CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Study of Anticancerous Compounds of *Nigella sativa* Effective Against Liver Cancer

by

Zabinfat Ul Haq

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

in the

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Dedicated to ALLAH ALMIGHTY,Hazrat Muhammad (PBUH),my parents and my respected Teachers who have been a constant source of motivation and encouragement during challenges and supporting me spiritually throughout my life.

CERTIFICATE OF APPROVAL

Study of Anticancerous Compounds of *Nigella sativa* Effective Against Liver Cancer

by

Zabinfat Ul Haq

(MBS 203034)

THESIS EXAMINING COMMITTEE

S. No.	Examiner	Name	Organization
(a)	External Examiner	Dr. Marriam Shabbir	NUST, Islamabad
(b)	Internal Examiner	Dr. Sohail Ahmed Jan	CUST, Islamabad
(c)	Supervisor	Dr. Erum Dilshad	CUST, Islamabad

Dr. Erum Dilshad
Thesis Supervisor
December, 2022

Dr. Syeda Marriam Bakhtiar
Head
Deptt. of Bioinfo. and Biosciences
December, 2022

Dr. Sahar Fazal
Dean
Faculty of Health and Life Sciences
December, 2022

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Abstract

In the current situation of stress, toxic waste and era of radiations, humans are more susceptible to different types of diseases. Researchers are looking for therapeutic compounds which can treat or minimized the onset of disease. *N.sativa* belongs to family Ranunculaceae and it is popular medicinal plant in history. Metabolites of *N.sativa* seeds can present the therapeutically interesting activity for the immune, cardiovascular, endocrine and respiratory systems as well as they have anti oxidant, anti cancer and anti ulcer activity. Identifying natural, plant based and non toxic, anti cancerous drugs is necessary for the treatment of different types of cancers. In the current study Topoisomerase I and Topoisomerase II α were selected as target proteins and α-pinene, anethol, myristic acid, nigeglaine, nigellaquinomine, nigellicine, palmitic acid, pyrogallol, salfredin B11 and salicylic acid were selected as ligands.

Molecular Docking was used to estimate the bond strength between a ligand and target protein through a special scoring function and to determine the correct structure of the ligand within the binding site. Docking used the 3D structure of the target proteins in Pdb format and structure of ligands in Sdf format.

Best ligand was selected on the basis of best docking score, logP value, hydrogen bond acceptor, hydrogen bond donor and molecular weight. Salfredin B11 was identified as the lead compound which showed the best docking score, hydrogen bonding and pharmacokinetic properties as compared to other ligands. Sorafenib is an FDA approved drug for the treatment of liver cancer. The comparison between Sorafenib and Salfredin B11 can help us to identify the better treatment for liver cancer. Both of them were compared using parameters like; ADMET properties and physiochemical properties. According to comparison, it is concluded that Salfredin B11 is better in activity as well as safety as compared to Sorafenib.
This identified active compound of *N.sativa*; Salfredin B11 can be used as medicine in near future.To prove its rank as a drug it can be used on mice for experiments and after these successful experiments, it can be introduced in the clinical trials for its validation.

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Abbreviations

ADMET	Absorption, distribution, metabolism, excretion and toxicity
FDA	Food and Drug Administration
HCC	Hepatocellular carcinoma
MRTD	Maximum recommended tolerated dose
N. sativa	*Nigella sativa*
WHO	World Health Organization

Chapter 1

Introduction

Nigella sativa is a member of family Ranunculaceae and it is popular medicinal plant in history. It is described in different types of historical as well as religious books. The seeds of this plant are called "Kalonji" in southern Asia, commonly known as "habbat us sauda" in middle east and in English it is mostly known as "black cumin" [1, 2]. Metabolites of *N.sativa* seeds can present the therapeutically interesting activity for the immune, endocrine, cardiovascular and respiratory systems [3]. Many activities of *N.sativa* are considered due to the existence of thymoquinone in the essential oil of these seeds [4]. *N.sativa* seeds are mostly used in different food dishes as condiment due to their specific aroma and bitter taste like peppers [5]. *N.sativa* is a green plant having finely divided leaves. It has flower in different colours i.e. pale blue and white colour containing 5-10 petals. Fruit is in the form of capsule which is further divided into three follicles and each follicle contains black oval seeds [6].

Liver is the substantial gland in the human body and one of the vital organs that metabolizes the nutrients and functions for the excretion of metabolites [7]. The liver is the reddish organ having two lobes of unequal size and shape. A human liver normal weight is approximately 1.5 kilogram and about 15cm wide. There is considerable variation in size among individuals i.e. 970-1860 grams in men while 600-1770grams for women [8]. In humans, its position is in the right upper quadrant of the abdomen, under the diaphragm. It play role in metabolism for

regulation of glycogen storage, for red blood cells decomposition and for the production of hormones [9]. The liver is an associated digestive organ which produces bile, an alkaline fluid is composed of cholesterol and bile acids which helps in the breakage of fat.

The gallbladder, a small sac like structure that is placed just under the liver, stores the bile which is later on moves to the small intestine completing digestion [10]. Primarily it functions to control the flow and safety of compounds absorbed from the digestive system before sending them to the circulatory system [11].

In addition to the above mentioned functions liver also perform biliary flow, carbohydrate metabolism, lipid metabolism, breakdown and excretion of waste materials, lymph production and blood reservoir as it is an expandable organ and can manage to hold extra volume of blood in some exceptional cases [12]. If liver totally lose its function, one can die in few minutes showing the greater importance of the liver [13].

Cancer is the term used for the disease which comprises of uncontrolled division of cells and they may invade other body cells through blood or lymph. It has become the second major cause of death in the world and over all cancer prevalence has raised [14]. Cancer is the dangerous health problem affecting human beings. At the tissue level it is a variety disease and this variety is huge challenge for the specific diagnosis followed by the treatment [15, 16].

Highest cancer type percentages in men occur in prostate, lung, bronchus, colon, rectum and urinary bladder respectively. While in women highest cancer prevalence is in breast, lung, bronchus, colon, rectum, uterine corpus and thyroid respectively. This information shows that breast and prostate cancer are the major types of cancer found in women and men respectively [17]. In children highest percentage of cancer type is blood cancer, cancer related to brain and lymph nodes cancer respectively [18, 19].

The question What causes cancer? got the attention of people over the generations. There are different causes of cancer which develop different type of cancer. WHO organized an international symposium in 1950, where the participants were intrigued by the variations in the variety of cancer found in the world [20]. It was

come to know that people who travelled to other countries developed the type of cancer common to their country of stay rather than their native country. It showed that most types of cancer are caused by exposures to the environment instead of inherited genes [21].

Cancer is mainly caused by a series of mutations in the genes and these mutations alter the cell function. Chemical compounds are responsible for gene mutations and production of cancer cells. Smoking leads to lung cancer because it is devoid of many carcinogens [22].Environmental chemical substances having carcinogenic properties effect the nucleus and cytoplasm of the cells directly and indirectly leading to gene mutations [23–26]. Bacteria , viruses and radiations contribute for 7% of all type of cancers [27].Generally cancer break the cellular relations leading to dysfunction of vital genes. This disturbance effect the cell cycle which result in abnormal proliferation [28, 29]. Proto-oncogenes which are essential for normal functioning of cell become oncogenes during mutations and this is very dangerous for the cell [30]. If there is no tumor suppressor gene it will trigger uncontrolled cell division [31].

Hepatocellular Carcinoma is a fourth leading cause of death due to cancer worldwide [32]. Contribution of HCC for primary liver cancer is about 80 % [33]. Every year it is reported that more than 800,000 people have liver cancer and there is huge variation in HCC incidence rates in different geographic regions of the world [34, 35]. HCC has more effects on men as compared to women having highest incidence in the age group 45 to 65 years [36, 37]. Globocan 2018 reported that HCC is the fifth most common type of cancer in men and ninth most common type of cancer in women [34].

The main risk factors of HCC are hepatitis B and C virus (HBV & HCV), obesity, non alcoholic fatty liver disease (NAFLD), alcoholic fatty liver disease (AFLD) and diabetes. Some other factors which also contribute for the development of HCC include food contaminants i.e. aflatoxins, genetic factors, tobacco smoking and some environmental toxins that are carcinogen in nature [38–40]. Incidence of HCC is started by hepatic inflammation which leads to necrosis and regeneration of the hepatocytes [41, 42].

1.1 Problem Statement

Hepatocellular Carcinoma is a fourth leading cause of death among all types of cancers worldwide. Contribution of HCC for primary liver cancer is about 80%. Every year it is reported that more than 800,000 people have liver cancer. HCC is the fifth most common type of cancer in men and ninth most common type of cancer in women. Medicines used for HCC treatment have many side effects and they does not inhibit any proper molecular target. We want to identify some compounds of plant origin having least side effects and more efficacy. So in this study we used *Nigella sativa* for compound identification which can be used to treat hepatocellular carcinoma.

1.2 Aim and Objectives

The main purpose of this research study was to identify potential compounds using molecular docking of *Nigella sativa* to treat the hepatocellular carcinoma which is a serious threat to life.

Objectives of this study were;

- To find the potential compound of *Nigella sativa* having anti cancerous properties.
- To study the interaction between ligand and protein complex through molecular docking.

Chapter 2

Literature Review

2.1 *Nigella sativa*

N.sativa has used as spices as well as flavoring agent in different food preparations i.e. pickles, sauces, salad etc. It has long been used in Europe, Arabian countries and Africa as traditional remedy [43]. The earlier herbalist consider the *N.sativa* as "The herb from heaven" [44]. The Holy Prophet (PBUH) had described the remedial powers of the black seeds in His Hadith as"Hold on to use this black seed, as it has a remedy for every illness except death" [45]. Avicenna has recommended the use of black seeds to enhance the body energy in his book "The Canon of Medicine" [46]. The morphology of *Nigella sativa* plant and seeds is shown in Figure 2.1. Taxonomic classification of this plant is given in Table 2.1.

FIGURE 2.1: Morphology of *Nigella sativa* seeds and plant [47].

Table 2.1: Taxonomic classification of *Nigella sativa* [48]

Sr.No.	**Domain**	**Eukarya**
1	Kingdom	Plantae
2	Subkingdom	Tracheobionata (Vascular plant)
3	Division	Spermatophyta
4	Class	Magnoliophyta
5	Subclass	Magnolidae
6	Order	Ranunculales
7	Family	Ranunculaceae
8	Genus	Nigella
9	Species	sativa

2.2 Cultivation and Collection of *Nigella sativa*

N.sativa is cultivated all over the world and it is grown as annual herb in Pakistan and India. It is grown as the same way like wheat during winter season. Land from where crops of green grams, black grams or corn are harvested can be used for its cultivation. Before cultivation 2-3 times plouging is enough to get good yield and to stop the weeds growth in the field.

Light soils can be prepared more easily as compared to heavy soils as they required more plouging. Distance between the seeds should be 30cm and to avoid the delayed germination, there should not be deep sowing of seeds. 12-15 kg per hectare seed is required. 3-5 irrigations are enough for the crop at different stages till its harvesting. Crop of *Nigella sativa* matures in April and May. When fruit turns yellowish, crop is harvested and it should be done early in the morning because late harvesting may leads to the scattering of seeds. After harvesting process and drying, crop is threshed by proper thresher or by tractor. After this threshing step seeds should be stored in the proper bags [49].

According to WHO more than 3/4th communities of less resource countries depend on medicinal plants for their basic health care because they are unable to buy or do not have access to the allopathic medicines [50, 51]. Now a day with the development of optimum nutrition; a new interest is being developed to use plants as food as well as medicine [52, 53]. Recently there is increased use of plant derive medicines not of due to easy access but the belief that phyto medicines have less side effects as compared to the synthetic medicines [54]. It is reported that about 300,000 herbal species exist in the world but only 15% of these species are examined for their pharmacological activity [55]. *N.sativa* is considered as the much nutritious herb among all herbs around the world and different scientific researches are going on to validate its traditional claim of uses [56, 57].

2.3 Nutritional Value of *Nigella sativa*

Different researchers reported its nutritional values as 38.20% of fats, 31.94% of total carbohydrates, 7-94% of fiber and 20-85% of protein. Different amino acids found in *Nigella sativa* are aspartate, glutamate and arginine whereas methionine and cysteine are the minor and major amino acids respectively. It also contains significant levels of copper, iron, zinc, phosphorus, thiamin, niacin, calcium and folic acid [56, 57].

2.4 Chemical Composition

As this plant is widely used in food as well as medicine, it is extensively studied to analyze the phytochemicals found in it. *N.sativa* seeds composed of different chemicals i.e. fixed oil, alkaloid, saponin, essential oil and proteins. They contain 0.4-2.5% essential oils and 28-36% fixed oil. Main component of fixed oil is unsaturated fatty acids which includes arachidonic, linoleic, eicosadienoic and linolenic acid. Stearic, myristic and palmitic acid are the components of saturated fatty acid of fixed oil [58]. Essential oil of *N.sativa* seeds was investigated through gas

chromatography-gas spectrometry (GC-MS). There are many compounds in the oil but pharmacologically most active component found in volatile oil are dithymoquinone, thymoquinone, thymol and thymohydroquinone. Chemical structures of these compounds are shown in Figure 2.2 [58, 59].

Dithymoquinone Thymoquinone

Thymohydroquinone Thymol

FIGURE 2.2: Chemical structure of active components of *Nigella sativa*[48]

In addition to these active compounds some other chemical compounds are also found in the *Nigella sativa* seeds which have different active components in them performing some specific function in the body when used as a remedy. Summary of these compounds is described in Table 2.2.

