CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Screening of Therapeutic Agents of *Allium sativum* Effective Against Lungs Cancer

by

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

in the

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Dedicated to my beloved sister who passed away due to lung cancer and left us in deep anguish!

CERTIFICATE OF APPROVAL

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Abstract

Lung cancer is the most common and leading cause of cancer-related deaths in men worldwide. And it is the fourth most common cancer in women, with the second highest mortality rate. About 25% of cancer-related deaths are due to lung cancer and are considered a life-threatening form of cancer as they are easily identified due to the large flow of blood to the lungs. The transformation of normal cancer cells involves three distinct phases, namely initiation, proliferation and progression. These abnormal cells do not grow into normal or healthy lung tissue; their fastest production causes tumors. Despite all its advances in medicine, its overall survival remains low. One of the reasons for such a negative clinical result is the delayed diagnosis of the disease and that ultimately results in metastasis. The main aim of this study is to predict potential inhibitors against Lungs Cancer by the use of molecular docking of active compounds found in *Allium sativum*. Ligands and proteins were docked using CB dock and visualized through PyMol and analyzed through LigPlot. These ligands were then screened out based on Lipinski rule and their ADMET properties were studied. Alliin was selected as leading compound against, K-Ras GTPase and B-Raaf protease receptors. The comparative results of selected lead compound with standard drug, Abraxane showed less toxicity and far more activity. However, further research has to be carried out to investigate its potential medicinal use.

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Abbreviations

AI:	Aliphatic Index
COPD:	Chronic obstructive pulmonary disease
CB:	Dock Cavity-detection guided Blind Docking
FDA:	Food Drug Authority
GBM CSC:	Glioblastoma multiforme stem cells
GRAVY:	Grand average of hydropathicity
HCMV:	Human cytomegalovirus
II:	Instability Index
KEGG:	Kyoto Encyclopedia of genes and genomes
MW:	Molecular weight
NSCLC:	Non-small cell lung cancer
NSAIDs:	Non Steroidal Anti-inflammatory Drugs
NR:	Total number of negatively charged residues (Asp + Glu)
PDB:	Protein Data Bank
pI:	Theoretical pI
PR:	Total number of positively charged residues (Asp + Glu))
SNPs:	Single nucleotide polymorphisms
SCC:	Squamous cell carcinoma
VDss:	Volume of Distribution
WHO:	World Health Orginization

Chapter 1

Introduction

1.1 Background

Cancer is a condition with a complicated pathogenesis that is characterised as uncontrolled cell proliferation. . Cancer can affect any social class [1]. Typical cancer cells have three separate phases of transformation: initiation, proliferation, and progression. These aberrant cells do not develop into normal or healthy lung tissue, and their rapid proliferation results in tumours. Among all malignancies, lung cancer has the second highest fatality rate. Even after diagnosis, the survival rate is extremely poor, hence the death rate rises year after year. The progression of lung cancer at the point of prognosis is related to the likelihood of survival.

However, when cancer cells are identified in their early stages, a person's chances of survival improve. [2]. Lung cancer, also known as cancer that begins malignancies arising from airway epithelioma, is the most common type of cancer diagnosed worldwide and the leading cause of cancer death. Every year, nearly 1.8 million incidence of lung cancer are diagnosed worldwide. Lung cancer killed around 1.6 million individuals in 2012, and the number of fatalities is expected to climb to 3 million by 2035 [3]. Lung cancer has a terrible prognosis, with 5-year survival ranging from 4% to 17% depending on disease stage at the time of diagnosis. [4] [5]. Oncogenic transformation is a multistep, extremely complicated process

including changes to the DNA and epigenome. Lung cancer has a higher tumour mutational burden (TMB) than other forms of cancer, which is likely connected to smoking behaviours and xenobiotic exposure from tobacco smoke cite6. textitKan et al. (2010) investigated 441 tumours, giving special emphasis to the prevalence of protein-altering mutations. They discovered that NSCLC adenocarcinomas and squamous cell carcinomas (SCCs) have mutation rates in adenocarcinomas of 3.5 and 3.9 per megabase (Mb), respectively, making it one of the malignancies with the highest rate of protein-altering mutations. Prostate cancer, on the other hand, had a modest mutation rate of 0.33 per Mb of DNA. [7].

The two types of cancer genes are oncogenes and tumour suppressor genes. Most oncogenes began as regulatory proteins called proto oncogenes, which are involved in cell growth, proliferation, or apoptosis inhibition. Unchecked cell growth and oncogenic cell transformation arise from oncogene activation. The MYC, RAS, and HER families of proto-oncogenes are the ones that are most frequently mutated in lung cancer. Anti-oncogenes, also known as tumour suppressor genes, are a class of genes that regulate cell growth by preventing cell proliferation and preserving genome stability. TP53, RB, and p16 mutations are the most frequent tumour suppressor gene alterations in lung cancer [8].

The World Health Organization has divided lung cancer into two types based on its characteristics: small-cell carcinoma of the lungs (SCLC) or non-small cellular lung cancer (NSCLC). SCLC is divided into three types: small cell tumor, small cell melanoma cell carcinoma, and small cell melanoma cite9. NSCLC accounts for 80% of lung cancer cases, and therapy may include surgery in some cases. Other kinds of lung cancer include squamous cell carcinoma (SCC), adenocarcinoma, bronchoalveolar carcinoma (BAC), and big cell carcinoma. [4]. Despite all of its medical improvements, overall survival remains poor cite5. One of the causes for such a dismal clinical outcome is the disease's delayed diagnosis, which eventually leads to metastasis. [9]. Most cancers, when diagnosed, are already advanced, making it difficult to treat with alternative therapies. Similarly, various respiratory tract infections are also not properly diagnosed or diagnosed in later stages of the disease when recovery in a rare condition is almost impossible. Overdose

is a phenomenon, associated with decreased lung function in asthma [7] and chronic obstructive pulmonary disease (COPD) [8], which is usually caused by community-acquired infections. Although, clinicians may be able to detect the increase in history and objective testing [9] but there are no ways to explain its patho-physiology that prevents its complete treatment. The clinical features of lower respiratory tract infections are also misleading and vary depending on viral load and bacteria, virulence and host response [10].

At various phases of lung cancer and lung illness, several serum proteins are discharged into the urine or bloodstream [11]. Careful examination of these serum proteins can aid in the early detection of these potentially fatal disorders, as well as the prospect of effective treatment. [12].

A major obstacle that many physicians face in treating various respiratory diseases and lung carcinoma is that their diagnosis is delayed or inaccurate. In many respiratory tract infections, the mechanism of infection is not fully understood. Similarly, various lung carcinoma are diagnosed early when not only are the chances of treatment reduced but the chances of survival are also reduced [13]. Despite great advances in the field of oncology, the treatment and outcome of lung cancer is far from improving. Understanding the biological mechanisms involved in lung cancer aetiology is necessary to identify important biomolecules that may have significant clinical value, either predictive, predictive or diagnostic symptoms, or as objectives for the development of new therapies for the disease, in addition. smoking cessation strategies [14]. Lung cancer, like all other forms of human cancer, is caused by an aberrant Genomic dna or exposure. Lung cancer is classified into two histopathological subgroups: non-small cellular lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC may now be classified based on 'driver' mutations seen in various oncogenes [14], [15] and [16]. However, great interest remains in the genetic predisposition to lung cancer associated with single nucleotide polymorphisms (SNPs) in germline [17]- [18], with the exception of somatic mutations from tumor. The risk of lung cancer has been clearly established in many studies, including family risk analysis [19] and segregation analysis [20]. However the genetic impact of lung cancer is moderate.

1.2 Problem Statement

Lung's cancer has been considered as major threat nowadays by WHO due to its high mortality rate, high transmission rate with increased abnormal mitotic division and lack of treatment. For this we need to discover and have to identify new compounds having anticancer properties with least side effects and whose availability is easy around the world to minimize the effect of the cancer. In this study, we will target the main genes "K-Ras" and "B-raaf" encode proteins that are encoded by oncogene with the active compounds having anticancer properties present in *Allium sativum* for the conduction of extensive computational studies through molecular docking.

1.3 Objectives of the Reserach

The objective of this study is to predict potential inhibitors against Lungs Cancer by the use of molecular docking of active compounds found in *Allium sativum*. The objectives of the study include:

1. To identify the probable inhibitory compounds with anticancer properties, present in *Allium sativum*.
2. To analyze the interaction between ligand and protein complex by performing molecular docking.
3. To find the best of the interacting molecules that shows inhibitory effects against the cancer.

1.4 Scope

The idea of favoured structures is well understood and often employed in medication design and development. Despite his hazy beliefs, his total utility remains

high. Special subdivisions have been recognised, and garlic organic compounds are an outstanding illustration of this structure. These organosulfur compounds change the activity of various active enzymes that activate or release toxins, carcinogens, and inhibit DNA adduct formation in a variety of target tissues. These chemicals, without a doubt, have a dramatic influence on anti-cancer medications and are now licenced for anti-cancer treatment, having been examined by over two dozen different persons. The moiety of these compounds provides a simple, understood framework for the development of novel medications. [21].

Chapter 2

Review of Literature

2.1 Lungs Cancer

Most body cells have specific functions and life spans. Cell death, on the other hand, is a natural event known as apoptosis. The cell gathers up sinking directions in order that the body may replace it with a better one. Cancer cells lack the ability to be trained to cease dividing and dying. Thus, they grow in the body, utilizing oxygen and nutrients that normally nourish other cells [22]. Cancer cells can form tumors, damage the immune system, and cause other defects that prevent the body from functioning normally.Lung cancer is a kind of aggressive lung cancer that is distinguished by uncontrolled cell proliferation in lung tissue. Lung leading cause of cancer of cancer fatalities. [23].

Lung cancer is the largest cause of cancer-related fatalities among males globally. It is also the fourth most frequent cancer in ladies, with both the greatest fatality rate. Lung cancer accounts for around 25% of cancer-related fatalities and is considered a life-threatening kind of cancer due to the massive flow of blood to the lungs [24]. Lung cancer is the third most frequent kind of cancer in the world and a leading cause of cancer-related fatalities in the United States. Although smoking is one of the leading causes, other viruses and toxins can also cause cancer. Lung cancer is dangerous and can result in early death and treatment.

Cancer cells can come out of the lungs into the bloodstream, or the lymph fluid that surrounds the lung tissue. Arteries of the lymphatic system get swelled, which draw lymph nodes located in the lungs and in the center of the chest cavity. Lung cancer usually spreads in the middle of the chest due to the normal flow of lymph in the middle of the chest from the lungs. It is usually classified as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Depending on the cellular factors, these may be assigned to other types of lung cancer. As a stage, there are usually four stages of lung cancer; I, II, III & IV. The lungs cancer stages are based on location and size of the tumor and area where lymph node is present. Currently, CT scan is found to be much effective than x-ray of the empty chest in detecting and diagnosing lung cancer [26]. Current treatment for lung cancer varies depending on the histological type and anatomic level, or stage, of the tumor. The type of small cell lung cells tends to spread earlier and more widely and is better treated with chemicals and radiation. Popular treatment for squamous carcinoma but this can occur in less than one-third of all cases [25]. Cancer cells can come out of the lungs into the bloodstream, or the lymph fluid that surrounds the lung tissue [26].

