0.763

Tiruc-allol	-0.568	No	Yes	1.906	0.788	No	No	0.736	-1.757
Dithy-moqu-inone	0.534	No	No	1.649	1.53	No	No	0.445	1.323
Loph-enol	-0.695	No	Yes	2.411	0.892	No	No	0.42	-1.856
Isoqu-inoline	0.694	No	No	2.126	2.189	No	Yes	0.148	0.972
Tara-xerol	-0.623	No	Yes	2.386	0.808	No	No	0.359	-1.511
Beta Amy-rin	-0.56	No	Yes	2.478	0.873	No	No	0.383	-1.345
Buty-rosper-mol	-0.668	No	Yes	2.239	0.796	No	No	0.523	-1.824
Cyclo-artenol	-0.46	No	Yes	2.627	0.806	No	No	0.305	-1.928
Alpha-hederin	-0.105	No	No	2.963	1.766	No	No	0.285	1.925
Alpha-pinene	0.48	No	No	1.77	2.262	No	No	0.45	1.159
Alpha-thujene	0.353	No	No	1.589	2.243	No	No	0.597	0.995
Beta-pinene	0.371	No	No	1.673	2.28	No	No	0.628	1.012
Boryl-acetate	0.841	No	No	2.596	1.196	No	No	1.335	-0.214
Carv-acol	1.007	No	No	2.074	2.212	Yes	Yes	0.387	1.213

Carvone	0.775	No	No	1.86	1.972	No	Yes	0.41	1.445
Gamma Terpinene	0.756	No	No	1.766	2.394	No	No	0.627	0.906
Myrcrene	0.617	No	No	1.643	2.406	No	No	0.894	0.736
Longifolene	0.072	No	No	1.58	1.353	No	No	1.367	0.371
Nigellicine	0.283	No	No	2.265	1.17	Yes	No	0.237	1.776
Limonene	0.777	No	No	1.88	2.336	No	Yes	0.579	1.203
Nigellidine	-0.425	No	No	2.423	1.081	Yes	No	1.437	1.17
P-Cymene	0.903	No	No	1.827	2.328	No	Yes	0.462	0.869
Sabinene	0.369	No	No	1.549	2.309	No	No	0.788	0.726
Thymohydroquinone	0.441	No	No	2.367	1.96	Yes	Yes	0.839	1.252
Thymoquinone	0.89	No	No	1.743	2.378	Yes	Yes	0.138	1.758
Gramisterol	-0.6	No	Yes	2.395	0.818	No	No	0.426	-1.973