TABLE 2.2: Chemical composition of *N. sativa* seeds including active components

Group	**Sub-group**	**Components**
Fixed oil (32-40 %) [60, 61]	Unsaturated fatty acids	Arachidonic, eicosadienoic linoleic, linolenic, oleic and almitoleic acid. Palmitic, stearic and myristic acid. Beta-sitosterol, cycloeucalenol, cycloartenol, sterol esters and sterol glucosides
Volatile oil(0.4-0.45%) [59, 62]	Saturated fatty acids	Thymoquinone, nigellone,thymol , carvacrol, dithymoquinone, thymohydroquinone,p-cymene d-limonene, α & β-pinene, d-citronellol, and 2-(2-methoxypropyl) -5-methyl-1,4-benzenediol
Alkaloids [63]	-	Nigellicine, nigellidine, nigellimine-N-oxide
Coumarins [63]	-	6-methoxy-coumarin, 7-oxy-coumarin, 7-hydroxy-coumarin
Saponins [64]	Triterpenes, Steroidal	Alpha-Hedrin, Steryl-glucosides, acetyl-steryl-glucoside
Minerals(1.79-3.74%) [61]	-	Calcium, phosphorous, potassium, sodium and iron
Carbohydrates (33.9%)	-	-
Fiber(5.5%), Water(6%) [65]		

2.5 Pharmacological Activities of *N.sativa*

There are so many pharmacological activities of *N.sativa* of which some are being discussed here.

2.5.1 Antioxidant Activity

Free radicals may be produced in many human diseases. *N.sativa* has antioxidant ability and to find the antioxidant compounds in the essential oil of *N.sativa*, it was tested. Essential oil along with other components of oil perform respectable radical scavenging property [66].

2.5.2 Anticancer Activity

Research shows that methanolic *Nigella sativa* seeds extract has strong cytotoxic effects on Elrich ascites carcinoma, sarcoma 180 and Daltons ascites lymphoma with least cytotoxic effects on normal lymphocytes [67]. Other research shows that alcoholic and aqueous extracts of *N.sativa* in pure form or along with hydrogen per oxide, as an oxidative stressor were found useful for inactivating MCF-7 breast cancer cells in vitro [68].

Hepatocellular carcinoma is a rapidly growing malignant disease and number of cases has rapidly raised in the previous decades globally. The cytotoxic action of *Nigella sativa* seeds was performed on human hepatoma HepG2 cell lines and then incubated for 24 hours with distinct quantities of the *N.sativa* extract [69]. Oral administration of thymoquinone was found useful to increase the actions of quinine reductase and glutathione transferase and acts as prophylactic source for the toxicity produced in chemical carcinogenesis and hepatic cancer [70].

2.5.3 Anti-ulcer Activity

Ulcer index induced by Aspirin can be reduced by the aqueous extract of *N. sativa* seeds by 36% [48]. Recent clinical studies support the use *Nigella sativa* for the eradication of Helicobactor pylori in non-ulcer dyspepsia patients [71].

2.5.4 Effects on Cardiovascular System

Nigella sativa is recommended alone or along with honey or garlic for treating hypertension. El-Tahir et al. [72] investigated the activity of volatile oil of *Nigella sativa* along its active compound; the thymoquinone on the heart of

anesthetized rats and arterial blood pressure.

2.5.5 Effects on Reproductive System

60 days administration of *N.sativa* seeds enhanced the sperm motility, sperm count in the testicular ducts and caudal epidydimides as well as increase in the weight of reproductive organs. Spermatogenesis increased at primary and secondary spermatocytes hence curing the male infertility [73, 74].

2.6 Liver Cancer

Most common primary liver cancer is called Hepatocellular carcinoma which is a main cause of cancerous deaths in the whole world. HCC is the ninth leading cause of cancerous death in the United States [75]. HCC incidence is more often in males as compared to females [76].

Incidence of liver cancer has raised from 1.6 per 100,000 individuals to 4.6 per 100,000 individuals among Alaskan, Indian and American natives followed by Hispanics, Whites and Black [77]. Globocan 2018 reported that HCC is the fifth most common type of cancer in men and ninth most common type of cancer in women [34]. The main risk factors of HCC are hepatitis B and C virus (HBV & HCV), obesity, nonalcoholic fatty liver disease (NAFLD), alcoholic fatty liver disease (AFLD) and diabetes. Some other factors which also contribute for the development of HCC include food contaminants i.e. aflatoxins, genetic factors, tobacco smoking and some environmental toxins that are carcinogen in nature [39].

2.7 Treatment of Liver Cancer

Liver cancer is a type of cancer that originates in the liver and is an aggressive tumor that usually occurs in the setting of cirrhosis and chronic liver disease. Despite improvement in its treatment, liver cancer remains one of the most challenging cancers to treat. Patients with early HCC, techniques that can provide

curative potential are surgery, local destructive therapies and liver transplantation. However, recurrence of HCC remains a major issue after curative treatment, extending an incidence of over 70% at 5 year [78]. Even in patients with early, small HCC (less than 3cm) receiving surgery, the five year survival rate is unsatisfactory (47% to 53%) [79].

Usually, HCC is often diagnosed at an advanced stage and curative therapies are unsuitable for many people with advanced stage. Moreover, traditional systemic chemotherapy shows less efficacy and little survival rate [80]. The approval of Sorafenib has shown some survival benefits in patients having advanced HCC and preserved function of liver highlighting an auspicious molecular targeted strategy for advanced hepatocellular carcinoma [81].

Now treatment of liver cancer is multidisciplinary, multi-model treatment options are usually chosen on an individualized basis, according to the complex interplay of tumor stage and the extent of underlying liver disease along with patient‘s overall general health. There are different recommendations for the management of liver cancer across the specialties and geographical area. Heterogeneity exists in management of liver cancer across the various guidelines from the Europe (European Association for the Study of the Liver-European Organization for Research and Treatment of Cancer EASLEORTC), Asia (consensus statement from the Asian Oncology Summit 2009 AOS) and the United States (National Comprehensive Cancer Network NCCN), [82]. Till now, proper molecular target for liver cancer can't be set as drug target.

Nanotechnology, which might be used to make or upgrade therapies that leads to improved results for carcinomas, can enhance the activity of least effective drugs in targeting & killing cancerous cells. This can be achieved by optimizing the size and surface properties of medications as well as using tissue specific homing devices to target sites that reduced the tendency of systemic toxicity and adverse effects [83]. Nanotechnology may change ongoing compound medication approaches and enhance permeability, confinement and pharmaco-kinetic figures and hence reduce the side effects [84, 85]. Nanoparticle approaches cater an auspicious future by treatment methods that couple the sovereign agents to improve

the drug effects [86, 87].

2.7.1 Treatment of Liver Cancer Through Chemotherapy

As the first line therapy for advanced HCC, oral administration of Sorafenib which is a multi-kinase inhibitor is recommended worldwide; supported by the results of many trials. [88]. This FDA approved compound overcome tumor angiogenesis, cellular division and cell proliferation by inhibiting MAP kinase cascade that causes apoptosis of cancerous cells. Sorafenib inhibit following proteins; serine-threonine kinase Raf-1, platelet derived growth factor receptor-β, c-KIT, FLT-3, VEGF receptor 2 & 3 and RET [89, 90]. FDA approved sorafenib in 2007 as a drug for treating hepatocellular carcinoma, yet the average survival time of patients expanded by only 3-5 months as compared to the placebo group. [81, 91]. Cancerous cells become resistant to the drug with extended administration of sorafenib, making it ineffective. Furthermore, sorafenib has adverse side effects in cancer patients. These side effects includes increased concentrations of serum lipase and amylase, hypertension, hemorrhage, leukopenia, lymphopenia, neuropathy, diarrhea, nausea, dyspnea and vomiting. Furthermore, 10% of the patients treated with sorafenib may develop cutaneous squamous cell carcinomas [92, 93]. A new treatment, transarterial chemoembolization plus sorafenib, is considered superior as compared to sorafenib alone [94].

Chapter 3

Materials and Methods

3.1 Selection of Disease

Hepatocellular Carcinoma is a fourth leading cause of death due to cancer worldwide [32]. Contribution of HCC for primary liver cancer is about 80%. Every year it is reported that more than 800,000 people have liver cancer and there is huge variation in HCC incidence rates in different geographic regions of the world [35]. Topoisomerase I and Topoisomerase II α enzymes are the main proteins playing major role in the proliferation of cancerous cells and for drug designing they provide a potential site for target [95].

3.2 Determination of Physiochemical Properties of Proteins

Chemical and physical properties of a protein has key role in the proteins function. ProtParam a tool of Expasy is used to determine theses properties of the proteins. Following physiochemical properties were studied; isoelectric point, number of amino acids present, molecular weight, instability index, grand average of

hydropathicity, number of positively charged residues (Lys+Arg) and negatively charged residues (Asp+Glu).

3.3 Cleaning of the Downloaded Protein

Extra constituents attached to the protein have to be removed after downloading protein structure. This was performed by using an open source system Pymol. Linear chain consisting of 1-306 amino acids was kept and referred as A chain. All other constituents were removed so further process can be done effectively [96].

3.4 Determination of Functional Domains of Target Proteins

To determine the functional domains of the target proteins, InterPro database was used. It can analyze the protein and provides protein information regarding functional sites, families and domains of the protein of interest [97].

3.5 Selection of Active Metabolic Ligands

Those active ligands were selected which have shown some anticancerous properties in the past. These active ligands were α-pinene, anethol, myristic acid, nigeglaine, nigellaquinomine, nigellicine, palmitic acid, pyrogallol, salfredin B11 and salicylic acid.

3.6 Ligand Preparation

3-dimensional structures of the selected ligands were downloaded using PubChem database which is running under National Centre of Biotechnology Information

(NCBI). It contains the information of chemical molecules which is stored with reference to their names, simple or 3-dimensional structure, their isomers, molecular formulas, canonical smiles and activities of molecules against the biological assays [97]. Structures of the ligands were searched from PubChem, downloaded and MM2 energy minimization was done using Chem3D ultra. When energy was minimized we selected the sdf format at the end to save the energy minimized structure of the ligand.

3.7 Molecular Docking

CB-dock (Cavity detection guided blind docking) was used to perform the molecular docking between the ligand and the target protein. CB-dock automatically finds the sites of docking. It is a method of protein and ligand docking which indicates about the sites of bonding, the size and the center is calculated. Docking was performed by adjusting the box size according to the ligand. The docking was performed through AutoDock Vina. As it is cavity binding focused docking so ratio of accuracy is higher [98]. To perform docking we uploaded 3D structure of ligand in sdf format and of target protein in pdb format. There were 5 different interaction poses as end result. Of these poses we selected the best pose on the basis of minimum vina score given in kJ/mol [99].

3.8 Visualization of Result via PyMol

Pymol has emerged as an efficient visualization molecular tool over the past few years. Its graphics and ability to visualize 3D structures is extraordinary [100]. The result of docking can be captured and somehow Pymol provides a plugin which have access to the result as well as make their visualization more clear so that docking result can be studied easily [101]. For whole process docking results were saved in pdb format and after visualization in the Pymol were also be saved in the pdb file format.

3.9 Analysis of Docked Complex via Ligplot

Once we got the docked complex with the minimum vina score, the next step was the analysis of the docked complex. This analysis was done by the software LigPlot. For the given pdb file format the schematic diagrams of the protein and ligand interactions were generated automatically. These interactions were modified by hydrogen bonds and through hydrophobic contacts. LigPlot provided the analysis of the hydrophobic and hydrogen bonding interactions. LigPlot plus generated the 2D representation of the protein-ligand complex [102].

3.10 Ligand ADMET Properties

As analysis had been done the next step was the study of pharmacokinetic and toxicity properties. The weak ligands of the drug were eliminated during preclinical ADMET. The remaining ligands were selected as potential drugs against the disease. Optimization of the ADMET which is absorption, distribution, metabolism, excretion and toxicity related to human body were done using the PkCSM online server [103].

3.11 Lead Compound Identification

Out of these potential ligands, lead compound was identified on the basis of Lipinski's rule of five and pharmocokinetic properties.

3.12 Comparison with the Standard Drug

Sorafenib is the drug which has shown anticancerous properties - used in chemotherapy for liver cancer, was selected as a standard drug for comparison with the anticancerous compound of *N.sativa*.

3.13 Drug Proposed Against Liver Cancer

Though much work has been done in making and the use of drugs against Liver cancer but still there is difficulty in the treatment and cure of this disease. The active compound derived from *Nigella sativa*, may be used for the liver cancer treatment.