2.2 Origin of Herb

It has been used as both a food flavoring and a conventional medicine since the time of the ancient Egyptians. It has a long history of human consumption and use, dating back several thousand years, and is a native of Central Asia and northeastern Iran. Among all cultivated plants, garlic (Allium sativum) is among the oldest. It is the most extensively studied medicinal plant and has been used as a spice, food, and folk medicine for more than 4000 years [27]. 22 therapeutic formulae in the Codex Ebbers, an Egyptian medical papyrus from around 1550 BC, mention garlic as a potent treatment for a range of diseases, including heart issues, headaches, bites, worms, and tumors [28]. The Bible claims that the Jewish slaves in Egypt were fed garlic and other allium vegetables, apparently to give them strength and to increase their productivity [29].

Garlic was taken in ancient Greece to alleviate pulmonary and gastrointestinal conditions [30]. Garlic has a long history of use in India as an antiseptic ointment for treating wounds and ulcers. Garlic was applied on troops' wounds during World War II [31].

Numerous researchers have studied the insecticidal, antimicrobial, antiprotozoal, and anticancer properties of garlic. Several spices and plants, including garlic, are said to have therapeutic capabilities, such as anti-thrombotic, hypolipidemic, and antihypertensive effects, in traditional Chinese medicine, Islamic medicine, folkloric medicine, and the Ayurvedic school of medicine [33], [34]. Garlic is also a useful treatment for many illnesses in the homoeopathic system.

Garlic tea has long been advocated in China as a treatment for fever, headaches, cholera, and dysentery. Miso soup with garlic is used in rural Japan as a treatment for the common cold, which includes headache, fever, and sore throat.

2.3 Taxonomic Hierarchy

Allium sativum is a perennial flowering plant growing throughout the year.

TABLE 2.1: Taxonomy of *Allium sativum* [36]

Sr. No.	**Domain**	**Eukarya**
1.	Kingdom:	Plantae
2.	Division:	Tracheophyta
3.	Super- Division:	Spermatophyta
4.	Class:	Equisetopsida
5.	Super Order:	Lilianae
6.	Order:	Asparagales
7.	Family:	Amaryllidaceae
8.	Genus:	*Allium*
9.	Specie:	*Allium sativum*

2.4 Botanical Description

A. sativum var. *sativum*, popularly known as softneck garlic, and *A. sativum var.* ophioscorodon, generally known as hardneck garlic, are the two subspecies of the *Allium sativum* plant. Both types consist of an underground bulb made up of cloves, which are prophylls that are covered in dry membrane skins and joined by a basal plate. Hard neck garlic differs from other varieties in that it has a bulb made up of six to eleven cloves arranged in a circle around a central woody stem. The scape on this particular species of garlic twists one to three times at the tip before being cut off. This is so that less energy will be sent toward the bulb if it continues to develop. The scape would eventually mature into bulbils that contained tiny cloves. Light purple or white blooms that are sterile are present in season with the bulbils. A softneck garlic bulb can have up to 24 cloves and does not have a flowering tip. The cloves are stacked, with longer ones on the exterior, while the stem is medial and soft, hence the name. The most common variety is *Allium sativum*; many research on garlic do not specify which subspecies is utilised, although the physiochemical and phytobiological actions of garlic are thought to be similar in some way. *Allium sativum* is produced from cloves asexually without the aid of a pollinator because it is sterile. It flourishes best in moderate climates, through hardneck varieties are better modified to survive in colder environments. *Allium sativum* is a perennial species that contains some strong organosulfur compounds which act as secondary metabolites as mentioned in the section entitled chemistry and pharmacology. These compounds are responsible for the very pervasive odour and flavour of raw garlic and functions as protection against predators [36].

2.5 Chemistry and Pharmacology

Polyphenolic compounds are the primary chemical ingredients responsible for the taste, smell, and biological effects of textitAllium sativum. Whenever a garlic cloves is intact, the major sulphur components are glutamyl cysteine [51]. Alliin is formed when they are hydrolyzed [51]. When cloves is crushed by chewing, cutting,

etc., the alliin quickly interacts with the enzymatic alliinase to generate allicin; the process is 97% complete after 30 seconds. Allicin is a diallyl thiosulfinate that contributes for 70-80 percent of the thiosulfinates found in *Allium sativum* [52]. When oxidised, allicin decomposes fast into sulphur compounds such as diallyl sulphide. [54].

2.6 Herbal Medicine

It is well known that more than 60% of anticancer drugs are made from plant materials, and plants have long been utilised to treat a number of deadly diseases, including cancer [55]. Numerous population studies have shown a link between consuming too much garlic and a lower chance of developing pancreatic, colon, stomach, esophageal, and breast cancers. A review of seven population studies found evidence that frequent usage of cooked or raw garlic may slow the growth of tumours [56]. Garlic will lower the risk of stomach and colon cancer [[61] to [63]].

2.7 Significance of Herbal Medicinal Plant

Numerous species of herbal plants have been documented to possess pharmacological characteristics because of their phytoconstituents, including glycosides, alkaloids, saponins, steroids, and flavonoids, terpenoids as well as tannins (e.g., monoterpenes, diterpenes, and sesquiterpenes). Currently, traditional medicines provide for a sizable portion of the basic health care for 80% of the world's population [38] to [45]. Numerous biological properties, including as virucidal, bactericidal, sedative, spasmolytic, fungicidal, anti-inflammatory, analgesic, and local anaesthetic activities, are also present in medicinal plant extracts and their constituents [37], [38]. It has been reported that numerous species of herbal plants with pharmacological properties due to their phytoconstituents such as glycosides, alkaloids, saponins, steroids, flavonoids, tannins, and terpenoids (e.g.,

monoterpenes, diterpenes, and sesquiterpenes). Currently, eighty percent population of the world pivot on traditional medicines as a significant source of their basic health care [38] to [45]. Medicinal plant extracts and their constituents also have numerous biological activities, including virucidal, bactericidal, sedative, spasmolytic, fungicidal, anti-inflammatory, analgesic and local anesthetic activities [37], [38].

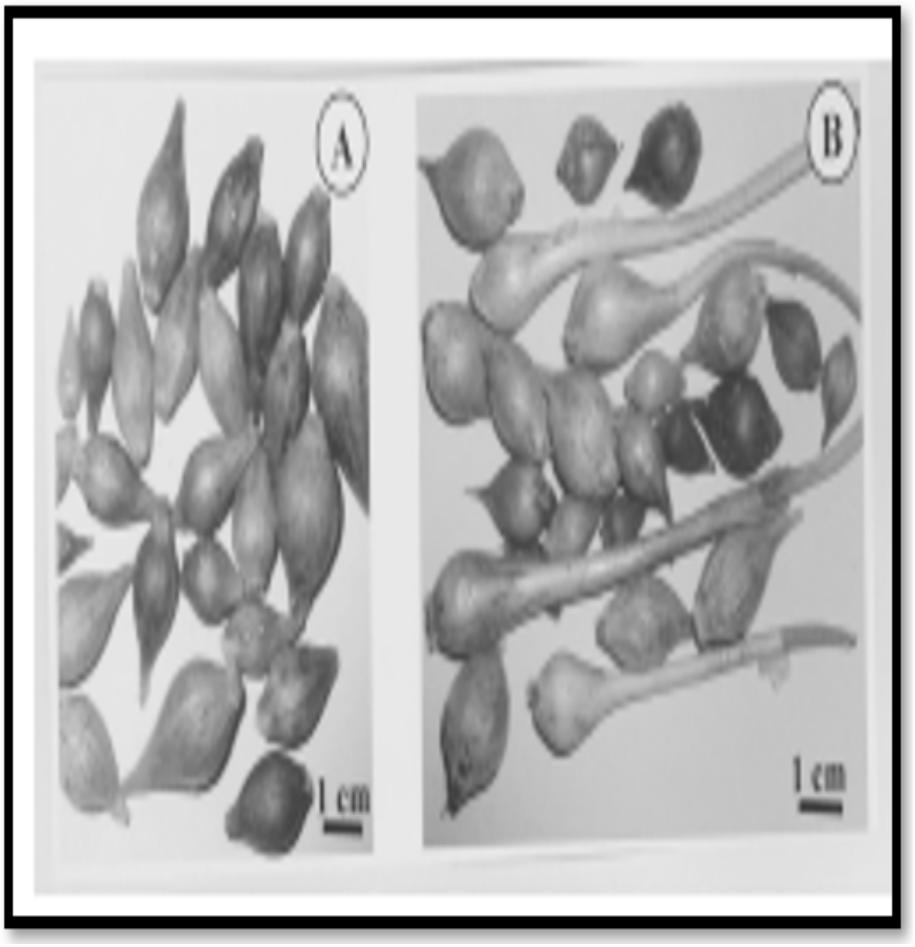


FIGURE 2.1: (A)bulbs obtained from garlic seedlings. (B) complete and incomplete bulbings from garlic seedlings [41].

2.8 *Allium sativum* (Garlic Plant)

Garlic (textitAllium sativum L.; Family: Amaryllidaceae) is a fragrant herbaceous annual spice that has been used as a traditional remedy from ancient times [72], [73]. It is the second most often used species of Allium, with onion (*Allium cepa*

L.) being utilised as a treatment for a variety of maladies including colds, flu, snakebite, and hypertensioncite [74]. *Allium* species and their active compounds have been shown in human clinical studies to reduce the likelihood of diabetes and cardiovascular disease, defend against infection by stimulating the immune system, and have antibacterial, antifungal, antiaging, and anticancer activities researches [75] to [78].

2.9 Therapeutic Significance

2.9.1 Antibacterial Activity

The antimicrobial activity of garlic is ascribed to the activity of allicin, which has been declared effective against a wide range of antibiotic-resistant microorganisms comprises of gram-positive and gram-negative bacteria such as *Escherichia coli, Shigella* [80]. Different garlic extracts (including aqueous, chloroform, methanol, and ethanol extracts) have been shown to selectively suppress the growth of a number of toxicl bacteria. For instance, a study found that extract of ethanolic garlic had a greater inhibitory impact than aqueous garlic extract did against *E. coli* and *Salmonella typhi* [84].

Garlic has been shown to inhibit the production of toxins brought on by bacterial infection in addition to its antibacterial properties [85]. Additionally, studies on allicin's effectiveness against methicillin-resistant *S. aureus* (MRSA) [83]. The chemical linkage between thiol-containing enzymes and allicin, such as thioredoxin reductase, RNA polymerase and alcohol dehydrogenase, which results in the oxidation of cysteine or glutathione residues in proteins, that gives allicin its antibacterial properties [86], [87].

2.9.2 Antifungal Activity

Garlic extracts displays wide range of fungicidal activity against a several species of fungi including *Trichophyton, Candida, Torulopsis, Rhodotorula, Trichosporon,*

Cryptococcus and Aspergillus species. Recently, essential compounds of Galic was found to suppress the germination and growth of *Meyerozyma guilliermondii* and *Rhodotorula mucilaginosa* [88]. The extract of garlic take action by invading the fungal cell wall and causing irreversible histochemical changes in the fungal cells that resulted in loss of integrity of its structural and damage germination ability. These changes in protoplasmic content led to damage to the nucleus and cell organelles, ultimately leading to cell death. In addition, allicin and garlic oil exhibited strong antifungal effects against *Candida albicans, Ascosphaera apisin,* and *A. niger* [81] and acted by penetrating the plasma membrane and membranes of organelles such as mitochondria, resulting in organelle destruction and ultimately leads to cell destruction [90]. DADS and DATS extracted from garlic essential oil displays antifungal activity against a significant fungal species.