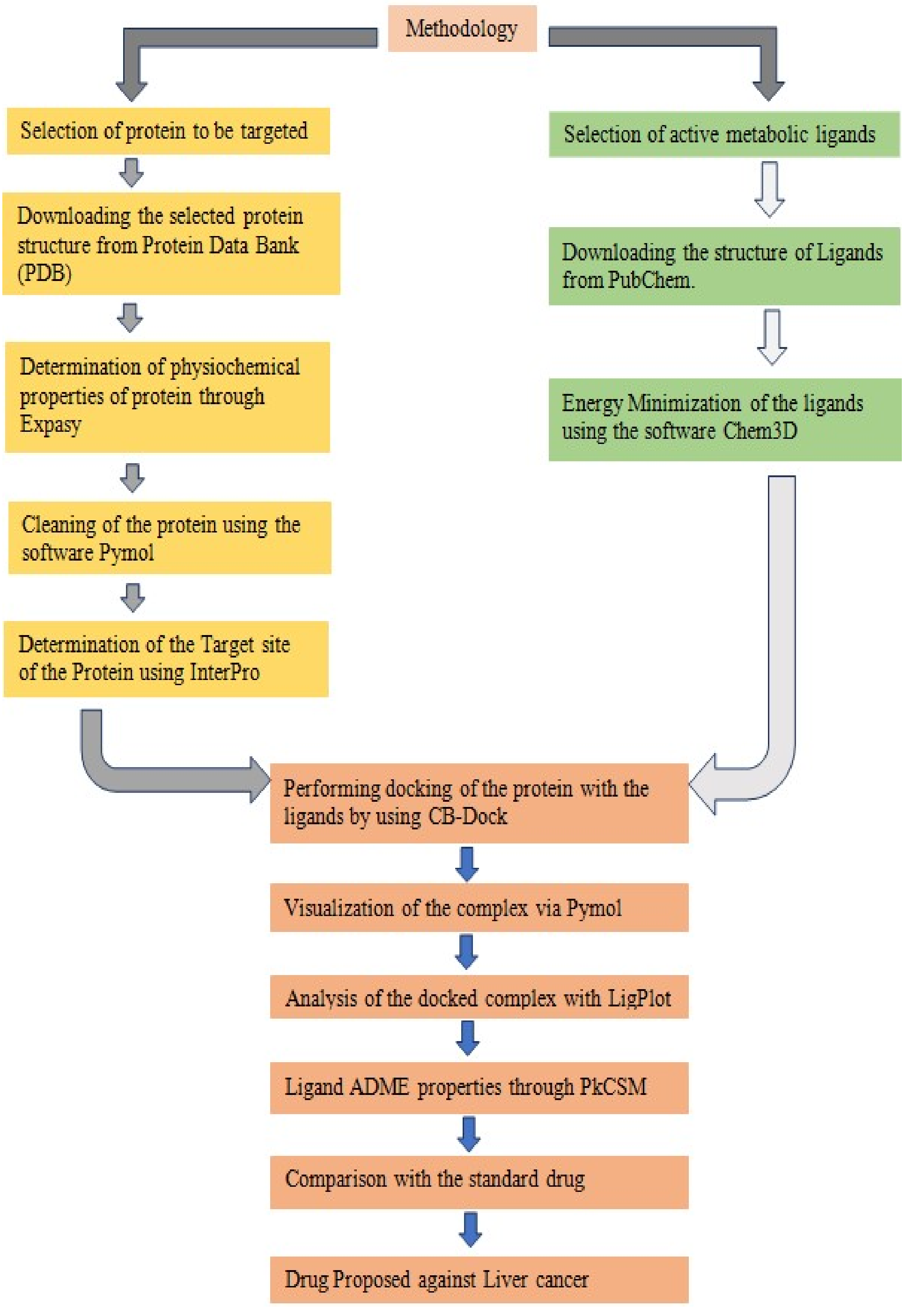

FIGURE 3.1: Flow chart of Methodology

Chapter 4

Results And Discussion

4.1 Structure Modeling

4.1.1 Primary Sequence Retrieval

Primary sequence of target proteins (Topoisomerase I and Topoisomerase II α) were taken in FASTA format from UniProt database (http://www.uniprot.org) under accession number P11387, P11388 and 764, 1531 residues length.

>sp P11387 TOP1_HUMAN DNA topoisomerase 1 OS=Homo sapiens OX=9606 GN=TOP1 PE=1 SV=2

```
MSGDHLHNDSQIEADFRLNDSHKHKDKHKDREHRHKEHKKEKDREKSKHSNSEHKDSEKK
HKEKEKTKHKDGSSEKHKDKHKDRDKEKRKEEKVRASGDAKIKKEKENGFSSPPQIKDEP
EDDGYFVPPKEDIKPLKRPRDEDDADYKPKKIKTEDTKKEKKRKLEEEEDGKLKKPKNKD
KDKKVPEPDNKKKKPKKEEEQKWKWWEEERYPEGIKWKFLEHKGPVFAPPYEPLPENVKF
YYDGKVMKLSPKAEEVATFFAKMLDHEYTTKEIFRKNFFKDWRKEMTNEEKNIITNLSKC
DFTQMSQYFKAQTEARKQMSKEEKLKIKEENEKLLKEYGFCIMDNHKERIANFKIEPPGL
FRGRGNHPKMGMLKRRIMPEDIIINCSKDAKVPSPPPGHKWKEVRHDNKVTWLVSWTENI
QGSIKYIMLNPSSRIKGEKDWQKYETARRLKKCVDKIRNQYREDWKSKEMKVRQRAVALY
FIDKLALRAGNEKEEGETADTVGCCSLRVEHINLHPELDGQEYVVEFDFLGKDSIRYYNK
VPVEKRVFKNLQLFMENKQPEDDLFDRLNTGILNKHLQDLMEGLTAKVFRTYNASITLQQ
QLKELTAPDENIPAKILSYNRANRAVAILCNHQRAPPKTFEKSMMNLQTKIDAKKEQLAD
ARRDLKSAKADAKVMKDAKTKKVVESKKKAVQRLEEQLMKLEVQATDREENKQIALGTSK
LNYLDPRITVAWCKKWGVPIEKIYNKTQREKFAWAIDMADEDYEF
```

>sp|P11388|TOP2A_HUMAN DNA topoisomerase 2-alpha OS=Homo sapiens OX=9606 GN=TOP2A PE=1 SV=3

```
MEVSPLQPVNENMQVNKIKKNEDAKKRLSVERIYQKKTQLEHILLRPDTYIGSVELVTQQ
MWVYDEDVGINYREVTFVPGLYKIFDEILVNAADNKQRDPKMSCIRVTIDPENNLISIWN
NGKGIPVVEHKVEKMYVPALIFGQLLTSSNYDDDEKKVTGGRNGYGAKLCNIFSTKFTVE
TASREYKKMFKQTWMDNMGRAGEMELKPFNGEDYTCITFQPDLSKFKMQSLDKDIVALMV
RRAYDIAGSTKDVKVFLNGNKLPVKGFRSYVDMYLKDKLDETGNSLKVIHEQVNHRWEVC
LTMSEKGFQQISFVNSIATSKGGRHVDYVADQIVTKLVDVVKKKNKGGVAVKAHQVKNHM
WIFVNALIENPTFDSQTKENMTLQPKSFGSTCQLSEKFIKAAIGCGIVESILNWVKFKAQ
VQLNKKCSAVKHNRIKGIPKLDDANDAGGRNSTECTLILTEGDSAKTLAVSGLGVVGRDK
YGVFPLRGKILNVREASHKQIMENAEINNIIKIVGLQYKKNYEDEDSLKTLRYGKIMIMT
DQDQDGSHIKGLLINFIHHNWPSLLRHRFLEEFITPIVKVSKNKQEMAFYSLPEFEEWKS
STPNHKKWKVKYYKGLGTSTSKEAKEYFADMKRHRIQFKYSGPEDDAAISLAFSKKQIDD
RKEWLTNFMEDRRQRKLLGLPEDYLYGQTTTYLTYNDFINKELILFSNSDNERSIPSMVD
GLKPGQRKVLFTCFKRNDKREVKVAQLAGSVAEMSSYHHGEMSLMMTIINLAQNFVGSNN
LNLLQPIGQFGTRLHGGKDSASPRYIFTMLSSLARLLFPPKDDHTLKFLYDDNQRVEPEW
YIPIIPMVLINGAEGIGTGWSCKIPNFDVREIVNNIRRLMDGEEPLPMLPSYKNFKGTIE
ELAPNQYVISGEVAILNSTTIEISELPVRTWTQTYKEQVLEPMLNGTEKTPPLITDYREY
HTDTTVKFVVKMTEEKLAEAERVGLHKVFKLQTSLTCNSMVLFDHVGCLKKYDTVLDILR
DFFELRLKYYGLRKEWLLGMLGAESAKLNNQARFILEKIDGKIIIENKPKKELIKVLIQR
GYDSDPVKAWKEAQQKVPDEEENEESDNEKETEKSDSVTDSGPTFNYLLDMPLWYLTKEK
KDELCRLRNEKEQELDTLKRKSPSDLWKEDLATFIEELEAVEAKEKQDEQVGLPGKGGKA
KGKKTQMAEVLPSPRGQRVIPRITIEMKAEAEKKNKKKIKNENTEGSPQEDGVELEGLKQ
RLEKKQKREPGTKTKKQTTLAFKPIKKGKKRNPWSDSESDRSSDESNFDVPPRETEPRRA
ATKTKFTMDLDSDEDFSDFDEKTDDEDFVPSDASPPKTKTSPKLSNKELKPQKSVVSDLE
ADDVKGSVPLSSSPPATHFPDETEITNPVPKKNVTVKKTAAKSQSSTSTTGAKKRAAPKG
TKRDPALNSGVSQKPDPAKTKNRRKRKPSTSDDSDSNFEKIVSKAVTSKKSKGESDDFHM
DFDSAVAPRAKSVRAKKPIKYLEESDEDDLF
```

Topoisomerase I and Topoisomerase II α were selected as the target proteins and α-pinene, anethol, myristic acid, nigeglaine, nigellaquinomine, nigellicine, palmitic acid, pyrogallol, salfredin B11 and salicylic acid were selected as ligands for the current study.

4.1.2 Physiochemical Characterization of Topoisomerase I and Topoisomerase II α

ProtParam is an online tool which let on the calculation of different physical and chemical properties for a given protein stored in Swiss-Prot or TrEMBL or for protein sequence entered by the user. The various parameters computed by ProtParam are molecular weight, theoretical PI, amino acid composition (positively and negatively charged), extinction coeffcient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY). The Calculated pI greater than 7 represents the basic nature of the protein while less than

7 represents the acidic nature of protein. Light absorption is represented by extinction coeffcient. Instability index if less than 40 indicates the stability of the protein while greater than 40 indicates the instability of protein. 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. Low GRAVY shows better interaction with water molecules. The Physiochemical properties of Topoisomerase 1 and Topoisomerase II alpha are shown in Table 4.1 and 4.2 respectively.

Table 4.1: Physiochemical Properties of Topoisomerase I

Sr.No.	**Parameters**	**Computed values**
1	Molecular weight	90594.53
2	Theoretical pI	9.33
3	Positively charged Residues	176
4	Negatively charged Residues	142
5	Extinction coefficient 1	103290
6	Extinction coefficient 2	102790
7	Estimated half-life	1.9 hrs (Mammals, in vitro) >20 hours (Yeast, in vivo) >10 hours (E. coli, in vivo)
8	Instability index	45.21
9	Aliphatic index	58.99
10	Grand average of hydropathicity (GRAVY)	-1.295

Topoisomerase I has theoretical pI greater than 7 which represents that it is basic in nature, Instability index is greater than 40 which shows instability of protein. It has low GRAVY values showing better interactions with water molecules.

TABLE 4.2: Physiochemical Properties of Topoisomerase II α

Sr.No.	Parameters	Computed values
1	Molecular weight	174385.11
2	Theoretical pI	8.82
3	Positively charged Residues	246
4	Negatively charged Residues	226
5	Extinction coefficient 1	163820
6	Extinction coefficient 2	163070
7	Estimated half-life	30hrs (Mammals, in vitro)
		20hrs (Yeast, in vivo)
		10hrs (E. coli, in vivo)
8	Instability index	40.29
9	Aliphatic index	75.62
10	Grand average of hydropathicity (GRAVY)	-0.695

As theoretical pI is greater than 7 which represents that Topoisomerase II α is basic in nature, Instability index is greater than 40 which shows that protein is unstable. It has low GRAVY values showing better interactions with water molecules.

4.1.3 Functional Domains Identication of Proteins

Active part of a protein is termed as the functional domain which is involved in the interaction of proteins with other compounds. Proteins can have more than one functional domain that shows different functions. Functional domains are identified using Interpro; an online server which uses FASTA format of protein as

input [104].

Functional domains of Topoisomerase I and Topoisomerase II α are shown in Figure 4.1 and 4.2 respectively. Topoisomerase I contains four functional domains i.e. First domain TopoI_DNA_bd_euk starting from residue number 215 and ends at residue number 429, second domain starting from residue number 432 and ends at 663 with name TopoI_cat_euk, third domain named TopoI_euk starts from residue number 360 and ends at 737. Fourth and last domain of Topoisomerase I is TopoI_C_dom starting from residue number 695 and ends at 765. These domains are enlisted in Table 4.3.

TABLE 4.3: Functional Domains of Topoisomerase I

Sr.No.	Name of protein	Functional Domains	Residues Length
1	Topoisomerase I	TopoI_DNA_bd_euk	215 To 429
2		TopoI_cat_euk	432 To 663
3		TopoI_euk	360 To 737
4		TopoI_C_dom	695 To 765

Topoisomerase II α has seven functional domains. First domain HATPase_C starting from residue number 79 and ends at 224, second domain Topo_IIA_bsu_dom starts from residue number 266 and ends at 426, third domain starting from residue number 455 and ends at 572 named TOPRIM_domain, fourth domain of this protein starts from residue number 455 and ends at 575 with name title TOPRIM_TopoII.

Fifth domain starting from residue number 573 and ends at 711 named TOPRIM_C, sixth domain Topo IIA dom A starts from residue number 693 and ends at 1174 and seventh domain DTHCT starts from residue number 1435 and ends at 1522. Functional domains of Topoisomerase II α are represented in Table 4.4.

TABLE 4.4: Functional Domains of Topoisomerase II α

Sr.No.	**Name of protein**	**Domain**	**Residues Length**
1	Topoisomerase II α	HATPase_C	79 To 224
2		Topo_IIA_bsu_dom	266 To 426
3		TOPRIM_domain	455 To 572
4		TOPRIM_TopoII	455 To 575
5		TOPRIM_C	573 To 711
6		Topo_IIA_dom_A	693 To 1174
7		DTHCT	1435 To 1522

Figure 4.1 and 4.2 are showing functional domains of Topoisomerase I and Topoisomerase II α respectively.

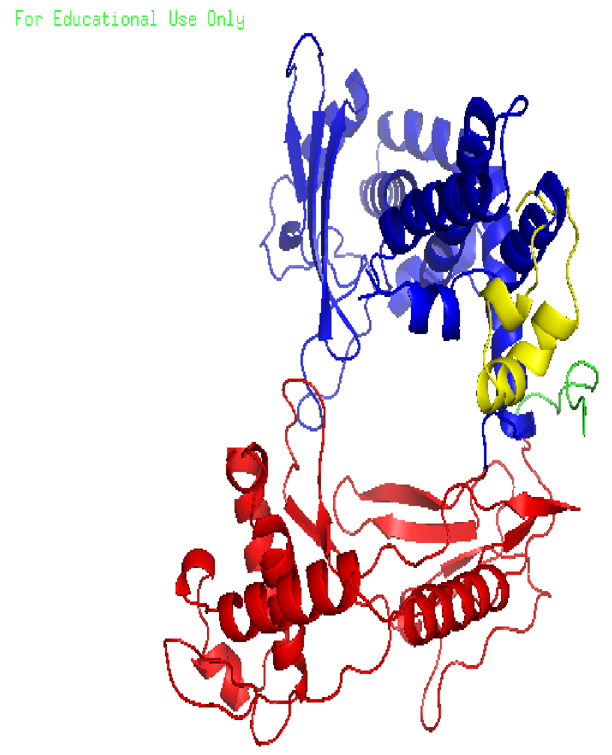

FIGURE 4.1: 3D structure of Topoisomerase I showing functional domains.

Topoisomerase I contains four functional domains i.e. First domain TopoI_DNA_bd_euk starting from residue number 215 and ends at residue number 429, second

domain starting from residue number 432 and ends at 663 with name TopoI_cat_euk, third domain named TopoI_euk starts from residue number 360 and ends at 737. Fourth and last domain of TopoisomeraseI is TopoI_C_dom starting from residue number 695 and ends at 765.

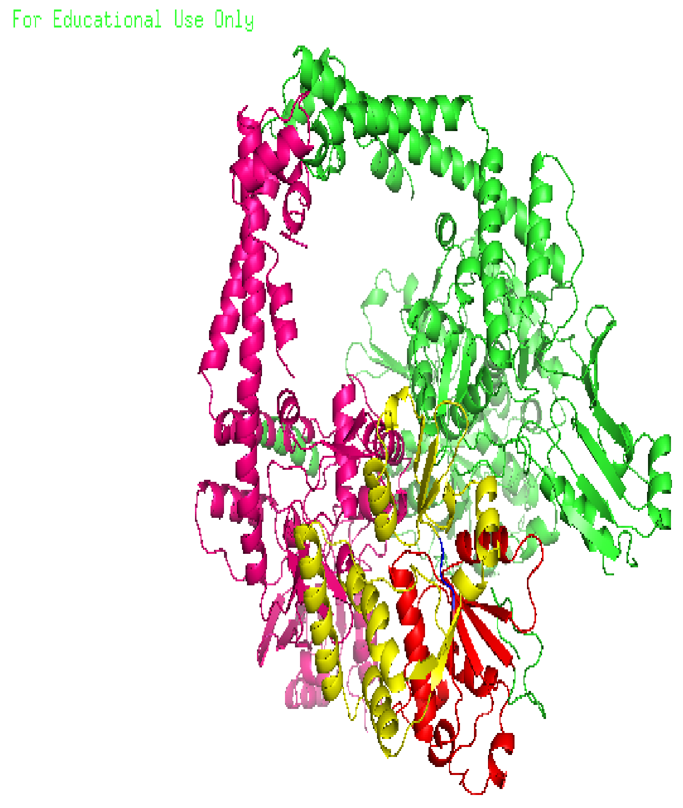

FIGURE 4.2: 3D structure of Topoisomerase II α showing functional domains.

Topoisomerase II α has seven functional domains. First domain HATPase_C starting from residue number 79 and ends at 224, second domain Topo_IIA_bsu_dom starts from residue number 266 and ends at 426, third domain starting from residue number 455 and ends at 572 named TOPRIM domain.

Fourth domain of this protein starts from residue number 455 and ends at 575 with name title TOPRIM TopoII, fifth domain starting from residue number 573 and ends at 711 named TOPRIM_C, sixth domain Topo_IIA_dom_A starts from residue number 693 and ends at 1174 and seventh domain DTHCT starts from residue number 1435 and ends at 1522.

4.1.4 Template Selection

When a list of possible templates was obtained by search method; it was important to select one or more templates that were particularly suitable for molecular docking. There are several factors which have to be kept in mind while selecting a template. The simplest rule used for template selection is to choose the structure that matches to the modeled sequence [105]. The structures of the selected templates are taken from the Protein Data Bank (PDB) and are listed in the Table 4.5.

TABLE 4.5: Selected PDB Template Structures

S.No.	Templates	Resolution	PDB ID	Structure
1	Crystal Structure of Human Topoisomerase I DNA Complex	2.60 Å	1EJ9	For Educational Use Only
2	Human Topoisomerase II α in complex with DNA and etoposide	3.15Å	5GWK	For Educational Use Only

These structures are representing the templates choosen for target proteins. Templates have ligands and water molecules attached to them and we had removed to refine the structure so that, this refined structure of protein can be used for molecular docking.

4.1.5 Structure of Proteins Refined for Docking

3D structure of proteins were refined by the use of pymol software. Refining was done by removing water molecules as well as other ligands attached to the proteins. Finally, refined structure of protein was obtained, which was used for Molecular Docking. Refined structures of Topoisomerase I and Topoisomerase II α are shown in Fig.4.3 and Fig.4.4 respectively.

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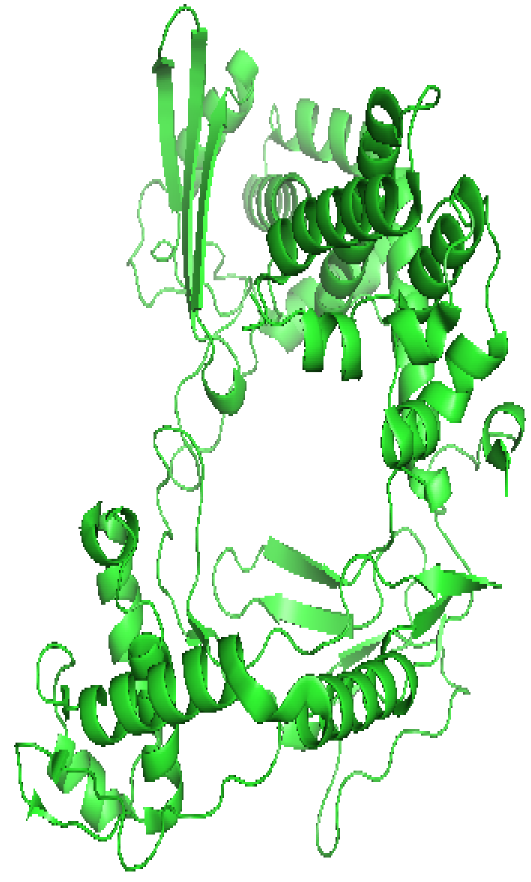

FIGURE 4.3: Refined structure of Topoisomerase I without any water molecule and extra ligands.

For Educational Use Only

FIGURE 4.4: Refined structure of Topoisomerase II α without any water molecule and extra ligands.

4.2 Ligand Selection

If we need to select a single protein-ligand complex for structure based drug designing, we need to look at the ligand present in the active site. There must be a good affinity between ligand and the receptor. Ligand should be able to adjust the function of proteins (interact with potential residues) and should be able to be used as a drug molecule or lead compound. On the basis of these characteristics we have selected best ligands. Bioactive compounds of *Nigella sativa* were selected as ligands for the present research and they are enlisted in Table 4.6.

TABLE 4.6: Detailed information about Selected Ligands

Sr.No	Name	Molecular formula	Molecular weight g/mol	Structure
1	α Pinene	$C_{10}H_{16}$	136.23	
2	Anethol	$C_{10}H_{12}O$	148.20	
3	Myristic acid	$C_{14}H_{28}O_2$	228.37	
4	Nigeglaine	$C_{12}H_{14}N_2O$	202.25	
5	Nigellaquinomine	$C_{19}H_{25}NO$	283.4	
6	Nigellicine	$C_{13}H_{14}N_2O_3$	246.26	
7	Palmitic acid	$C_{16}H_{32}O_2$	256.42	
8	Pyrogallol	$C_6H_6O_3$	126.11	
9	Salfredin B11	$C_{13}H_{12}O_4$	232.23	
10	Salicylic acid	$C_7H_6O_3$	138.12	

The selected ligands were α-pinene, anethol, myristic acid, nigeglaine, nigellaquino mine, nigellicine, palmitic acid, pyrogallol, salfredin B11, salicylic acid. PubChem

is a public repository for experimental data that identifies the biological activity of small molecules. The structure of ligands and other information related to ligands were obtained from PubChem database.

4.3 Virtual Screening and Toxicity Prediction

PkCSM is an online tool used for toxicity prediction of ligands. It was used to find out the (ADMET) properties of the ligands; absorption, distribution, metabolism, excretion and toxicity. In this study, the Lipinskis rule has employed for screening of ligands. Application of Lipinskis rule on ligands is mentioned in Table 4.7. All ligands followed Lipinskis rule of five.

TABLE 4.7: Application of Lipinskis Rule on Ligands

Sr. No.	Ligands	LogP Value	Molecular Weight	H-Bond Acceptor	H-Bond Donor
1	α Pinene	2.9987	136.23	0	0
2	Anethol	2.7283	148.20	1	0
3	Myristic acid	4.7721	228.37	1	1
4	Nigeglaine	1.85682	202.25	3	0
5	Nigellaquinomine	4.4277	283.4	2	0
6	Nigellicine	1.55502	246.26	4	1
7	Palmitic acid	5.5523	256.42	1	1
8	Pyrogallol	0.8034	126.11	3	3
9	Salfredin B11	2.2468	232.23	4	1
10	Salicylic acid	1.0904	138.12	2	2

According to Lipinski‘s rule, the number of Hydrogen bond donor must be less than 5, the maximum number of Hydrogen bond acceptors must be 10, the logp value must be limited to 5 and the molecular weight must be less than 500 g/mol.

4.3.1 Toxicity Prediction

PkCSM is an online tool which provides an integrated plat form for rapid evaluation of pharmacokinetics and toxicity properties of drugs. So this tool was used for the toxicity measurements of ligands against Topoisomerase I and Topoisomerase II α which were the target proteins in the present study. Mutagenic potential of a compound is being checked through AMES toxicity test using bacteria. If it show positive result, then ligand is mutagenic and can act as a carcinogenic compound

and vice versa [106]. The maximum recommended tolerated dose (MRTD) is a measure of toxic chemical limits on individuals. It is helpful for directing first recommended dose of the treatment in phase 1 clinical trials. MRTD is expressed in terms of logarithm (log mg/kg/day). For a given compound MRTD value is considered to be lower if it is less than or equal to 0.477 log (mg/kg/day) and higher if its value is greater than 0.477 log (mg/kg/day) [107].

Models hERG I and II inhibitors are used to determine the potential of a compound to cause the inhibition of potassium channels induced by the hERG (human ether-a-go-go gene). An inhibitor of these channels may develop chronic QT syndrome and sudden death. Many items of the pharmaceutical market had withdrawn due to the inhibitor of hERG channels [108].

Oral Rat Acute Toxicity (LD50) is the quantity of a compound that causes the death of 50% experimental rats. The LD50 (mol/kg) predicts toxicity of a probable compounds while Oral Rat Chronic Toxicity (LOAEL) identify the lowest dose of a compound with a significant adverse effect.It is expressed in log (mg/kg-bw/day). Exposure to low to moderate chemical dose for a long time is very important in medicine. Hepatotoxicity is the measure of liver damage due to drug and is a major safety concern for drug designing. Potential negative effect of skin care products are determined by skin sensitization. T. pyriformis is a protozoan whose toxin is often used as toxic end point (IGC50) which inhibits 50% growth. Predicted value of T.pyriformis> -0.5 log ug/L is considered toxic. The concentration of compound required to cause the death of 50% of Fathead Minnows (small bait fishes), termed as lethal concentration (LC50). Minnow toxicity values less than 0.5mM are considered to be high acute toxicity [109].