2.9.3 Antiviral Activity

In vitro experiment revealed the antiviral properties of garlic extract and showed that garlic displays shielding activity for influenza viruses by promoting the production of antibodies that neutralizes when administered to mice, and this procedure was formed on the presence of several phytochemicals, named ajoene, allicin, allylmethylthiosulfinate, and biotin [97]. Allicin acts by inhibiting numerous thiol enzymes, while the antiviral activity of ajoene was due to the limitation of adhesive interlinkage and bonding of leukocytes. In addition, DATS was impactful against HCMV replication and viral instantaneous premature gene expression and works with enhancing the activity of natural killer cells (NK cells), which demolish virus-infected cells [91].

2.9.4 Anti-Inflammatory Activity

Aged black garlic (ABG) displays strong antioxidant activities and these actions may be responsible for its anti-inflammatory activity. Chloroform extract of ABG acts by reducing tumor necrosis factor-α (TNF-α)-induced activation of NF-κB

in human umbilical vein endothelial cells. In addition, the methanolic extract of ABG has been reported to prevent the production of cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) by inactivating NF-κB [107]. Allicin has been demonstrated as a defense mechanism in opposition to pathogens through its ability to amplify the activity of immune cells and controls signaling pathways linked up with these immune cells. In addition, allicin acts on T-cell lymphocytes by inhibiting the chemokine SDF1, which is linked to a weakened dynamic actin cytoskeleton structure [109], more over it leads to the inhibition of neutrophil trans endothelial migration.

2.9.5 Anticancer Activity

As compared to 33 different extracts obtained from raw vegetable against various cancer cells only raw garlic extract was found to be the most productive and highly targeted drug against cancer cells without affecting non-cancerous cells [112]. Allicin obtaineded from garlic extracts has been reported to suppress the metastasis of colorectal cancer by intensifying function of immune system and preventing tumor vessel formation, as well as surviving gene expression to increase cancer cell apoptosis. By reversing gene silencing and limiting proliferation of cancer cell, garlic may also help to improve treatment of pancreatic cancer [113]. Allicin has been shown in several studies to have the ability to stop proliferation of cell [114], [115] by targeting tubulin, which forms the mitotic spindle and put a stop to cell division [116]. In addition, many researchers have examined the antitumor and cytotoxic effects of *Allium sativum* and its associated components in vitro and in vivo [102].

2.10 Bioactive Compound

The anticarcinogenic effect of Allium vegetables, including garlic, is called organosulfur compounds (OSCs), which are very effective in boosting immunity against

cancer in animal models that are stimulated with a variety of different chemical compounds [79]. Theses organosulfur compounds includes Campesterol, allyl Mercaptan, e-Ajoene, Allyl methyl Trisulfide, Biotin. Alliin, Mettiin, Chlorogenin, beta-sitosterol, Qurectin, sapogenin and p-hydroxybenzoic Acid.

Chapter 3

Research Methodology

3.1 Methodology Flowchart

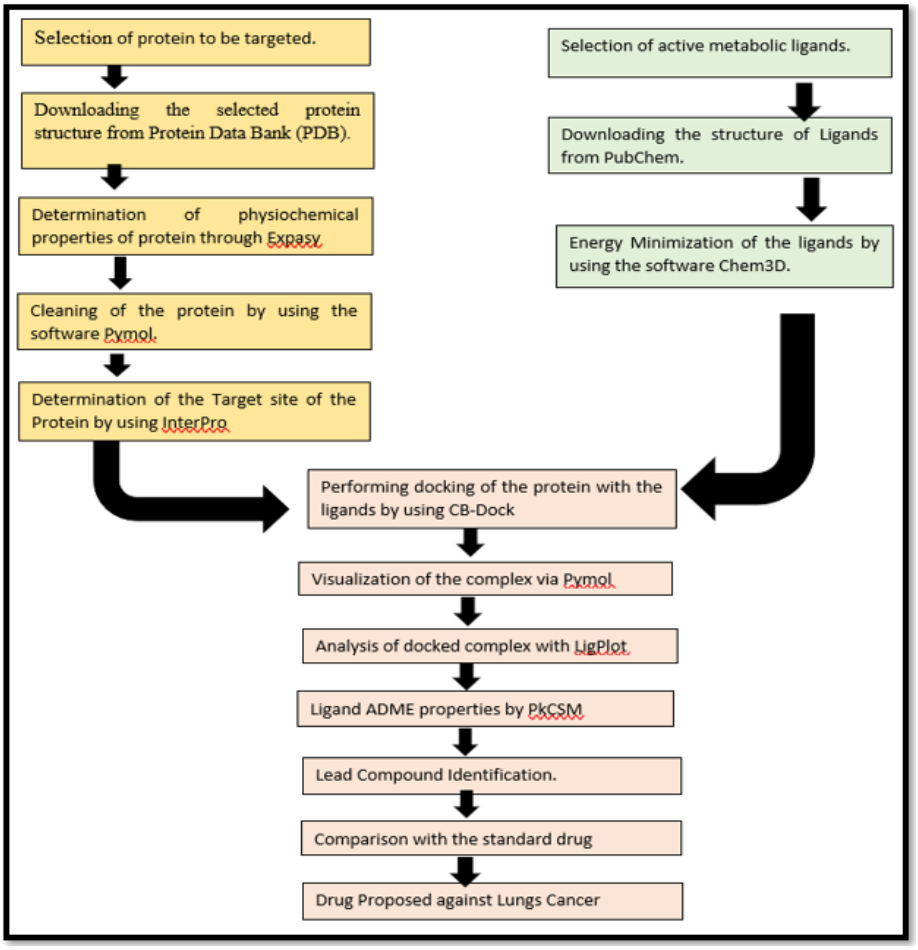


FIGURE 3.1: The flowchart of research methodology.

3.2 Selection of Disease

In the Western world, lung cancer is the main reason for cancer-related deaths. Because of the strong correlation between its incidence and cigarette smoking and the eventual diagnosis of lung cancer in 10% of long-term smokers, tobacco control strategies must be enhanced. The environmental or hereditary causes of lung cancer are typically unknown in the 10% of people who get the disease without ever smoking. Even in high-risk populations, there is no proven screening tool for lung cancer, and the five-year survival rate overall has not altered appreciably over the previous 20 years. However, significant progress has been made in our understanding of this illness, and we are now starting to see the clinical application of this information [112].

3.3 Selection of Protein

One of the most common oncogenes in human cancer is Ras. Roughly 30% of human malignancies have activating Ras gene mutations described [122]. Ras induces a number of downstream effector pathways, which in cancer cells harbouring oncogenic Ras are constitutively activated in a growth factor-independent manner [123]. Targeted therapies targeting Ras were mainly ineffective in clinical trials despite significant effort [124], and as a result, pharmacological suppression of Ras effector pathways may be a more practical technique to treat Ras mutant tumours. Ras is one of the most prevalent oncogenes in human cancer. Activating mutations in Ras genes have been reported in approximately 30% of human cancers [122].

3.4 Determination of Physiochemical Properties of Proteins

The study and determination of a protein's physical and chemical properties is critical in determining its function. ProtParam, an ExPAsy tool, has been used for

it. The molecular weight, isoelectric point, number of amino acids present, grand average of hydropathicity, instability index, and number of negatively charged (Asp+Glu) and positively charged (Arg+Lys) residues have been identified [125].

3.5 Cleaning of the Downloaded Protein

After downloading the protein structure, the extra constituents attached to the protein were removed using the open source system Pymol. The linear chain containing 1-301 amino acids was referred to as the A chain, and the remaining protein constituents was eliminated to ensure that the subsequent process runs smoothly [126].

3.6 Determination of Functional Domains of Target Proteins

InterPro, a database that can analyse a protein, was used to determine the domains of the target protein. It provides information about the families, functional sites, and domains of the protein under study [127]. By inserting the main protease's FASTA sequence, I have obtained polypeptide binding sites and homodimer interfaces.

3.7 Selection of Active Metabolic Ligands

The active compounds present in the *Allium sativum* were selected on the basis of their anti-bacterial, anti-fungal, anti-viral, anti-protozoal, anti-oxidants, anti-inflammatory and anti-cancer properties. These ligands includes Allyl methyl sulfide, Allyl methyl trisulfide, S-methyl cysteine, Diallyl sulfide, Alliin, E-ajoene, p-hydroxybenzoic acid, Sapogenins, Beta-chlorogenin, Allyl mercaptan, Apigenin,

Thiosulfinates, Methiin, Isoalliin, B-sitosterol, Campesterol, Biotin, Nicotinic acid, Quercetin and Myricetin.

3.8 Ligand Preparation

The 3-dimensional structure of the above-mentioned ligands have been downloaded from the PubChem database. PubChem is a database maintained by the National Center for Biotechnology Information (ncbi) that contains information about chemical molecules. The information saved is associated with chemical names and molecular formulas. 3D or simple structures, their isomers, canonical similes, and information about the molecules' activities in biological assays [128]. The structure of the ligands obtained from PubChem was downloaded, and the MM2 energy of the ligands was minimized using Chem3D ultra. If the selected ligand structure was not available, then next attempt was to download the canonical similes from PubChem, then i inserted them in the software ChemDraw, and later repeat the energy minimization step using Chem3D ultra after obtaining the 3D structure [129]. Finally, the sdf format was chosen to save the ligand's with energy-minimized structure.

3.9 Molecular Docking

CB-dock (Cavity detection guided blind docking) was used to perform molecular docking between the protein and the ligand. CB dock automatically locates docking locations. CB-Dock is a protein and ligand docking method that calculates the size and location of the bonding sites. The box size is adjusted based on the ligand, and docking was then performed. AutoDock Vina was used to dock the device. Because it focused on cavity binding, the accuracy ratio is higher. I uploaded the 3D structure of the protein in pdb format and the 3D structure of the ligand in sdf format for docking. The end result of this docking was five different poses of interaction. To choose the best pose, i considered the minimum vina score, which

was expressed in KJ/m-1. CB -Dock has displayed results in 5 different poses in an interactive 3D visualization. The best pose was chosen based on the lowest vina score (kJ/m-1) [130].

3.10 Visualization of Docking Result via PyMol

PyMol was used to display the docked complex of ligand and protein. It is a free open-source molecular visualization tool that can generate high-quality 3D images of proteins, small molecules, nucleic acids, and electron densities, among other things. This is capable of editing molecules, ray tracing, and creating movies. Docking poses generated by CB-Dock was visualized and saved as a molecule in.pdb format in a single file for further analysis [131].

3.11 Analysis of Docked Complex via LigPlot

Once we had the docked complex with the lowest vina score, the complex was analyzed. The complex was saved in pdb format. This analysis was carried out with the help of the software LigPlot.

The schematic diagrams of protein and ligand interactions was generated automatically for the given pdb file format. Hydrogen bonds and hydrophobic contacts influence these interactions. LigPlot analysed the hydrophobic and hydrogen bonding interactions [132].

3.12 Ligand ADMET Properties

Following the analysis, the next step was to investigate the pharmacokinetic and toxicity properties. During preclinical ADMET, the drug's weak candidates were eliminated. The remaining candidates were chosen as potential anti-cancer drugs. The PkCSM was used to optimize the ADMET of the human body [133].

3.13 Lead Compound Identification

After all the work was performed the next step was to find the lead compound. The lead compound was identified after applying the rule of 5 which includes

1. The log value of the drug-like compound must be limited to 5.
2. The molecular weight should also be lesser than 500.
3. Hydrogen bond acceptors maximum number should be 10.
4. Hydrogen bond donors' maximum number should be 5.
5. Number of rotatable bonds should be less than 10.