Insilico toxicology is the measure of toxicity assessment through computational approach to analyze or predict the toxicity of chemicals. Toxicity test predict the harmful effects of a compound on humans, animals or plants through single or multiple exposures. Many factors are involved in determining the toxicity of chemicals like dose, duration, frequency and ADME properties including absorption, distribution, metabolism, and excretion [110]. The Toxicity Values of ligands are described in Table 4.8

TABLE 4.8: The Toxicity Values of ligands

Sr. No.	Model Name	α-Pinene	Anethol	Myristic acid	Nigeg-laine	Nigellaqu-inomine	Nigellicine	Palmitic acid	Pyrog-allol	Salfredin B11	Salicylic acid
1	AMES toxicity	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
2	Max.tolerated dose (Human)	0.48	0.824	-0.559	0.19	-0.138	0.283	-0.708	-0.269	-0.051	0.61
3	hERG I inhibitor	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
4	hERG II inhibitor	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
5	Oral Rat Acute Toxicity	1.77	1.798	1.477	2.094	1.853	2.265	1.44	2.049	1.701	2.282
6	Oral Rat Chronic Toxicity	2.262	2.217	3.034	1.037	0.872	1.17	3.181	2.374	2.419	2.483
7	Hepatotoxicity	NO	NO	NO	NO	NO	YES	NO	NO	NO	NO
8	Skin sensitisation	NO	YES	YES	NO	NO	NO	YES	NO	NO	NO
9	*T.pyriformis* toxicity	0.45	0.807	0.978	1.016	1.456	0.237	0.84	0.127	0.494	0.263
10	Minnow toxicity	1.159	0.869	-0.601	1.432	0.488	1.776	-1.083	2.734	1.492	1.812

All ligands are non carcinogenic. α-pinene, Anethol and Salicylic acid show high maximum tolerated dose. All ligands are supporter of potassium channels. Except Nigellicine, all ligands are non hepatotoxic. Anethol, Myristic acid and Palmitic acid are skin sensitive and other show no skin sensitivity. Minnow toxicity values of myristic acid, nigellaquinomine and palmitic acid predicted them toxic.

4.4 Molecular Docking

Molecular Docking is technique used to estimate the bond strength between a ligand and a target protein through a special scoring function and used to determine the correct structure of the ligand within the target binding site. It also helps in the recognition of new small molecular compounds, revealing the essential properties, such as high interaction between binding with target protein having reasonable absorption, distribution, metabolism and excretion which help in the selection of lead compound for the target [111].

The 3D structure of the target proteins in Pdb format and the ligands in Sdf format was used as input for molecular docking. It represents a frequently used approach in structure-based drug designing. The docking was performed among Topoisomerase I, Topoisomerase II α and ligands which are α-pinene, anethol, myristic acid, nigeglaine, nigellaquinomine, nigellicine, palmitic acid, pyrogallol, salfredin B11 and salicylic acid. Ligands with best binding score values with Topoisomerase I, Topoisomerase II α are represented in Table 4.9 and 4.10 respectively.

To automatically predict binding modes without information about binding sites, used a user friendly blind docking web server called CB Dock, which predicted and estimated a binding site for a given protein and calculated centersand sizes with a novel rotation cavity detection method and performed docking with the popular docking program named Auto dock Vina [110]. CB Dock gave 5 best interacting confirmations for each ligand molecule. These confirmations were arranged based on binding affinity and then finest confirmation selection was made on the basis of highest affinity score of protein-ligand interaction.

TABLE 4.9: Ligands having Best Binding Score Values with Topoisomerase I

Sr. No.	**Parameters**	**α-Pinene**	**Anethol**	**Myristic acid**	**Nigeg-laine**	**Nigellaq-uinomine**	**Nigellicine**	**Palmitic acid**	**Pyrog-allol**	**Salfredin B11**	**Salicylic acid**
1	Binding score	-5.8	-5.9	-5.8	-6.1	-7.1	-7	-5.6	-6	-7.1	-6.6
2	Cavity size	963	1940	1940	256	1940	1940	1940	1940	1940	1940
3	HBD	0	0	1	0	0	1	1	3	1	2
4	HBA	0	1	1	3	2	4	1	3	4	2
5	logP	2.9987	2.7283	4.7721	1.85682	4.4277	1.55502	5.5523	0.8034	2.2468	1.0904
6	Molecular weight (g/mol)	136.23	148.20	228.37	202.25	283.4	246.26	256.42	126.11	232.23	138.12
7	Rotatable bonds	0	2	12	0	0	1	14	0	0	1
8	Grid map	24	33	33	39	33	33	33	33	33	33
9	Min.Energy Kcal/mol	0.1965	0.0005	0.3900	5.1561	-0.4090	11.5810	0.3901	-2.2322	2.9755	2.9688
10	Max.Energy Kcal/mol	38.7381	5.6305	5.9051	33.2906	25.5747	50.7663	7.1828	-15.3188	19.2704	5.6109

TABLE 4.10: Ligands having Best Binding Score Values with Topoisomerase II α

Sr. No.	Parameters	α-Pinene	Anethol	Myristic acid	Nigeg-laine	Nigellaq-uinomine	Nigellicine	Palmitic acid	Pyrog-allol	Salfredin B11	Salicylic acid
1	Binding score	-6.1	-6.1	-5.4	-6.8	-8.2	-7.2	-5	-5.6	-7.4	-6.5
2	Cavity size	1448	1448	1448	1448	45026	1448	45026	1448	1448	1448
3	HBD	0	0	1	0	0	1	1	3	1	2
4	HBA	0	1	1	3	2	4	1	3	4	2
5	logP	2.9987	2.7283	4.7721	1.85682	4.4277	1.55502	5.5523	0.8034	2.2468	1.0904
6	Molecular weight (g/mol)	136.23	148.20	228.37	202.25	283.4	246.26	256.42	126.11	232.23	138.12
7	Rotatable bonds	0	2	12	0	0	1	14	0	0	1
8	Grid map	25	25	24	25	35	25	35	25	25	25
9	Min.Energy Kcal/mol	0.1965	0.0005	0.3900	5.1561	-0.4090	11.5810	0.3901	-2.2322	2.9755	2.9688
10	Max.Energy Kcal/mol	38.7381	5.6305	5.9051	33.2906	25.5747	50.7663	7.1828	-15.3188	19.2704	5.6109

The Docked structures after docking process were selected for further analysis; on the basis of docking score,binding energy, cavity size and Grid map, we selected best docked structure.

Molecular docking was performed using Topoisomerase I, Topoisomerase II α as receptors and 15 selected compounds as ligands. Out of which only 10 ligands showed interactions with target proteins i.e. Topoisomerase I and Topoisomerase II α and these compounds were taken for further proceedings. Data of all these ligands was arranged after performing molecular docking. Docking result gave five confirmations based and arranged on the basis of binding score and we had selected the confirmation with minimum binding score. All these ligands are showing minimum binding score in their corresponding confirmations.

4.5 Ligands Interaction with Target Proteins

For interpretation of docked results, interaction of the active pockets of the ligands and proteins were calculated.Two types of interactions were studied to analyse the docking results; hydrogen bonding and hydrophobic interaction using Ligplot plus version v.1.4.5. Hydrogen bonding and hydrophobic interactions of active ligands are shown in Table 4.11 and 4.12.

4.5.1 Interactions of Ligands with Topoisomerase I

TABLE 4.11: Hydrogen and Hydrophobic Interactions of Topoisomerase I

Sr. No.	**Ligands**	**Binding Energy**	**No. of HBs**	**Hydrogen Bonding Amino acids**	**Hydrogen Bonding :Bond length**	**Hydrophobic Interactions Amino acids**
1	α-Pinene	-5.8	0	—-		Trp203, Glu209, Glu208, Glu438, Tyr211, Arg434 and Asp344
2	Anethol	-5.9	0	—-		Gly503, Cys504, Leu487, Gly531, Ile535, Cys630, Asn631, Arg590, Arg488,Phe529

Sr. No.	Ligands	Binding Energy	No. of HBs	Hydrogen Bonding Amino acids:Bond length	Hydrophobic Interactions Amino acids
3	Myristic acid	-5.8	1	Arg590:2.81	Ala486, Leu629, Cys630, Tyr537, Arg488, Lys532, Gln633, Asp533, His632,Ile535, Gly531,Asn631
4	Nigeglaine	-6.1	1	Phe240:2.95	Pro235, Glu254, Leu234, Val238, Lys239, Met247, Glu236,Lys248 and Val246
5	Nigellaquinomine	-7.1	0	—-	Val502, Ser506, Arg508, Asp500, Gly496, Thr498, Ala499, Glu494 and Lys493
6	Nigellicine	-7	6	Arg364:2.88, Lys493:2.94, Asp533:3.10 His367:2.95, Ala499:3.33, Ser534 :2.99 :3.11	Thr498, Gly363, Lys532, Thr501
7	Palmitic acid	-5.6	0	—-	Arg590, Cys630, Gly531, Tyr537, Lys532, Lys493, Asn491, Gly490, Gly503, Arg488, Phe529, Asn631 and Val502
8	Pyrogallol	-6	2	Arg590:2.94, Arg488:3.04	Cys630, Asn631, Tyr537, Phe529, Gly531, Ala486 and Leu487
9	Salfredin B11	-7.1	4	His367:3.20, Thr501:3.20, Ser534:2.73 Arg364 :2.85 :3.08	Phe361, Gln421, Asp533, Lys493, Ala499, Thr498
10	Salicylic acid	-6.6	3	Ile535:2.70, Arg488:2.91 :3.05 Cys630:2.71	Gly531, Asn631, Ala486, Arg590, Leu487,Phe529 and Tyr537

α-Pinene made 6 hydrophobic interactions with Topoisomerase I and no hydrogen bonding as illustrated in figure 4.5. Anethol made 10 hydrophobic interactions with Topoisomerase I and no hydrogen bonding as illustrated in figure 4.6. Myristic acid made 12 hydrophobic interactions with Topoisomerase I and 1 hydrogen bond with Arg590 residue as illustrated in figure 4.7. Nigeglaine made 09 hydrophobic interactions with Topoisomerase I and 1 hydrogen bond with Phe240 residue as illustrated in figure 4.8. Nigellaquinomine made 09 hydrophobic interactions with Topoisomerase I and no hydrogen bonding as shown in figure 4.9. Nigellicine made 04 hydrophobic interactions with Topoisomerase I and 06 hydrogen bonds with Arg364, His367, Lys493, Ala499, Asp533 and Ser534 residues as shown in figure 4.10. Palmitic acid made 13 hydrophobic interactions with Topoisomerase I and no hydrogen bonding as shown in figure 4.11. Pyrogallol made 07 hydrophobic interactions with Topoisomerase I and 02 hydrogen bonds with Arg590 and Arg488 residues as illustrated in figure 4.12. Salfredin B11 made 06 hydrophobic interactions with Topoisomerase I and 04 hydrogen bonds with His367, Arg364, Ser534 and Thr501 residues as illustrated in figure 4.13. Salicylic acid made 07 hydrophobic interactions with Topoisomerase I and 03 hydrogen bonds with Arg488, Cys630 and Ile535 residues as illustrated in figure 4.14.

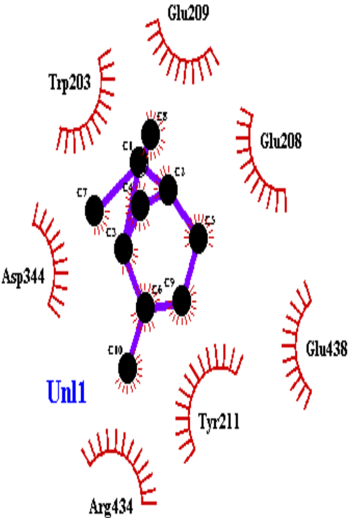

FIGURE 4.5: Interaction of α-Pinene with Topoisomerase I

α-Pinene made 07 hydrophobic interactions with Trp203, Glu209, Glu208, Glu438, Tyr211, Arg434 and Asp344 residues and no Hydrogen bond.

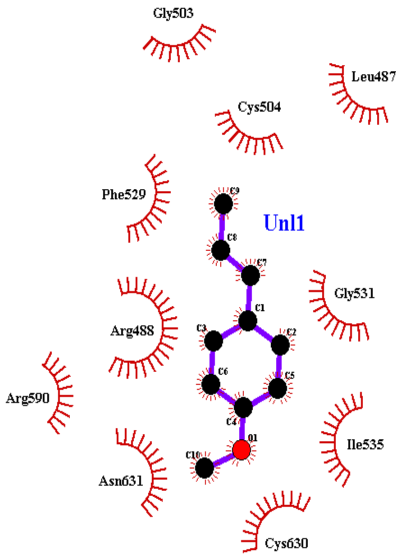

FIGURE 4.6: Interaction of Anethol with Topoisomerase I

Anethol made 10 hydrophobic interactions with Gly503, Cys504, Leu487, Gly531, Ile535, Cys630, Asn631, Arg590, Arg488 and Phe529 residues and no Hydrogen bond.

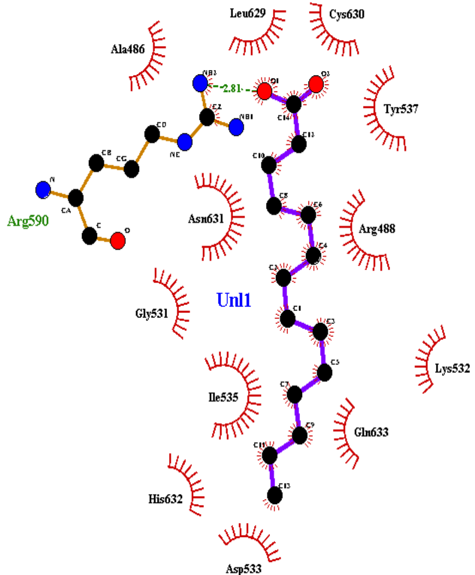

FIGURE 4.7: Interaction of Myristic acid with Topoisomerase I

Myristic acid made 12 hydrophobic interactions with Ala486, Leu629, Cys630, Tyr537, Arg488, Lys532, Gln633, Asp533, His632,Ile535, Gly531 and Asn631 residues and 1 Hydrogen bond with Arg590 residue having bond length of 2.81.

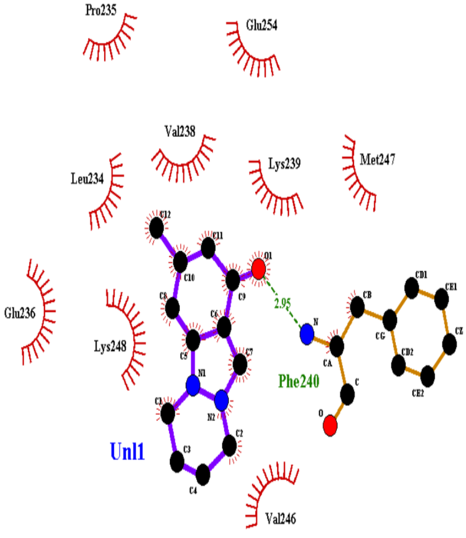

FIGURE 4.8: Interaction of Nigeglaine with Topoisomerase I

Nigeglaine made 09 hydrophobic interactions with Pro235, Glu254, Leu234, Val238, Lys239, Met247, Glu236,Lys248 and Val246 residues and 01 Hydrogen bond with Phe240 residue having bond length of 2.95.

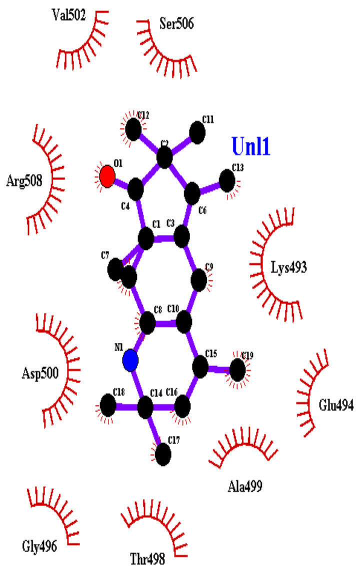

FIGURE 4.9: Interaction of Nigellaquinomine with Topoisomerase I

Nigellaquinomine made 09 hydrophobic interactions with Val502, Ser506, Arg508, Asp500, Gly496, Thr498, Ala499, Glu494 and Lys493 residues and no Hydrogen bond.

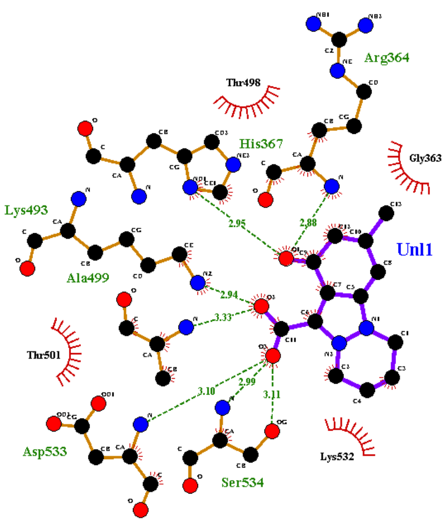

FIGURE 4.10: Interaction of Nigellicine with Topoisomerase I

Nigellicine made 04 hydrophobic interactions with Thr498, Gly363, Lys532 and Thr501 residues and 06 Hydrogen bonds with residues Arg364 having bond length of 2.88, His367 having bond length of 2.95, Lys493 having bond length of 2.94, Ala499 having bond length of 3.33, Asp533 having bond length of 3.10 and Ser534 having bond lengths of 2.99 and 3.11.

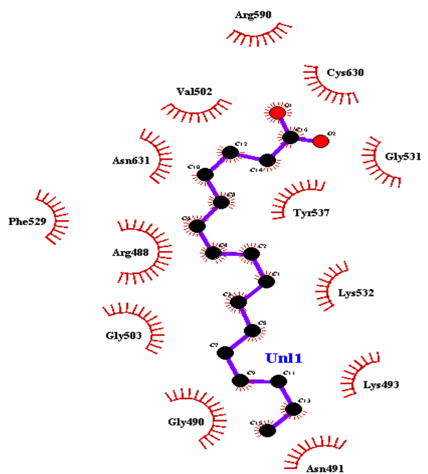

FIGURE 4.11: Interaction of Palmitic acid with Topoisomerase I

Palmitic acid made 13 hydrophobic interactions with Arg590, Cys630, Gly531, Tyr537, Lys532, Lys493, Asn491, Gly490, Gly503, Arg488, Phe529, Asn631 and Val502 residues and no Hydrogen bond.

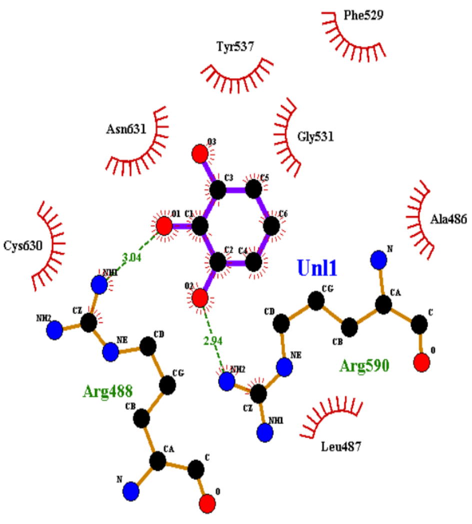

FIGURE 4.12: Interaction of Pyrogallol with Topoisomerase I

Pyrogallol made 07 hydrophobic interactions with Cys630, Asn631, Tyr537, Phe529, Gly531, Ala486 and Leu487 residues and 02 Hydrogen bonds with Arg590 having bond length of 2.94 and Arg488 having bond length of 3.04.

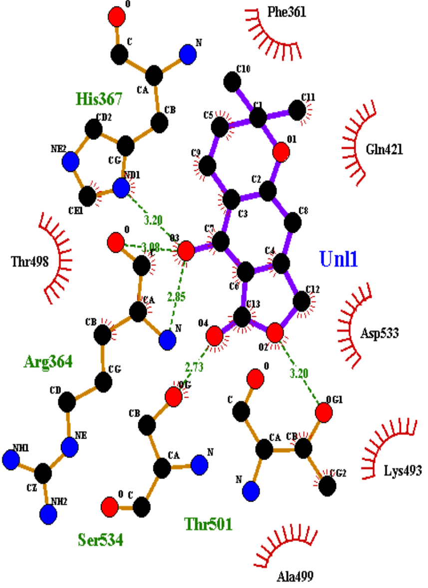

FIGURE 4.13: Interaction of Salfredin B11 with Topoisomerase I

Salfredin B11 made 06 hydrophobic interactions with Phe361, Gln421, Asp533, Lys493, Ala499 and Thr498 residues and 04 Hydrogen bonds with residues His367 having bond length of 3.20, Thr501 having bond length of 3.20, Ser534 having bond length of 2.73 and with Arg364 having bond lengths of 2.85 and 3.08.

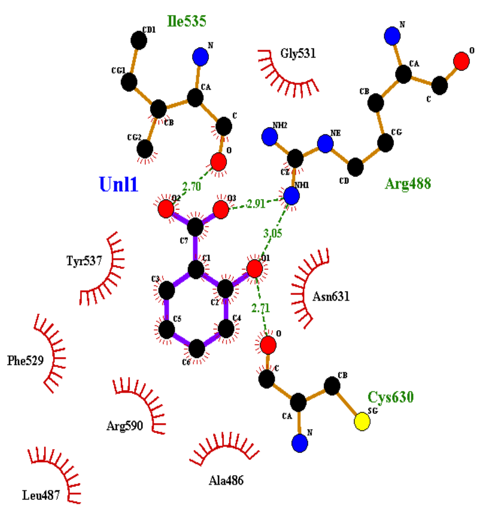

Figure 4.14: Interaction of Salicylic acid with Topoisomerase I

Salicylic acid made 07 hydrophobic interactions with Gly531, Asn631, Ala486, Arg590, Leu487,Phe529 and Tyr537 residues and 03 Hydrogen bonds with Ile535 having bond length of 2.70, Arg488 having bond lengths of 2.91 and 3.05 and with residue Cys630 having bond length of 2.71.

4.5.1 Interactions of Ligands with Topoisomerase II α

Table 4.12: Hydrogen and Hydrophobic Interactions of Topoisomerase II α

Sr. No.	Ligands	Binding Energy	No. of HBs	Hydrogen Bonding Amino:Bond acids length	Hydrophobic Interactions Amino acids
1	α-Pinene	-6.1	0	NIL:NIL	Phe1003, Glu712, Pro724, Pro716, Ser717 and Ile715
2	Anethol	-6.1	0	NIL:NIL	Arg673, Gly1007, Pro724, Ile715, Arg727, Leu722, Ser717, Pro716, Phe1003,Glu712

Sr. No.	Ligands	Binding Energy	No. of HBs	Hydrogen Bonding Amino acids:Bond length	Hydrophobic Interactions Amino acids
3	Myristic acid	-5.4	2	Arg727:3.07 :3.15 Leu722:2.77	Val1006, Glu839, Gly1007, Leu829, Pro724, Glu712, Ser717, Lys723, Pro716, Phe1003, Ile715, Asp1004 and Trp840
4	Nigeglaine	-6.8	1	Trp840:3.20	His1005, Glu839, Val1006, Gly1007, Glu712, Phe1003 and Ser717
5	Nigellaquinomine	-8.2	0	NIL:NIL	Thr618, Phe807, Tyr805, Gly617, Gly615,Lys614
6	Nigellicine	-7.2	3	Trp840:3.10, Asp1004:2.68 Ser717:3.17 :3.32	His1005, Phe1003, Glu712, Gly1007, Val1006, Lys676 and Glu839
7	Palmitic acid	-5	2	Thr618:2.70 Gly617:3.16	Lys614, Gly615, Tyr805, Ala801, Leu616, Gln789, Leu468, Thr467, Ser619,Ser464
8	Pyrogallol	-5.6	1	Arg727:3.30 :2.82 :3.09	Pro724, Ile715, Phe1003, Glu712, Glu839,Ser717
9	Salfredin B11	-7.4	2	Glu839:2.85 Arg727:3.22	Trp840, Val1006, Gly1007, Glu712, Phe1003, Pro724 and Ser717
10	Salicylic acid	-6.5	1	Arg727:3.06 :2.89	Pro724, Ser717, Pro716, Ile715, Glu712, Phe1003 and Gly1007

α-Pinene made 06 hydrophobic interactions with Topoisomerase II α and no hydrogen bonding as illustrated in figure 4.15. Anethol made 10 hydrophobic interactions with Topoisomerase II α and no hydrogen bonding as illustrated in figure 4.16. Myristic acid made 13 hydrophobic interactions with Topoisomerase II α and 02 hydrogen bonds with Arg727 and Leu722 residues as illustrated in figure 4.17. Nigeglaine made 07 hydrophobic interactions with Topoisomerase II α and 01 hydrogen bonds with Trp840 residue as illustrated in figure 4.18. Nigellaquinomine made 06 hydrophobic interactions with Topoisomerase II α and no hydrogen bonding as illustrated in figure 4.19. Nigellicine made 07 hydrophobic interactions with Topoisomerase II α and 03 hydrogen bonds with Trp840, Asp1004 and Ser717 residues as shown in figure 4.20. Palmitic acid made 10 hydrophobic interactions with Topoisomerase II α and 02 hydrogen bonds with Gly617 and Thr618 residues

as shown in figure 4.21. Pyrogallol made 06 hydrophobic interactions with Topoisomerase II α and 01 hydrogen bond with Arg727 residue as shown in figure 4.22. Salfredin B11 made 07 hydrophobic interactions with Topoisomerase II α and 02 hydrogen bonds with Arg727 and Glu839 residues as shown in figure 4.23. Salicylic acid made 07 hydrophobic interactions with Topoisomerase II α and 01 hydrogen bond with Arg727 residue as illustrated in figure 4.24.

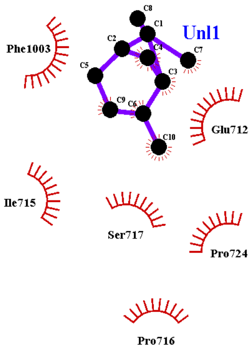

FIGURE 4.15: Interaction of α-Pinene with Topoisomerase II α

α-Pinene made 06 hydrophobic interactions with Phe1003, Glu712, Pro724, Pro716, Ser717 and Ile715 residues and no Hydrogen bond.

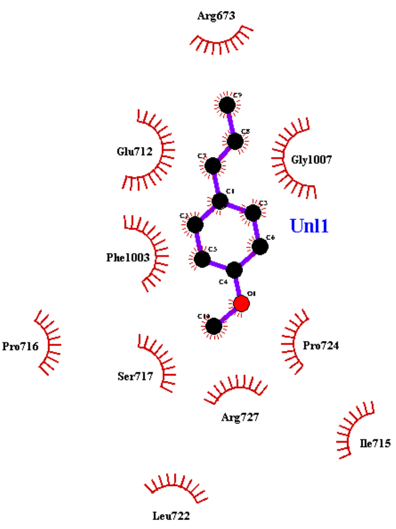

FIGURE 4.16: Interaction of Anethol with Topoisomerase II α

Anethol made 10 hydrophobic interactions with Arg673, Gly1007, Pro724, Ile715, Arg727, Leu722, Ser717, Pro716, Phe1003 and Glu712 residues and no Hydrogen bond.