Once the compound fulfills the above mentioned rules it has been selected as our lead compound. The selected compound was our lead compound [134].

3.14 Inflammatory Drug Identification

The inflammatory drug identification refers to the identification of drugs that are used for inflammatory diseases, treatment purpose. KEGG stands for Kyoto Encyclopedia of genes and genomes. This online database is used for drug identification because it helps to analyze the disease in details with its pathway and drugs [135].

3.15 Inflammatory Drug Selection

The identified drugs must be filtered in order to select the most effective drug. This is done through a detailed study of identified drugs and most effective drug is identified setting parameters; physiochemical properties, effective ADMET properties, effective mechanism of action and minimal side effects. Physical Chemical Properties, ADMET Properties and mechanisms of action with drug side effects are collected from PubChem, Drug Bank, PKCSM, and KEGG databases [107].

3.16 Anti-Inflammatory Drug Docking

The identified drug was then docked with K-Ras GTPase and B-raaf protein to identify the inhibition efficiency. CB dock (Cavity-detection guided Blind Docking) is an online docking server which is used to perform docking. It can simplify docking procedures and improve accuracy by predicting target protein binding sites [108].

3.17 Comparison of Anticancer Drug Against Lungs Cancer and Lead Compound

Abraxane a drug with anti-cancer properties was used against lungs cancer and certain other mutagens were selected to compare with the lead compound. Abraxane has been used against tumor replication proteins and has shown effective results. The name of drug is based on US brand and it is FDA approved drug.

Despite the fact that much work has been done in developing drugs with medicinal plant, there is still a gap in the treatment and cure of this disease. The active compounds derived from Allium species that were chosen as the lead compound and shows more positive results when compared to the existing drug can be the future of medicinal drug against Lungs Cancer [136].

Chapter 4

Results and Discussions

4.1 Sequence Retrieval of Protein

The Primary sequence of target proteins (AChE and BChE) was downloaded in FASTA format from Uniprot using the accession number P22303 and P06276 respectively.

>spP01116RASK-HUMAN GTPase KRas OS=Homo sapiens OX=9606 GN=KRAS PE=1 SV=1 MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVV IDGETCLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQ IKRVKDSEDVPMVLVGNKCDLPSRTVDTKQAQDLARSYGIPFIETSAKTRQRVE DAFYTLVREIRQYRLKKISKEEKTPGCVKIKKCIIM

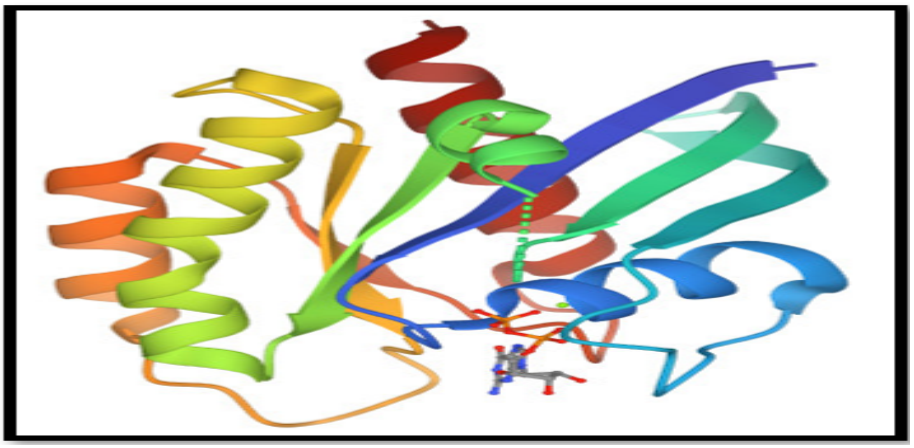


FIGURE 4.1: Structure of K-Ras Protein from PDB.

The structure of K-Ras protein which is available in PDB is shown in Figure 4.1 (https://www.rcsb.org/structure/4M22).

The structure of B-Raaf protein which is available in PDB is shown in Figure 4.2 (https://www.rcsb.org/structure/1UWJ).
>1UWJ-1Chains A, B—B-RAF PROTO-ONCOGENE SERINE/THREONINE-PROTEIN KINASEHOMO SAPIENS (9606)
DDWEIPDGQITVGQRIGSGSFGTVYKGKWHGDVAVKMLNVTAPTPQQLQA
FKNEVGVLRKTRHVNILLFMGYSTKPQLAIVTQWCEGSSLYHHLHIIETKFE
MIKLIDIARQTAQGMDYLHAKSIIHRDLKSNNIFLHEDLTVKIGDFGLATEKSR
WSGSHQFEQLSGSILWMAPEVIRMQDKNPYSFQSDVYAFGIVLYELMTGQLP
YSNINNRDQIIFMVGRGYLSPDLSKVRSNCPKAMKRLMAECLKKKRDERPLF
PQILASIELLARSLPK

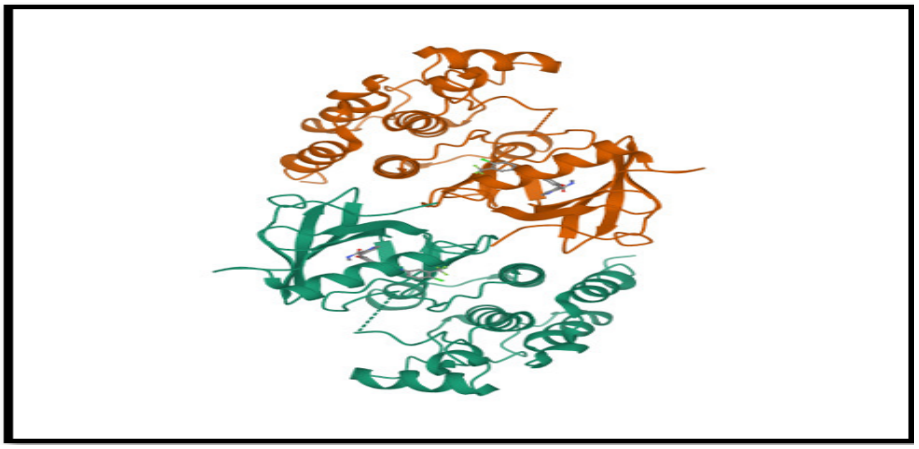


FIGURE 4.2: Structure of B-Raf Protein from PDB.

4.2 Analysis of Physiochemical Properties of Proteins

A tool of ExPASY named as ProtParam was used to study the properties of proteins AChE and BChE. It is an online program used to compute different physical and chemical properties of proteins stored in Swiss-prot or TrEMBL or

for the sequence of proteins that are entered by users. The parameters computed include molecular weight, atomic composition, proteins amino acid composition, estimated half-life, extinction co efficient, instability index, theoretical pI, aliphatic index and lastly grand average of hydropathicity (GRAVY). The pI of the protein represents acidity and basicity values [137]. pI greater means protein is basic in nature and less than 7 shows the acidic nature. Extinction coefficient shows absorption of light whereas instability index represents stability level of protein if it is lesser than 40 means protein is stable and values greater than 40 shows instability of protein [138].

The aliphatic index shows the aliphatic content of protein. The high level indicates the thermo stability of a protein. The molecular weight (MW) represents the values of both positive and negatively charged amino acid residues. PR shows positively charged residues (Arg+Lys) and NR indicates negatively charged residues (Asp+Glu). Low GRAVY shows better interaction of water molecules. All the above parameters were taken into consideration while performing research work. The physiochemical properties of the selected protein K-Ras and B-Raaf are shown in Table 4.1 and 4.2 respectively.

4.2.1 Physiochemical Properties of K-Ras Protein

Analysis of physicochemical parameters revealed that the K-Ras GTPase polypeptide is 189 amino-acid long with a molecular weight of 21655.83g/mol and a GRAVY score of -0.432, which gives the protein a stable, hydrophilic molecule capable of forming hydrogen bonds Table 4.1.

TABLE 4.1: Physiochemical properties of K-Ras G-12C Protein.

Parameters	**K-Ras G-12C protein**
Mol. Weight	21655.83
No. of amino acids	189
Theoretical pI	6.33
Instability index (II)	38.56

TABLE 4.1: Physiochemical properties of K-Ras G-12C Protein.

Parameters	**K-Ras G-12C protein**
No. of negatively charged residues (Asp + Glu)	29
No. of positively charged residues (Arg + Lys)	28
Aliphatic index	85.03
Grand average of hydropathicity (GRAVY)	-0.432
Atomic composition	Carbon-953, Hydrogen-1533, Nitrogen-261, Oxygen-293, Sulfur-10
Total number of atoms	3050
Amino acid composition	Ala-9(4.8%), Arg-12(6.3%), Asn-4(2.1%), Asp-14(7.4%), Cys-5(2.6%), Gln-10(5.3%), Glu-15(7.9%),Gly-11(5.8%), His-3(1.6%), Ile-15(7.9%), Leu-12(6.3%),Lys-16(8.5%), Met-5(2.6%), Phe-6(3.2%), Pro-5(2.6%), Ser-9(4.8%), Thr-13(6.9%),Trp-0(0.0%), Tyr-9(4.8%), Val-16(8.5%), Pyl-0(0.0%),Sec-0(0.0%)

4.2.2 Physiochemical Properties of K-Ras Protein

Analysis of physicochemical parameters revealed that the Serine/threonine-protein kinase B-raaf is 766 amino-acid long with a molecular weight of 84436.89 g/mol and a GRAVY score of -0.360, which gives the protein a stable, hydrophilic molecule capable of forming hydrogen bonds Table 4.2.

TABLE 4.2: Physiochemical properties of Serine/threonine-protein kinase B-raaf Protein.

Parameters	**B-Raf protein Kinase**
Mol. Weight	84436.89
No of amino acids	766
Theoretical pI	7.29
Instability index (II)	52.36
No. of negatively charged residues (Asp + Glu)	81
No. of positively charged residues (Arg + Lys)	81
Aliphatic index	78.45
Grand average of hydropathicity (GRAVY)	-0.360
Atomic composition	Carbon-3731, Hydrogen-5879, Nitrogen-1043, Oxygen-1131, Sulfur-31
Total number of atoms	11815

TABLE 4.2: Physiochemical properties of Serine/threonine-protein kinase B-raaf Protein.

Parameters	**B-Raf protein Kinase**
Amino acid composition	Ala-52(6.8%), Arg-40(5.2%), Asn-27 (3.5%), Asp-39(5.1%), Cys-13(1.7%), Gln-41(5.4%), Glu-42(5.5%), Gly-56 (7.3%), His-20(2.6%), Ile-43(5.6%), Leu-68(8.9%), Lys-41(5.4%), Met-18 (2.3%), Phe-33(4.3%), Pro-51(6.7%), Ser-78(10.2%), Thr-39(5.1%), Trp-8(1.0%), Tyr-17(2.2%), Val-40(5.2%), Pyl-0(0.0%), Sec-0(0.0%)

4.3 Structure of Protein Refined for Docking

PyMol is used to refine the selected protein before it is used in molecular docking. Refined 3D structure of K-Ras GTPase and B-Raf is shown in Figure 4.3 and 4.4 respectively.