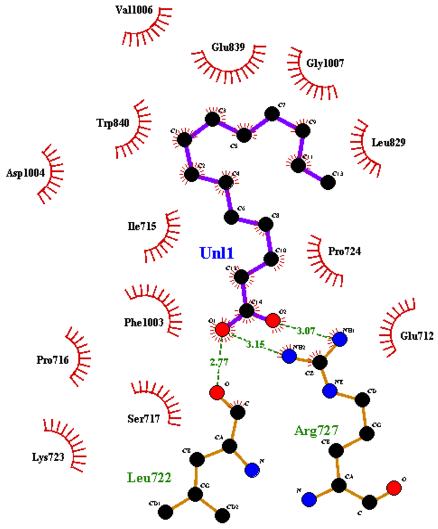

Figure 4.17: Interaction of Myristic acid with Topoisomerase II α

Myristic acid made 13 hydrophobic interactions with Val1006, Glu839, Gly1007, Leu829, Pro724, Glu712, Ser717, Lys723, Pro716, Phe1003, Ile715, Asp1004 and Trp840 residues and 02 Hydrogen bonds with Arg727 having bond lenghts of 3.07 and 3.15 and with residue Leu722 having bond length of 2.77.

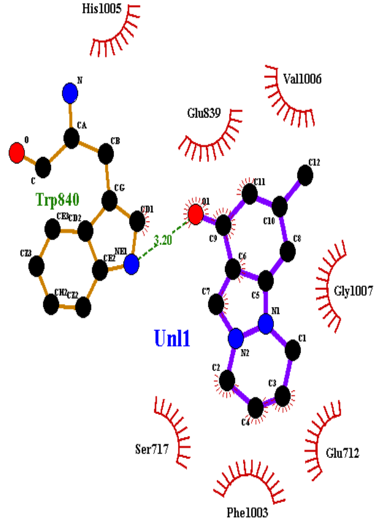

Figure 4.18: Interaction of Nigeglaine with Topoisomerase II α

Nigeglaine made 07 hydrophobic interactions with His1005, Glu839, Val1006, Gly1007, Glu712, Phe1003 and Ser717 residues and 01 Hydrogen bond with Trp840 residue having bond length of 3.20.

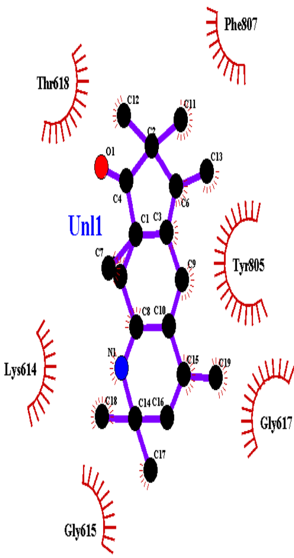

FIGURE 4.19: Interaction of Nigellaquinomine with Topoisomerase II α

Nigellaquinomine made 06 hydrophobic interactions with Thr618, Phe807, Tyr805, Gly617,Gly615 and Lys614 residues and no Hydrogen bond.

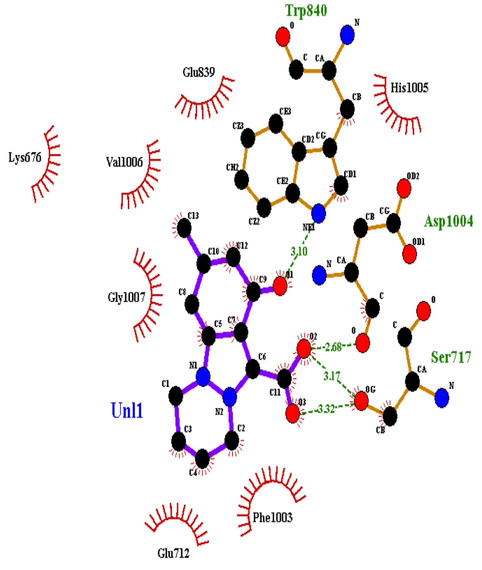

FIGURE 4.20: Interaction of Nigellicine with Topoisomerase II α

Nigellicine made 07 hydrophobic interactions with His1005, Phe1003, Glu712, Gly1007, Val1006, Lys676 and Glu839 residues and 03 Hydrogen bonds with Trp840 having bond length of 3.10, Asp1004 having bond length of 2.68 and with residue Ser717 having bond length of 3.17 and 3.32.

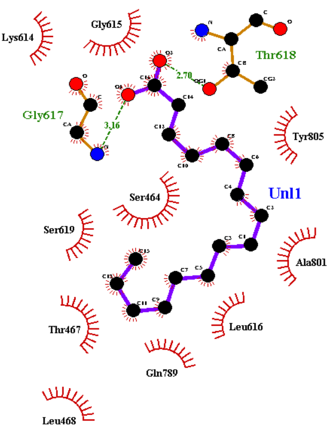

FIGURE 4.21: Interaction of Palmitic acid with Topoisomerase II α

Palmitic acid made 10 hydrophobic interactions with Lys614, Gly615, Tyr805, Ala801, Leu616, Gln789, Leu468, Thr467, Ser619 and Ser464 residues and 02 Hydrogen bonds with Thr618 having bond length of 2.70 and with Gly617 having bond length of 3.16.

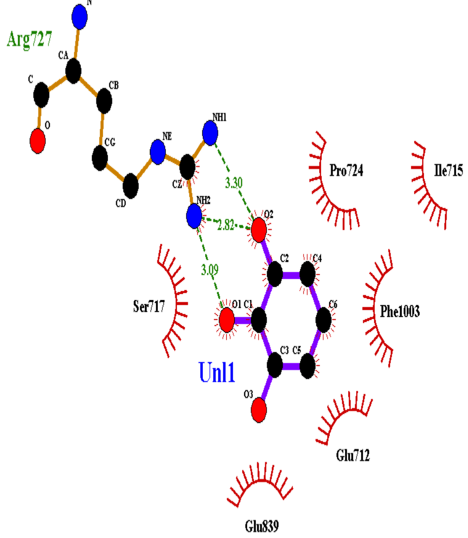

FIGURE 4.22: Interaction of Pyrogallol with Topoisomerase II α

Pyrogallol made 06 hydrophobic interactions with Pro724, Ile715, Phe1003, Glu712, Glu839 and Ser717 residues and 01 Hydrogen bond with residue Arg727 having bond lengths of 3.30,2.82 and 3.09.

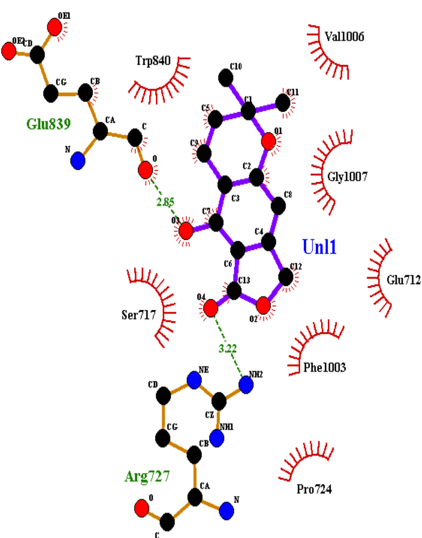

FIGURE 4.23: Interaction of Salfredin B11 with Topoisomerase II α

Salfredin B11 made 07 hydrophobic interactions with Trp840, Val1006, Gly1007, Glu712, Phe1003, Pro724 and Ser717 residues and 02 Hydrogen bonds with Glu839 having bond length of 2.85 and with residue Arg727 having bond length of 3.22.

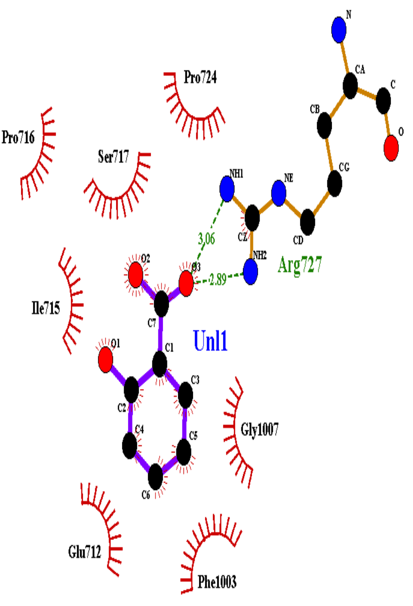

FIGURE 4.24: Interaction of Salicylic acid with Topoisomerase II α

Salicylic acid made 07 hydrophobic interactions with Pro724, Ser717, Pro716, Ile715, Glu712, Phe1003 and Gly1007 residues and 01 Hydrogen bond with Arg727 having bond lengths of 3.06 and 2.89.

4.6 ADME Properties of Ligands

Ligands toxicity and ADME properties are obtained from pkCSM online tool. Canonical SMILES of the ligands obtained from PubChem and are used as input for pkCSM server. Toxicity measurements provide information regarding the nature of ligands, which must be considered before drug designing. Toxicity of a compound must be tested to use it as a therapeutic agent. ADME properties of the selected ligands extracted from this server are as follows.

4.6.1 Absorption

In pharmacology absorption is reffered to as, the transportation of drug from the bloodstream to the tissues. Chemical composition of a drug as well as environment into which a drug is placed, work together to determine the extent and rate of drug absorption. It is necessary for a drug to cross cellular barriers for example epithelial or endothelial cells etc. so that it can be absorbed into the tissues. Mostly drugs cross cellular barriers through passive transport that is, drugs move from higher concentration area to an area of lower concentration diffusing through cell membranes. Only a few drugs move across cellular barriers in an active way; that is, transportation that requires energy in the form of ATP and moves the drug from an area of lower concentration to an area of higher concentration.

Absorption is one of the ADME properties which consists of 7 models that are Water solubility,$Caco_2$ permeability, Intestinal absorption, Skin permeability, P-glycoprotein substrate and P-glycoprotein I and II inhibitors. Water solubility of a compound represents its solubility in water at 25C. It is presented as logarithm of molar concentration (log mol/L). In water, water soluble drugs are more soluble

as compared to lipid soluble drugs [112].

The $Caco_2$ permeability model represents the logarithm of apparent permeability coefficient (log Papp; log cm/s). A compound has a high $Caco_2$ absorbency if it has a Papp value> 0.9 in terms of pkCSM predicted value. Intestinal absorption is the percentage that will enter in the small intestine of a person. A compound is less absorbent if it has absorption value less than 30%. The Skin permeability represents the absorbency in terms of log Kp value, it plays a key role in transdermal drug designing. The compound having log Kp value>-2.5 has less skin penetration.

The P-glycoprotein substrates act as a natural barrier and are helpful in the removal of toxins from the cell. This model predicts about given compound that either it is a P-glycoprotein substrate or not. A compound may show low oral absorption if it is P-glycoprotein substrate. P-glycoprotein I and II inhibitor models predict that a compound either P-gp I and II inhibitor or not. P-gp inhibitors reduce the activity of P-gp and have high absorption. Ligands absorption properties are shown in Table 4.13.

All these ligands enlisted in table 4.13 showed lower water solubility and $Caco_2$ permeability of all ligands is within the normal range except nigellicine whose value is less than the recomended range. The intestinal absorption values of all these ligands are good and are more than 90%. Nigellicine showed highest intestinal absorption among these ligands which is 100%. Only Myristic acid has Skin permeability value within the range showing high skin penetration. Nigellaquinomine is predicted as P-glycoprotein I inhibitor.

4.6.2 Distribution

In pharmacology distribution is a branch of pharmacokinetics which deals with the study of movement of the drug within the body from one site to another site. When drug enters the systemic circulation through direct administration or via absorption, it must be distributed into intracellular and interstitial fluids [113].

TABLE 4.13: Absorption Properties of Ligands

Sr. No.	Model Name	α-Pinene	Anethol	Myristic acid	Nigeglaine	Nigellaquinomine	Nigellicine	Palmitic acid	Pyrogallol	Salfredin B11	Salicylic acid
1	Water solubility	-3.733	-2.936	-4.952	-3.052	-5.129	-2.148	-5.562	-1.408	-3.081	-1.808
2	$Caco_2$ permeability	1.38	1.669	1.56	1.217	1.42	0.453	1.558	1.122	1.201	1.151
3	Intestinal absorption (human)	96.041	95.592	92.691	99.353	97.773	100	92.004	83.549	94.058	83.887
4	Skin permeability	-1.827	-1.139	-2.705	-2.497	-2.472	-2.73	-2.717	-2.751	-3.236	-2.723
5	P-glycoprotein substrate	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
6	P-glycoprotein I inhibitor	NO	NO	NO	NO	YES	NO	NO	NO	NO	NO
7	P-glycoprotein II inhibitor	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO

Distribution properties of ligands are presented in Table 4.14. Distribution as one of the ADME property includes four models namely as Volume of distribution in human (VDss expressed as log L/kg), Function unbound in humans (Fu), Blood brain barrier (BBB) permeability expressed as log BB [114]. Model-I explains the theoretical volume that the total amount of drug will need to be evenly distributed to provide the same concentration in blood plasma. VDss is considered low, if it is less than 0.71 L/kg and higher if it is above 2.81 L/kg. 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 cross BBB and if value <-1 then no harm to brain. Log PS is the product of blood-brain permeability and surface area and its value >-2 considered to penetrate the Central Nervous System (CNS) and <-3 considered as safe.
All ligands showed low VDss (human) values indicating more drug is distributed to plasma as compared to tissues. Fraction unbound (human) values are within the recommended range. BBB permeability values of α-pinene, anethol, nigeglaine, nigellaquinomine and salfredin B11 are greater than 0.3 showing that these compounds can harm the brain. CNS permeability values of anethol, myristic acid and palmitic acid are greater than -2 indicating that these compounds can penetrate the CNS [114].

4.6.3 Metabolism

Metabolism is a process of converting one form of compound into another form; that is conversion of complex to simpler compounds and vice versa. Most of the drug metabolism occures in liver, blood plasma, lungs and intestine. Metabolism generally convert the drug into more water soluble compound by increasing its polarity. Cytochrome P450 is an important cleansing enzyme found in the liver has different isoforms whose models are included in metabolism of ADME properties which are CYP1A2,CYP2C19,CYP2C9,CYP2D6 and CYP3A4.