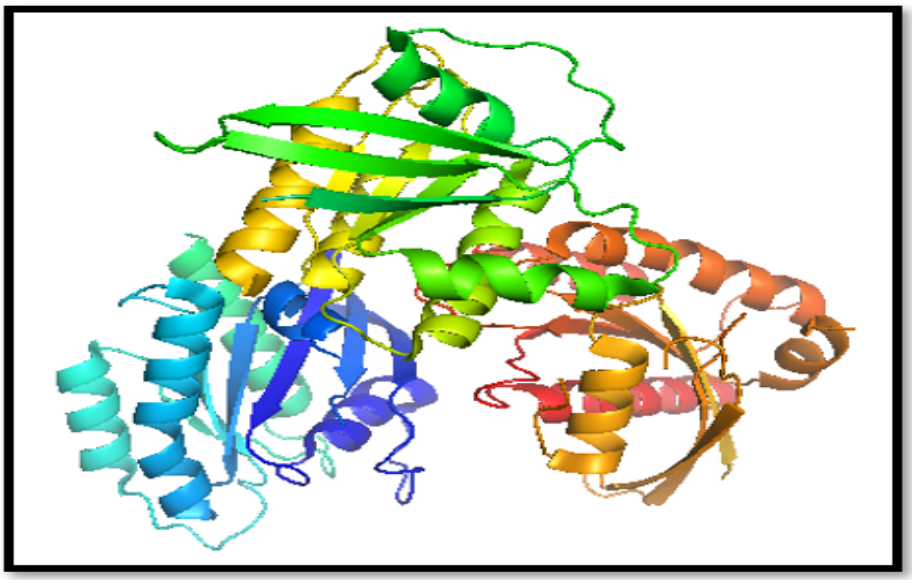


FIGURE 4.3: Refined structure of K-Ras GTPase for Docking.

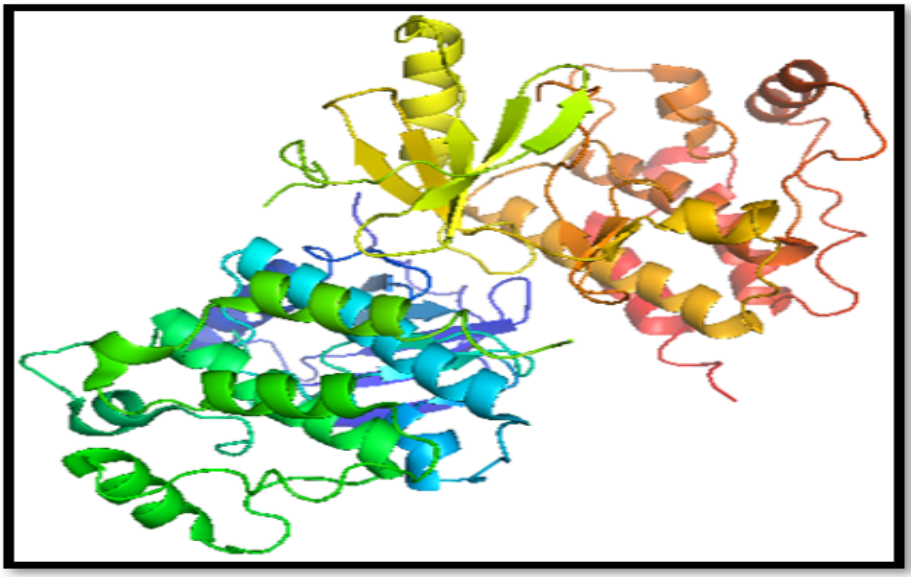


FIGURE 4.4: Refined structure of B-Raaf protease for Docking.

4.4 Identification of Functional Domains

Many proteins are made up of several functional domains, which are active parts of the protein that interact with other compounds [139]. Functional domain of proteins can be identified using InterPro. Proteins can have more than one domain performing different functions. Functional domains are major part of a protein and are sites utilized by proteins to interact with other protein or other substances. InterPro is an online database of protein families that helps in functional analysis

of proteins and classifies them into families by identifying domains and other important sites.

TABLE 4.3: Identification of functional domains.

S. No	Name	Domain	Start	End
1.	K-Ras GTPase	Small-GTP-bd-dom	2	60
2.	B-raaf	Ser-Thr/Tyr-kinase-cat-dom	11	265
3.	B-raaf	Prot-kinase-dom	10	270

The job ID for finding functional domain of K-Ras is https://www.ebi.ac.uk/interpro/result/InterProScan/iprscan5-R20220918-180704-0991-45557229\-p2m/

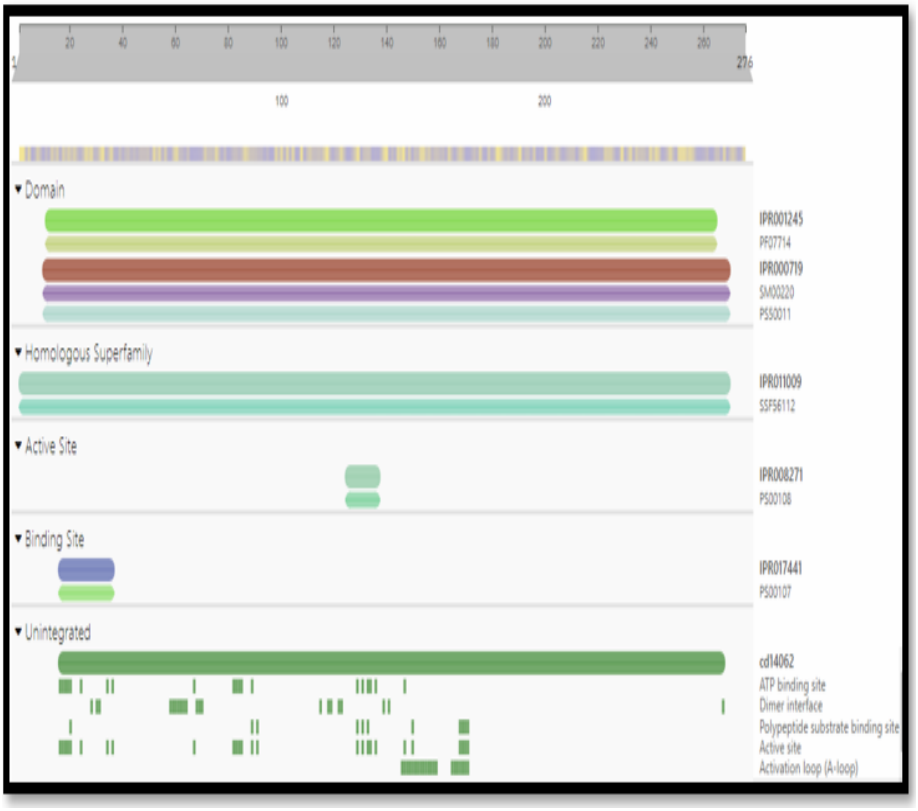


FIGURE 4.5: Functional domains of K-Ras Protein

The job ID for finding functional domain of B-Raaf is https://www.ebi.ac.uk/interpro/result/InterProScan/iprscan5-R20220809-181808-0328-14287152\-p2m/

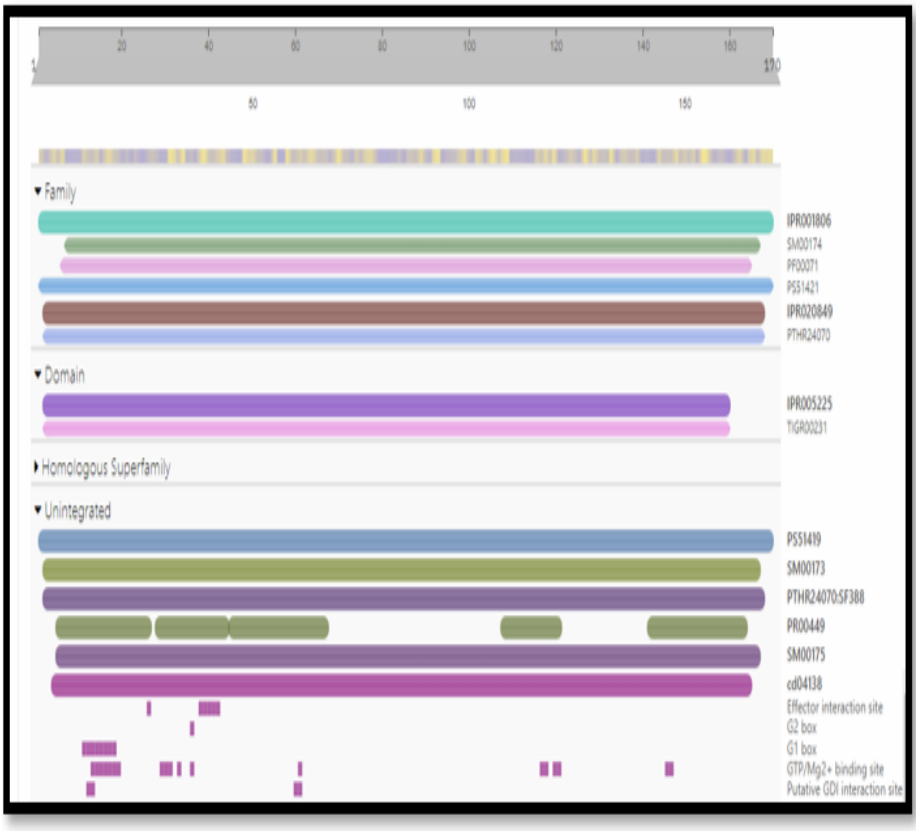


FIGURE 4.6: Functional domains of B-Raaf Protein

4.5 Ligand Selection

The PDB (Protein data Bank) contains abundant data related to protein ligand complexes. For this reason, the selection of ligand was based on its resolution structure with chemical class of the protein and their best binding affinities. This selection process required selective binding of ligand with the conformer strengthening it and increasing its population with respect to the population of the protein.

Several bioactive compounds obtained from *Allium* sativum show potential targets against receptor proteins K-Ras and B-Raaf. These inhibitory compounds were searched from world's largest chemical databank-PubChem (https://pubchem.ncbi.nlm.nih.gov). The 3D structures of these ligands were downloaded in sdf format. After downloading the structures of selected ligands the energy of ligands was minimized in the next step. This is important step as simple downloaded structures of the ligands cannot be used because the instability of ligands can affect the vina scores while docking.

TABLE 4.4: Ligands from *Allium sativum*

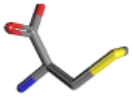
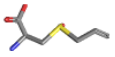
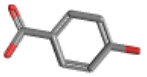
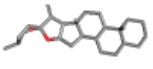
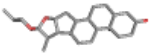
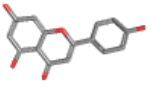
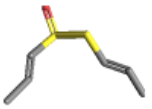
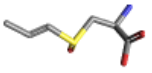
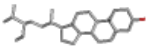
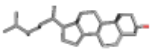
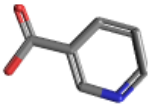
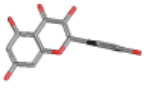
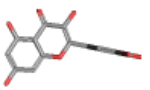
Compound	**Molecular Formula**	**Molecular Weight**	**Structure**
Allyl methyl sulfide	C_4H_8S	88.17g/mol	
Allyl methyl trisulfide	$C_4H_8S_3$	152.3g/mol	
S-methyl cysteine	$C_4H_9NO_2S$	135.19g/mol	
Diallyl sulfide	$C_6H_{10}S$	114.21g/mol	
Alliin	$C_6H_{11}NO_3S$	177.22g/mol	
E-ajoene	$C_9H_{14}OS_3$	234.4g/mol	
p-hydroxy-benzoic acid	$C_7H_6O_3$	138.12g/mol	
Sapogenins	$C_{27}H_{44}O_2$	400.6g/mol	
Beta-chlorogenin	$C_{27}H_{44}O_4$ C27H44O4	432.6g/mol	
Allyl mercaptan	C_3H_6S	74.15g/mol	

Table 4.4 continued from previous page

Compound	Molecular Formula	Molecular Weight	Structure
Apigenin	$C_{15}H_{10}O_5$	270.24g/mol	
Thiosulfinates	$C_6H_{10}OS_2$	162.3g/mol	
Methiin	$C_4H_9NO_3S$	151.19g/mol	
Isoalliin	$C_6H_{11}NO_3S$	177.22g/mol	
B-sitosterol	$C_9H_{50}O$	414.7g/mol	
Campesterol	$C_{28}H_{48}O$	400.7g/mol	
Biotin	$C_{10}H_{16}N_2O_3S$	244.31g/mol	
Nicotinic acid	$C_6H_5NO_2$	123.11g/mol	
Quercetin	$C_{15}H_{10}O_7$	302.23g/mol	
Myricetin	$C_{15}H_{10}O_8$	318.23g/mol	

4.6 Virtual Screening

The most important issue during drug development is safety, which includes a variety of toxicities and unfavorable drug effects that should be evaluated in the preclinical and clinical phases [140]. Selected ligands from the PubChem database follow the Lipinski rule, as shown in Table 4.5. The log p value of the molecule should be limited to 5, the molecular weight should be less than 500, the maximum number of H bond acceptors should be 10, and the maximum number of H bond donors should be 5 [141].

TABLE 4.5: Applicability of Lipinski Rule on Ligands of *Allium sativum*

Ligand	**Log P Value**	**Molecular weight (g/mol)**	**H-bond Acceptor**	**H-bond Donnor**	**Rotatable bonds**
Allyl methyl sulfide	1.5354	88.17	1	0	2
Allyl methyl trisulfide	2.8318	152.3	3	0	4
S-methyl cysteine	-0.2387	135.19	4	2	3
Diallyl sulfide	2.61	114.21	1	0	4
Alliin	-0.667	177.22	5	2	5
E-ajoene	3.0022	234.4	4	0	8
p-hydroxy benzoic acid	1.58	138.12	3	2	1
Sapogenins	6.823	400.6	2	0	0
Beta-chlorogenin	4.7646	432.6	4	2	0

TABLE 4.5: Applicability of Lipinski Rule on Ligands of *Allium sativum*

Ligand	**Log P Value**	**Molecular weight (g/mol)**	**H-bond Acceptor**	**H-bond Donnor**	**Rotatable bonds**
Allyl mercaptan	1.1022	74.15	1	1	1
Apigenin	3.02	270.24	5	3	1
Thiosulfinates	2.4505	162.3	3	0	3
Methiin	-1.2232	151.19	5	2	3
Isoalliin	-0.3194	177.22	5	2	4
B-sitosterol	8.0248	414.7	1	1	6
Campesterol	7.6347	400.7	1	1	5
Biotin	0.5	244.31	4	3	5
Nicotinic acid	0.147	123.11	3	1	1
Quercetin	1.48	302.23	7	5	1
Myricetin	1.42	318.23	8	6	1

4.7 ADMET Properties of Ligands

ADMET properties of ligands were identified via pkCSM online tool by putting input (ligands) as SMILES. ADMET properties describes the influence of drug level, kinetics and pharmacological activity of a compound that would be used as drug [142]. ADMET properties of selected compounds are shown in tables below.

4.7.1 Absorption

Normally, a compound must enter the bloodstream before reaching a tissue, often via mucous surfaces such as the digestive tract (intestinal absorption), before passing through the target cells [143]. Absorptive properties of selected compounds

are given in Table 4.6 and 4.7.

TABLE 4.6: Absorptive properties of ligands of *Allium sativum*

Ligand/ Properties	**Water solubility**	**Caco2 permeability**	**Intestinal absorption**	**Skin permeability**
Allyl methyl sulfide	-1.624	1.391	96.323	-1.953
Allyl methyl trisulfide	-2.796	1.397	92.885	-1.652
S-methyl cysteine	-2.887	0.589	83.271	-2.737
Diallyl sulfide	-2.695	1.394	96.268	-1.488
Alliin	-2.888	0.619	76.495	-2.735
E-ajoene	-3.54	1.329	95.186	-1.745
p-hydroxy benzoic acid	-1.877	1.151	83.961	-2.723
Sapogenins	-5.609	1.364	99.318	-2.416
Beta-chlorogenin	-5.213	1.263	96.823	-3.999
Allyl mercaptan	-0.887	1.385	100	-2.202
Apigenin	-3.329	1.007	93.25	2.735
Thio-sulfinates	-2.043	1.329	94.704	-1.776
Methiin	-2.888	0.504	79.681	-2.735
Isoalliin	-2.888	0.569	76.556	-2.735
B-sitosterol	-6.773	1.201	94.464	-2.783
Campesterol	-7.068	1.223	94.543	-2.86

TABLE 4.6: Absorptive properties of ligands of *Allium sativum*

Ligand/ Properties	**Water solubility**	**Caco2 permeability**	**Intestinal absorption**	**Skin permeability**
Biotin	-2	0.698	71.182	-2.727
Nicotinic acid	-2.134	1.17	94.099	-2.735
Quercetin	-2.925	-0.229	77.207	-2.735
Myricetin	-2.915	0.095	65.93	-2.735

TABLE 4.7: Absorptive properties of ligands of *Allium sativum*

Ligand/ Properties	**P-glyco protein substrate**	**P-glycoprotein I inhibitor**	**P-glycoprotein II inhibitor**
Allyl methyl sulfide	Yes	No	No
Allyl methyl trisulfide	Yes	No	No
S-methyl cysteine	No	No	No
Diallyl sulfide	No	No	No
Alliin	No	No	No
E-ajoene	No	No	No
p-hydroxy benzoic acid	No	No	No
Sapogenins	No	Yes	Yes

TABLE 4.7: Absorptive properties of ligands of *Allium sativum*

Ligand/ Properties	**P-glyco protein substrate**	**P-glycoprotein I inhibitor**	**P-glycoprotein II inhibitor**
Beta-chlorogenin	Yes	Yes	Yes
Allyl mercaptan	Yes	No	No
Apigenin	Yes	No	No
Thio-sulfinates	No	No	No
Methiin	No	No	No
Isoalliin	No	No	No
B-sitosterol	No	Yes	Yes
Campesterol	No	Yes	Yes
Biotin	No	No	No
Nicotinic acid	No	No	No
Quercetin	Yes	No	No
Myricetin	Yes	No	No

4.7.2 Distribution

In pharmacology, distribution refers to the movement of a drug from one location within the body to another. When a drug enters the bloodstream via direct administration or absorption, it must be distributed into intracellular and interstitial fluid [144]. The distribution properties of compounds are shown in Table 4.8.

TABLE 4.8: Distributive properties of ligands of *Allium sativum*

Ligand/ Properties	**VDss**	**Fraction unbound**	**BBB permeability**	**CNS permeability**
Allyl methyl sulfide	0.084	0.65	0.167	-2.237
Allyl methyl trisulfide	0.112	0.574	0.437	-2.435
S-methyl cysteine	-0.557	0.46	-0.363	-3.433
Diallyl sulfide	0.202	0.552	0.69	-2.102
Alliin	-0.553	0.462	-0.271	-3.472
E-ajoene	0.083	0.395	0.703	-2.178
p-hydroxy benzoic acid	-1.557	0.592	-0.334	-3.21
Sapogenins	-0.1	0	0.772	-1.8
Beta-chlorogenin	0.192	0.037	0.004	-1.592
Allyl mercaptan	0.055	0.691	0.113	-2.307
Apigenin	0.822	0.147	-0.734	-2.061
Thio-sulfinates	-0.073	0.541	0.574	-2.206
Methiin	-0.548	0.477	-0.25	-3.486
Isoalliin	-0.554	0.463	-0.242	-3.445
B-sitosterol	0.193	0	0.781	-1.705

TABLE 4.8: Distributive properties of ligands of *Allium sativum*

Ligand/ Properties	**VDss**	**Fraction unbound**	**BBB permeability**	**CNS permeability**
Campesterol	0.427	0	0.774	-1.758
Biotin	-0.933	0.58	-0.679	-3.541
Nicotinic acid	-1.015	0.776	-0.323	-2.869
Quercetin	1.559	0.206	-1.098	-3.065
Myricetin	1.317	0.238	-1.493	-3.709

4.7.3 Metabolism

Metabolism is the term used to describe the catabolic and anabolic reactions of compounds in the body that are carried out by enzymes. In general, metabolism occurs in the plasma of the blood, the liver, the intestine, and the lungs [145]. The metabolic properties of selected compounds are shown in Table 4.9.

TABLE 4.9: Metabolic properties of ligands of *Allium sativum*

Ligand/ Properties	**CYP 2D6 substrate**	**CYP 3A4 substrate**	**CYP 1A2 inhibitor**	**CYP 2C19 inhibitor**	**CYP 2C9 inhibitor**	**CYP 2D6 inhibitor**	**CYP 3A4 inhibitor**
Allyl methyl sulfide	No	No	No	No	No	No	No
Allyl methyl trisulfide	No	No	No	No	No	No	No
S-methyl cysteine	No	No	No	No	No	No	No

TABLE 4.9: Metabolic properties of ligands of *Allium sativum*

Ligand/ Properties	**CYP 2D6 substrate**	**CYP 3A4 substrate**	**CYP 1A2 inhibitor**	**CYP 2C19 inhibitor**	**CYP 2C9 inhibitor**	**CYP 2D6 inhibitor**	**CYP 3A4 inhibitor**
Diallyl sulfide	No	No	No	No	No	No	No
Alliin	No	No	No	No	No	No	No
E-ajoene	No	No	No	No	No	No	No
p-hydroxy benzoic acid	No	No	No	No	No	No	No
Sapogenins	No	Yes	No	No	No	No	No
Beta-chlorogenin	No	Yes	No	No	No	No	No
Allyl mercaptan	No	No	No	No	No	No	No
Apigenin	No	No	Yes	Yes	No	No	No
Thio-sulfinates	No	No	No	No	No	No	No
Methiin	No	No	No	No	No	No	No
Isoalliin	No	No	No	No	No	No	No
B-sitosterol	No	Yes	No	No	No	No	No
Campe-sterol	No	Yes	No	No	No	No	No
Biotin	No	No	No	No	No	No	No
Nicotinic acid	No	No	No	No	No	No	No
Quercetin	No	No	Yes	No	No	No	No
Myricetin	No	No	Yes	No	No	No	No

4.7.4 Excretion

In pharmacology, excretion refers to the removal of compounds and their metabolites via the kidneys or the faeces. Drug excretion occurs in three stages: renal excretion via the kidneys, faecal excretion via the liver, and gaseous excretion via the lungs [146]. Excretory properties of compounds are shown in Table 4.10.