Table 4.14: Distribution Properties of Ligands

Sr.No.	Model Name	VDss (human)	Fraction unbound (human)	BBB permeability	CNS permeability
1	α-Pinene	0.667	0.425	0.791	-2.201
2	Anethol	0.343	0.266	0.529	-1.659
3	Myristic acid	-0.578	0.171	-0.027	-1.925
4	Nigeglaine	0.312	0.414	0.673	-2.362
5	Nigellaquinomine	0.527	0.225	0.498	-2.645
6	Nigellicine	-0.801	0.489	-0.144	-2.944
7	Palmitic acid	-0.543	0.101	-0.111	-1.816
8	Pyrogallol	0.13	0.712	-0.441	-3.252
9	Salfredin B11	0.363	0.465	0.747	-2.827
10	Salicylic acid	-1.57	0.563	-0.334	-3.21

Table 4.15: Metabolic Properties of Ligands

Sr. No.	Model Name	CYP2D6 substrate	CYP3A4 substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
1	α-Pinene	NO	NO	NO	NO	NO	NO	NO
2	Anethol	NO	NO	YES	NO	NO	NO	NO
3	Myristic acid	NO	NO	NO	NO	NO	NO	NO
4	Nigeglaine	NO	NO	NO	NO	NO	NO	NO
5	Nigellaquinomine	NO	YES	NO	YES	NO	NO	NO
6	Nigellicine	NO	NO	NO	NO	NO	NO	NO
7	Palmitic acid	NO	YES	NO	NO	NO	NO	NO
8	Pyrogallol	NO	NO	NO	NO	NO	NO	NO
9	Salfredin B11	NO	NO	NO	NO	NO	NO	NO
10	Salicylic acid	NO	NO	NO	NO	NO	NO	NO

This enzyme reacts to xenobiotics to facilitate them to release. It triggers some drugs while most drugs are neutralized by it [109]. Table 4.15 show the metabolic properties of ligands.

Out of the ligands mentioned in Table 4.15, Nigellaquinomine and palmitic acid act as the substrate of isoform CYP3A4, anethol act as inhibitor of CYP1A2 isoform and Nigellaquinomine act as inhibitor of CYP2C19 isoform. No other ligand act as inhibitor or substrate of any other isoform.

4.6.4 Excretion

Liver and kidney are mainly involved in the excretion of a drug. Some other organs may also help in drug excretion such as lungs excrete gaseous products of the drug. Drugs can also be excreted via tears, sweat and saliva. Models of excretory properties includes total clearance (CL tot) expressed in terms of log CL tot. in ml/min/kg and renal OCT2 substrate whose results are predicted as Yes/No. OCT2 (organic cation transporter 2) is a renal uptake transporter that plays role in renal clearance of drugs [115].

Negative result of total clearance show poor clearance of drug while positive result show good clearance. Ligands excretory properties are given in Table 4.16. All ligands in Table 4.16 exhibit negative results for renal OCT2 substrate and shown positive values of total clearance depicting good drug clearance.

4.7 Lead Compound Identification

Physiochemical properties or Lipinskis rule of five acts as a primary filter and then pharmacokinetics properties sorts further potential compounds as drug or non drug. After analysing all ligands carefully, Salfredin B11 has selected as lead compound because it is active of all the ligands.

TABLE 4.16: Excretory Properties of Ligands

Sr.No.	Model Name	Total clearance	Renal OCT2 substrate
1	α-Pinene	0.043	NO
2	Anethol	0.268	NO
3	Myristic acid	1.693	NO
4	Nigeglaine	0.526	NO
5	Nigellaquinomine	0.903	NO
6	Nigellicine	0.55	NO
7	Palmitic acid	1.763	NO
8	Pyrogallol	0.104	NO
9	Salfredin B11	0.481	NO
10	Salicylic acid	0.607	NO

4.8 Drug Identification Against Liver Cancer

FDA approved different drugs for the treatment of liver cancer. These drugs are used alone or in combination depending upon the condition of the patients. These drugs work by inhibiting variety of signal transduction pathways or by inhibiting the production of blood vessels in the cancerous tissues. One of the FDA approved drug is Sorafenib which is used world wide. There are different side effects of sorafenib but its use is increased due to its efficacy as compared to other drugs used for liver cancer [116].

4.9 Sorafenib

Sorafenib is a drug approved by FDA for the treatment of advanced renal cell carcinoma. It is marketed by Bayer with brand name Nexavar. It has also got ”Fast Track” designation by the FDA for the treatment of advanced HCC and has since

performed better in Phase III trials. Sorafenib is a small molecular inhibitor of Raf kinase, PDGF (platelet-derived growth factor), VEGF receptor 2 & 3 kinases and c-Kit; the receptor for Stem cell factor. An expanding number of drugs target mostly these pathways. The originality of Sorafenib lays in its targeting of the Raf/Mek/Erk pathway at the same time [81].

FIGURE 4.25: 2D structure of Sorafenib Drug from Pubchem Database

Physiochemical properties of Sorafenib drug are shown in Table 4.17.

TABLE 4.17: Physiochemical properties of Sorafenib Drug

log P value	**Rotatable Bonds**	**H-Bond acceptor**	**H-Bond donor**	**Molecular formula**	**Molecular weight**
5.5497	5	4	3	$C_{21}H_{16}CIF_3N_4O_3$	464.8

4.10 ADMET Properties of Drug

pkCSM is used to predict the ADMET properties of Sorafenib drug. Predicted values of sorafenib are given in Table 4.18.

Table 4.18: ADMET Properties of Drug

Property	Sr.No.	Model Name	Predicted Values
	1	Water solubility	-4.255
	2	$Caco_2$ permeability	0.762
	3	Intestinal absorption (human)	85.494
Absorption	4	Skin permeability	-2.74
	5	P-glycoprotein substrate	YES
	6	P-glycoprotein I inhibitor	YES
	7	P-glycoprotein II inhibitor	YES
	1	VDss (human)	-0.009
	2	Fraction unbound (human)	0
Distribution	3	BBB permeability	-1.473
	4	CNS permeability	-2.025
	1	CYP2D6 substrate	NO
	2	CYP3A4 substrate	YES
	3	CYP1A2 inhibitor	NO
Metabolism	4	CYP2C19 inhibitor	YES
	5	CYP2C9 inhibitor	YES
	6	CYP2D6 inhibitor	NO
	7	CYP3A4 inhibitor	YES
Excretion	1	Total Clearance	-0.213
	2	Renal OCT2 substrate	NO
	1	AMES toxicity	NO
	2	Max.tolerated dose (Human)	0.253
	3	hERG I inhibitor	NO
	4	hERG II inhibitor	YES
Toxicity	5	Oral Rat Acute Toxicity	2.14
	6	Oral Rat Chronic Toxicity	1.068
	7	Hepatotoxicity	YES
	8	Skin sensitisation	NO
	9	$T.pyriformis$toxicity	0.307
	10	Minnow toxicity	-0.515

4.11 Mechanism of Action of Sorafenib

Sorafenib target multiple intracellular (CRAF, BRAF and mutant BRAF) and cell surface kinases (KIT, FLT-3, VEGFR-2, VEGFR-3 and PDGFR-β). Several of these kinases are considered to be involved in angiogenesis, hence sorafenib reduces the flow of blood to the tumor. Sorafenib is exclusive in targeting the Raf/Mek/Erk pathway. Angiogenesis is inhibited by inhibiting these kinases, genetic transcription which involves cell proliferation [117].

4.12 Sorafenib Effects on Body

Sorafenib has been associated with a low rate of transient elevations in serum aminotransferase levels during therapy that are generally mild and asymptomatic. Sorafenib has also been linked to rare instances of clinically apparent liver injury which can be severe and even fatal. The highest recommended dose of sorafenib studied clinically is 800mg two times a day. The side effects of Sorafenib observed at this dose are primarily diarrhea and dermatologic events [118].

4.13 Comparison Between Sorafenib and Lead Compound Salfredin B11

Comparison between Sorafenib and Salfredin B11 is done to identify the better treatment for Liver cancer. Comparison is done on the basis of physiochemical properties and ADMET properties of Sorafenib and Salfredin B11. Application of Lipinski rule of five on Sorafenib and Slfredin B11 is shown in Table 4.19.

TABLE 4.19: Application of Lipinski rule of five on Sorafenib and Slfredin B11

Sr.No.	Compound	LogP value	Molecular weight	H-Bond Acceptor	H-Bond Donor
1	Sorafenib	5.5497	464.8	4	3
2	Salfredin B11	2.2468	232.23	4	1

LogP value, molecular weight and hydrogen bond donor of Salfredin B11 is less than Sorafenib.

4.14 ADMET Properties Comparison

ADMET properties of drug includes Absorption, distribution, metabolism, excretion and toxicity which help us to determine the activity and efficacy of drug. Comparison between Sorafenib and Salfredin B11 is given in Table 4.20.

TABLE 4.20: Comparison of ADMET Properties

Property	Sr. No.	Model Name	Sorafenib	Slafredin B11
	1	Water solubility	-4.255	-3.081
	2	$Caco_2$ permeability	0.762	1.201
	3	Intestinal absorption (human)	85.494	94.058
Absorption	4	Skin permeability	-2.74	-3.236
	5	P-glycoprotein substrate	YES	NO
	6	P-glycoprotein I inhibitor	YES	NO
	7	P-glycoprotein II inhibitor	YES	NO
	1	VDss (human)	-0.009	0.363
	2	Fraction unbound (human)	0	0.465
Distribution	3	BBB permeability	-1.473	0.747
	4	CNS permeability	-2.025	-2.827
	1	CYP2D6 substrate	NO	NO
	2	CYP3A4 substrate	YES	NO
	3	CYP1A2 inhibitor	NO	NO
Metabolism	4	CYP2C19 inhibitor	YES	NO
	5	CYP2C9 inhibitor	YES	NO
	6	CYP2D6 inhibitor	NO	NO
	7	CYP3A4 inhibitor	YES	NO
Excretion	1	Total Clearance	-0.213	0.481
	2	Renal OCT2 substrate	NO	NO
	1	AMES toxicity	NO	NO
	2	Max.tolerated dose (Human)	0.253	-0.051
	3	hERG I inhibitor	NO	NO
	4	hERG II inhibitor	YES	NO
Toxicity	5	Oral Rat Acute Toxicity	2.14	1.701
	6	Oral Rat Chronic Toxicity	1.068	2.419
	7	Hepatotoxicity	YES	NO
	8	Skin sensitisation	NO	NO
	9	$T.pyriformis$toxicity	0.307	0.494
	10	Minnow toxicity	-0.515	1.492

Both are showing low water solubility, $Caco_2$ permeability value of salfredin B11 is greater than 0.9 indicating that it has high absorbancy, intestinal absorption and skin permeability of salfredin B11 is higher than sorafenib. As sorafenib is P-glycoprotein substrate depicting its low oral absorption. Both of these are showinglow VDss (human) but value of salfredin B11 is higher, fraction unbound (human) of salfredin is also greater than sorafenib making salfredin B11 more effective. BBB permeability value of sorafenib is good as compared to salfredin B11. CNS permeability values of both compounds are in recommended range. Total clearance value of salfredin B11 is positive which shows good drug clearance while of sorafenib, it is negative indicating poor drug clearance and none is OCT2 substrate. According to toxicity values both of the compounds are non carcinogenic, MRTD of sorafenib is high as compared to salfredin B11. Sorafenib act as hERG II inhibitor which is more dangerous as it is known to us that many drugs have had withdrawn from the market just because of hERG inhibitor which cause the dysfunction of potassium channels. Sorafenib is hepatotoxic which means it damage the liver and it is the main concern of safety for drug designing because mostly drug metabolism is done in the liver. Sorafenib also indicate minnow toxicity while salfredin B11 does not.

4.14.1 Comparison of Physiochemical Properties

The comparison between physiochemical properties of Sorafenib and Salfredin B11 is important step that help us to find out the drug activity manner and biochemical reactivity. Comparison between physiochemical properties of Sorafenib and Salfredin B11 is shown in Table 4.21.

Table 4.21: Comparison between physiochemical properties of Sorafenib and Salfredin B11

Sr.No.	**Compound**	**logP value**	**Rotatable Bonds**	**H-Bond Acceptor**	**H-Bond Donor**	**Molecular Weight**
1	Sorafenib	5.5497	5	4	3	464.8
2	Salfredin B11	2.2468	0	4	1	232.23

LogP value, rotatable bonds, hydrogen bond donor and molecular weight of salfredin B11 is less than sorafenib.

Chapter 5

Conclusions and Future Prospects

The aim of this study was to identify a compound using computational approach for treating liver cancer that can be use in near future as an efficient drug. After performing data mining studies on literature databases ten ligands were selected for current research work. The proteins used for virtual screening were Topoisomerase I and Topoisomerase IIα proteins. CB Dock automated version of Auto Dock vina is used for the molecular docking. Protein ligand interactions were analyzed using Ligplot plus version v.1.4.5. After the detailed analysis of binding score, physiochemical properties and ADMET properties, Salfredin B11 was identified as a potent inhibitor for liver cancer. From the above mentioned physiochemical and ADMET values comparison, it is concluded that the Salfredin B11 is better in activity as well as safety as compared to Sorafenib. All the softwares and tools used in the current study are reliable and authenticated.

5.1 Future Prospects

This identified active compound of *Nigella sativa*;Salfredin B11 can be used as medicine in near future. To prove its rank as a drug it can be used on mice for experiments and after these successful experiments, it can be introduced in the clinical trials for its validation.

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