TABLE 4.10: Excretion properties of ligands of *Allium sativum*

Ligand/ Properties	**Total clearance**	**Renal OCT2 substrate**
Allyl methyl sulfide	0.443	No
Allyl methyl trisulfide	0.347	No
S-methyl cysteine	0.467	No
Diallyl sulfide	0.555	No
Alliin	0.365	No
E-ajoene	0.538	No
p-hydroxybenzoic acid	0.593	No
Sapogenins	0.311	No
Beta-chlorogenin	0.346	Yes
Allyl mercaptan	0.359	No
Apigenin	0.566	No
Thiosulfinates	0.106	No
Methiin	0.281	No
Isoalliin	0.242	No
B-sitosterol	0.628	No
Campesterol	0.572	No
Biotin	0.368	No
Nicotinic acid	0.652	No
Quercetin	0.407	No
Myricetin	0.422	No

4.7.5 Toxicity

PkCSM is an online tool which provides an integrated platform to rapidly evaluate pharmacokinetics and toxicity properties of a drug. So this tool is used to find out the toxicity measurements of ligands against K-Ras and B-Raf , which are the targeted proteins. Toxicity of different ligands are shown in Table 4.11 and 4.12.

TABLE 4.11: Toxicity prediction of ligands of *Allium sativum*

Ligand/ Properties	**AMES toxicity**	**Max. tolerated dose**	**hERG I inhibitor**	**hERG II inhibitor**	**Oral Rat Acute Toxicity (LD50)**
Allyl methyl sulfide	No	0.946	No	No	2.182
Allyl methyl trisulfide	No	0.727	No	No	2.845
S-methyl cysteine	No	1.134	No	No	1.998
Diallyl sulfide	No	0.782	No	No	2.028
Alliin	No	1.164	No	No	2.051
E-ajoene	No	0.462	No	No	2.472
p-hydroxybenzoic acid	No	0.846	No	No	2.255
Sapogenins	No	0.024	No	Yes	2.285
Beta-chlorogenin	No	-0.678	No	No	1.976
Allyl mercaptan	No	1.164	No	No	2.213

TABLE 4.11: Toxicity prediction of ligands of *Allium sativum*

Ligand/ Properties	**AMES toxicity**	**Max. tolerated dose**	**hERG I inhibitor**	**hERG II inhibitor**	**Oral Rat Acute Toxicity (LD50)**
Apigenin	No	0.328	No	No	2.45
Thiosulfinates	No	0.818	No	No	2.504
Methiin	No	1.18	No	No	2.031
isoalliin	No	1.172	No	No	2.052
B-sitosterol	No	-0.621	No	Yes	2.552
Campesterol	No	-0.458	No	Yes	2.08
Biotin	No	0.11	No	No	1.876
Nicotinic acid	No	0.907	No	No	2.24
Quercetin	No	0.499	No	No	2.471
Myricetin	No	0.51	No	No	2.497

TABLE 4.12: Toxicity prediction of ligands of *Allium sativum*

Ligand/ Properties	**Oral Rat Chronic Toxicity (LOAEL)**	**Hepato-toxicity**	**Skin Sensiti-sation**	**T. Pyriformis toxicity**	**Minnow toxicity**
Allyl methyl sulfide	1.723	No	No	0.039	1.722
Allyl methyl trisulfide	1.728	No	Yes	1.416	1.051

TABLE 4.12: Toxicity prediction of ligands of *Allium sativum*

Ligand/ Properties	**Oral Rat Chronic Toxicity (LOAEL)**	**Hepato-toxicity**	**Skin Sensiti-sation**	**T. Pyriformis toxicity**	**Minnow toxicity**
S-methyl cysteine	2.569	No	No	0.119	2.613
Diallyl sulfide	1.812	No	Yes	0.63	1.154
Alliin	1.9	No	No	0.268	2.598
E-ajoene	0.899	No	Yes	2.197	0.155
p-hydroxy-benzoic acid	2.483	No	No	0.268	1.812
Sapogenins	1.786	No	No	0.319	-0.995
Beta-chlorogenin	1.29	No	No	0.355	1.027
Allyl mercaptan	1.622	No	No	-0.63	2.101
Apigenin	2.298	No	No	0.38	2.432
Thio-sulfinates	1.764	No	Yes	0.779	1.157
Methiin	1.89	No	No	0.263	3.182
Isoalliin	1.853	No	No	0.268	2.559
B-sitosterol	0.855	No	No	0.43	-1.802
Campesterol	0.892	No	No	0.631	-1.94
Biotin	2.05	Yes	No	0.3	2.183
Nicotinic acid	2.652	No	No	0.055	2.222
Quercetin	2.612	No	No	0.288	3.721
Myricetin	2.718	No	No	0.286	5.023

4.8 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. 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. 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 for the target [147]. CB-dock (Cavity detection guided blind docking) was used to perform molecular docking between the protein and the ligand. CB dock automatically locates docking locations. CB-Dock is a protein and ligand docking method that calculates the size and location of the bonding sites. The box size is adjusted based on the ligand, and docking was then performed.

We used the user-friendly blind docking web server CB Dock, which predicts and estimates a binding site for a given protein, calculates centres and sizes with a novel rotation cavity detection method, and performs docking with the well-known docking programme Auto dock Vina [148]. This allowed us to automatically predict binding modes without knowledge of binding sites. Each ligand molecule receives 5 best interacting confirmations from CB dock. These confirmations are organised according to binding affinity, and the best confirmations are chosen based on the interaction between the protein and the ligand that has the highest affinity score. [148].

After docking process the dock structure are selected for further analysis, on the basis of docking score, cavity size, Grid map, binding energy, we can select best docked structure. Table 4.13 displays the ligands with highest binding scores.

TABLE 4.13: Ligands of *Allium sativum* with Best Binding Score Values with K-Ras GTPase

Ligand	**Binding score (kJ/ m-1)**	**Cavity size**	**H B D**	**H B A**	**Log P**	**Molecular weight (g/mol)**	**Rotatable bond**
Allyl methyl sulfide	-2.9	4973	0	1	1.53	88.1	2
Allyl methyl trisulfide	-3.2	542	0	3	2.83	152.3	4
S-methyl cysteine	-4.5	1382	2	3	-0.23	135.1	3
Diallyl sulfide	-3.6	4973	0	1	2.09	114.2	4
Alliin	-5.1	4973	2	3	-0.66	177.2	5
E-ajoene	-5	1382	0	3	3.002	234.4	8
p-hydroxy 6CH	-6	4773	2	2	1.09	138.1	1
Sapogenins	-8.8	542	0	2	6.8	400.6	0
Beta-chlorogenin	-9	1382	2	4	4.76	432.6	0
Allyl mercaptan	-2.6	4973	1	1	1.10	74.1	1
Apigenin	-8.8	542	3	5	2.57	270.2	1
Thiosulfinat	-4.8	4973	0	2	2.4505	162.279	3
Methiin	-5.1	1382	2	3	-1.2	151.1	3
Isoalliin	-5.4	4973	2	3	-0.3	177.2	4
B-sitosterol	-9	1382	1	1	8.02	414.7	6
Campesterol	-9.1	1382	1	1	7.63	400.6	5
Biotin	-6.4	4973	3	3	0.79	244.3	5
Nicotinic acid	-5.6	4973	1	2	0.77	123.1	1
Quercetin	-8.9	542	5	7	1.9	302.2	1
Myricetin	-9.1	1382	6	8	1.69	318.2	1

TABLE 4.14: Ligands of *Allium sativum* with Best Binding Score Values with B-Raf protein kinase

Ligand/ Properties	**Binding score (kJ/ m-1)**	**Cavity size**	**H B D**	**H B A**	**Log P**	**Molecular weight (g/mol)**	**Rotatable bond**
Allyl methyl sulfide	-3.1	3919	0	1	1.5	88.1	2
Allyl methyl trisulfide	-3.4	1974	0	3	2.8	152.3	4
S-methyl cysteine	-4.7	3919	2	3	-0.2	135.1	3
Diallyl sulfide	-3.8	365	0	1	2.0	114.2	4
Alliin	-5.2	3919	2	3	-0.6	177.2	5
E-ajoene	-5.1	3919	0	3	3.0	234.4	8
p-hydroxy 6CH	-6.1	1974	2	2	1.0	138.1	1
Sapogenins	-10.9	3919	0	2	6.8	400.6	0
Beta-chlorogenin	-10.7	3919	2	4	4.7	432.6	0
Allyl mercaptan	-2.7	1974	1	1	1.1	74.1	1
Apigenin	-9.4	3919	3	5	2.5	270.2	1
Thiosulfinates	-4.7	3919	0	2	2.4	162.2	3
Methiin	-5	1974	2	3	-1.2	151.1	3
Isoalliin	-5.3	1974	2	3	-0.3	177.2	4
B-sitosterol	-11.6	1974	1	1	8.0	414.7	6
Campesterol	-9.9	1974	1	1	7.6	400.6	5
Biotin	-7	1974	3	3	0.7	244.3	5
Nicotinic acid	-5.4	1974	1	2	0.7	123.1	1
Quercetin	-9.5	1974	5	7	1.9	302.2	1
Myricetin	-9.6	3919	6	8	1.6	318.2	1

4.9 Interaction of Ligands and Targeted Protein

The interaction of the active pockets of the ligand and the protein are calculated for the interpretation of docking results. Two types of interactions are studied; hydrogen bonding and hydrophobic bonding interaction. Using Ligplot plus (version v.1.4.5) the protein ligand interactions are studied [149]. By using PDBsum the interaction of active confirmation of ligands and the target protein has been identified. The saved conformations for ligand receptor complex of each molecule are analyzed in detail. This software automatically generates schematic diagrams of the protein-ligand interactions of the given ligands in the PDB file. The docked files are uploaded in PDBsum to get hydrogen and hydrophobic bonding. Hydrogen bonding and hydrophobic interactions of active ligands are shown in Table 4.20. A significant number of hydrophobic and hydrogen bond interactions are observed between the twenty ligands and the two target proteins. Ligand-receptor complex shows strong hydrogen bonding, hydrophobic interactions and van der Waal forces [150]. 2D representation of ligands from Allium Sativum with K-Ras protein docked complexes are shown in Figures 4.7 to 4.17.

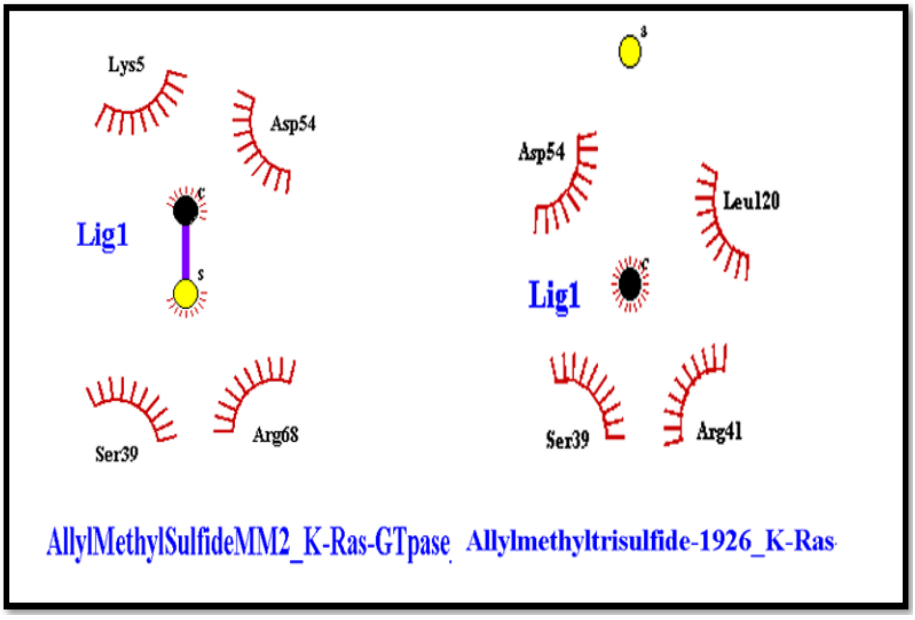


FIGURE 4.7: Interaction of allyl methyl sulfide and allyl methyl trisulfide with K-Ras GTPase.

Interaction of allyl methyl sulfide and allyl methyl trisulfide with K-Ras GTPase.

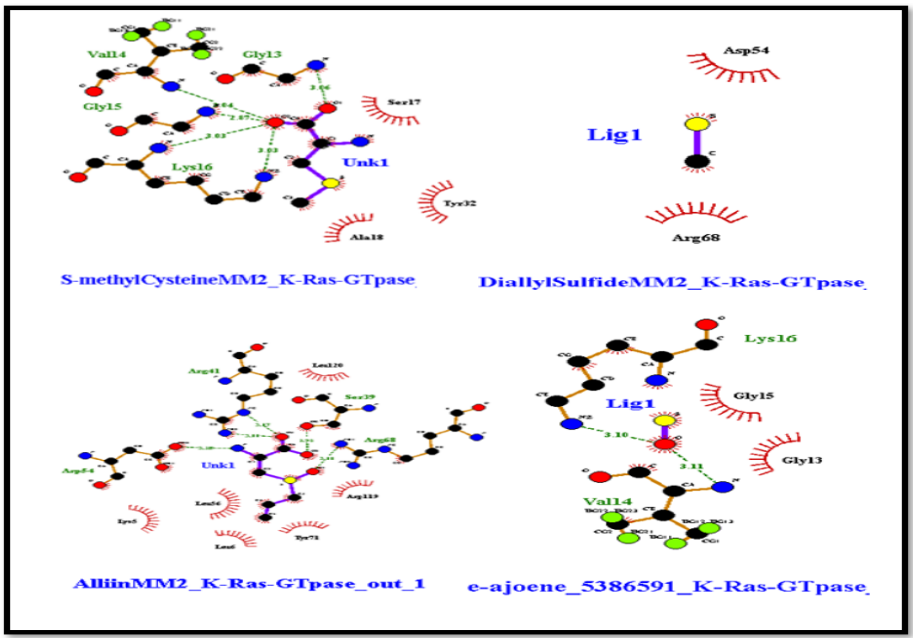


FIGURE 4.8: Interaction of S-methyl cysteine, Diallyl sulfide, Alliin and e-Ajoene with K-Ras GTPase.

Interaction of S-methyl cysteine, Diallyl sulfide, Alliin and e-Ajoene with K-Ras GTPase.

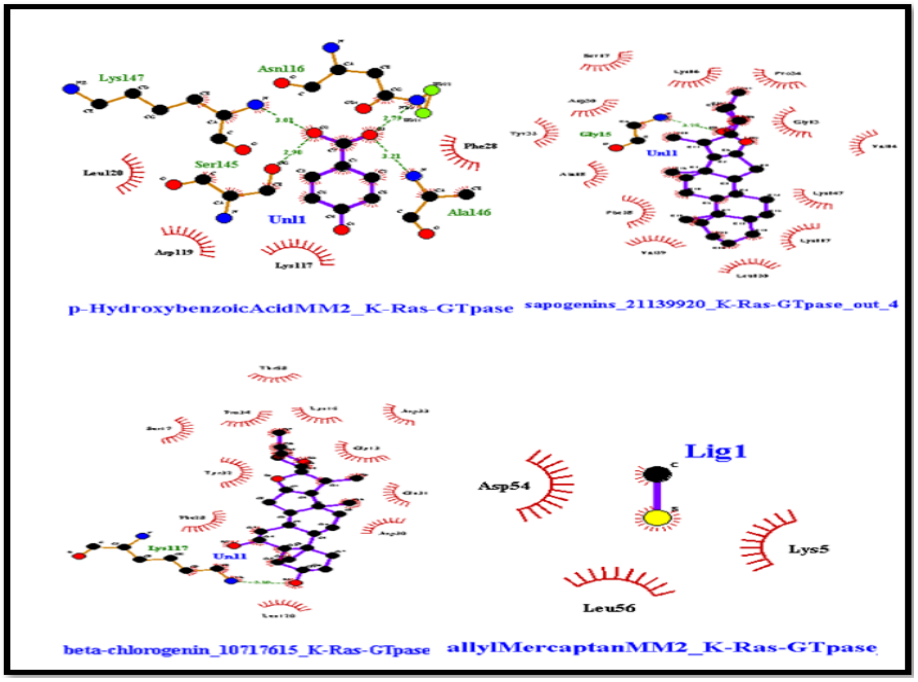


FIGURE 4.9: Interaction of p-hydroxybenzoic Acid, sapogenin, beta-chlorogenin and Allyl mercaptan with K-Ras GTPase

Interaction of p-hydroxybenzoic Acid, sapogenin, beta-chlorogenin and Allyl mercaptan with K-Ras GTPase

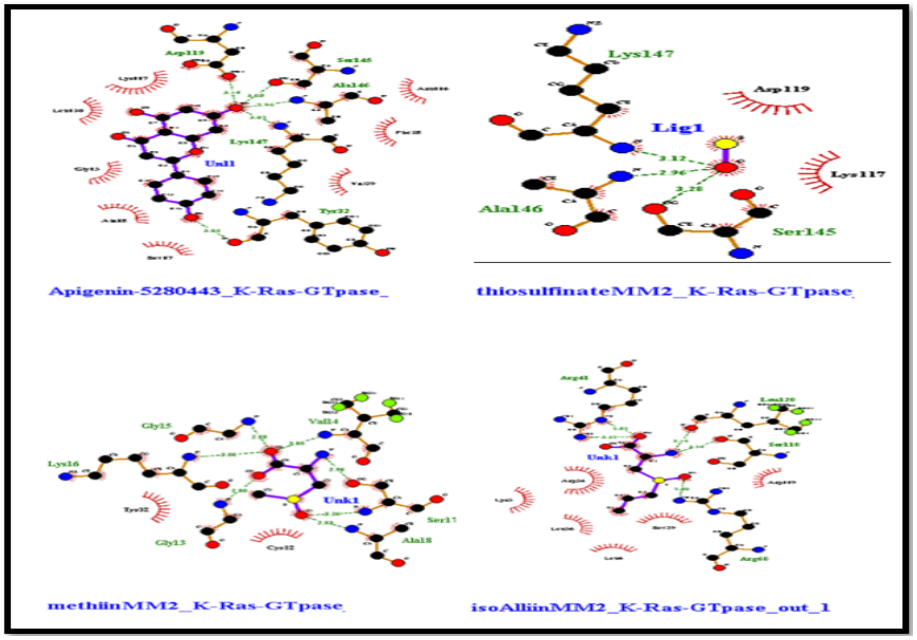


FIGURE 4.10: Interaction of apigenin, thiosulfinate, methiin and isoAlliin with K-Ras GTPase

Interaction of apigenin, thiosulfinate, methiin and isoAlliin with K-Ras GTPase

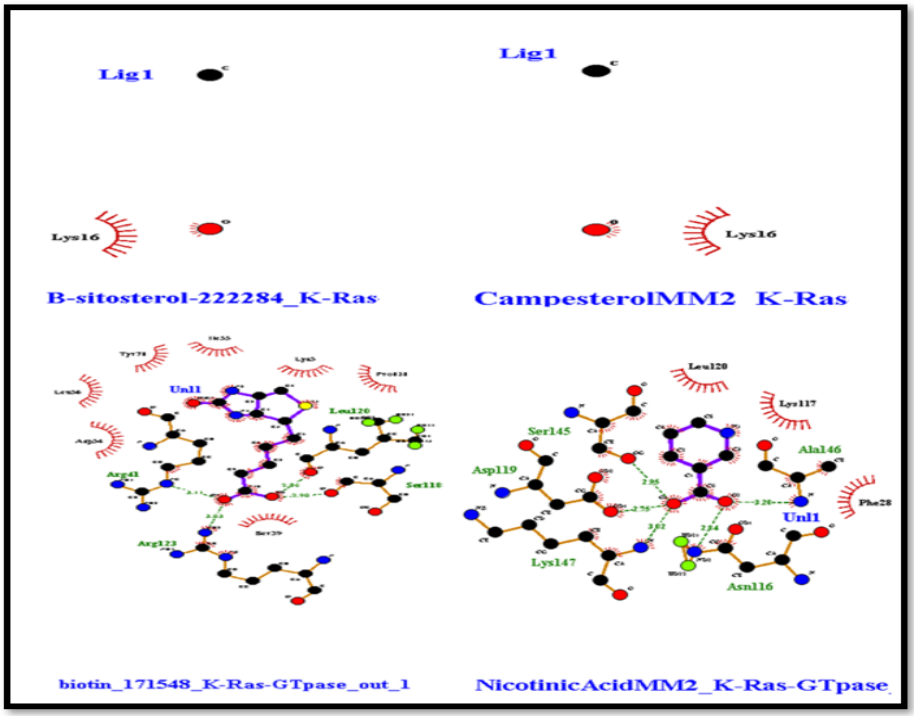


FIGURE 4.11: Interaction of B-sitosterol, campesterol, biotin and Nicotinic acid with K-Ras GTPase.

Interaction of B-sitosterol, campesterol, biotin and Nicotinic acid with K-Ras GTPase.

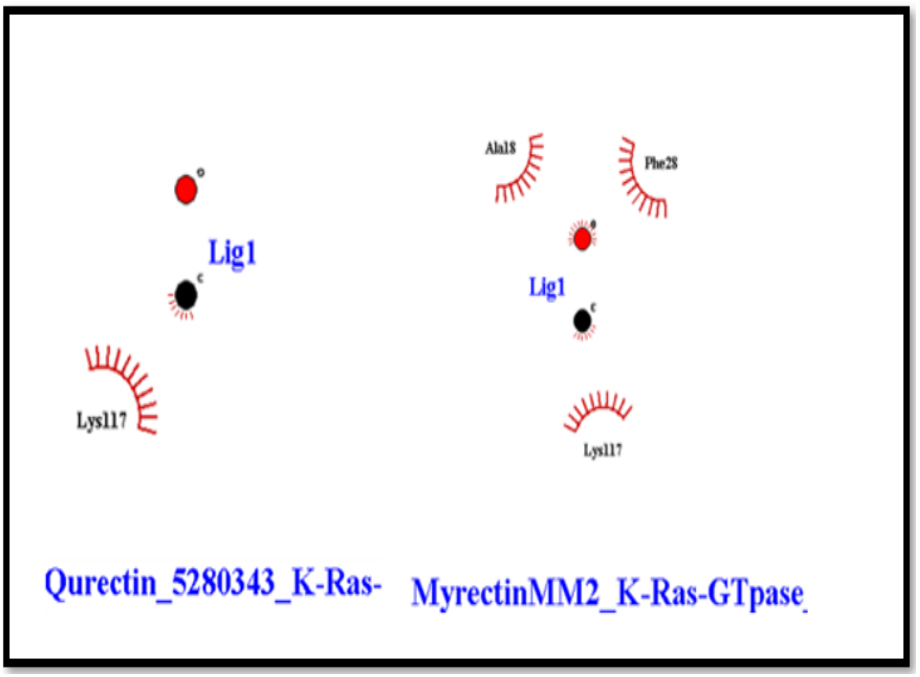


FIGURE 4.12: Interaction of qurectin and Myrectin with K-Ras GTPase

Interaction of qurectin and Myrectin with K-Ras GTPase.

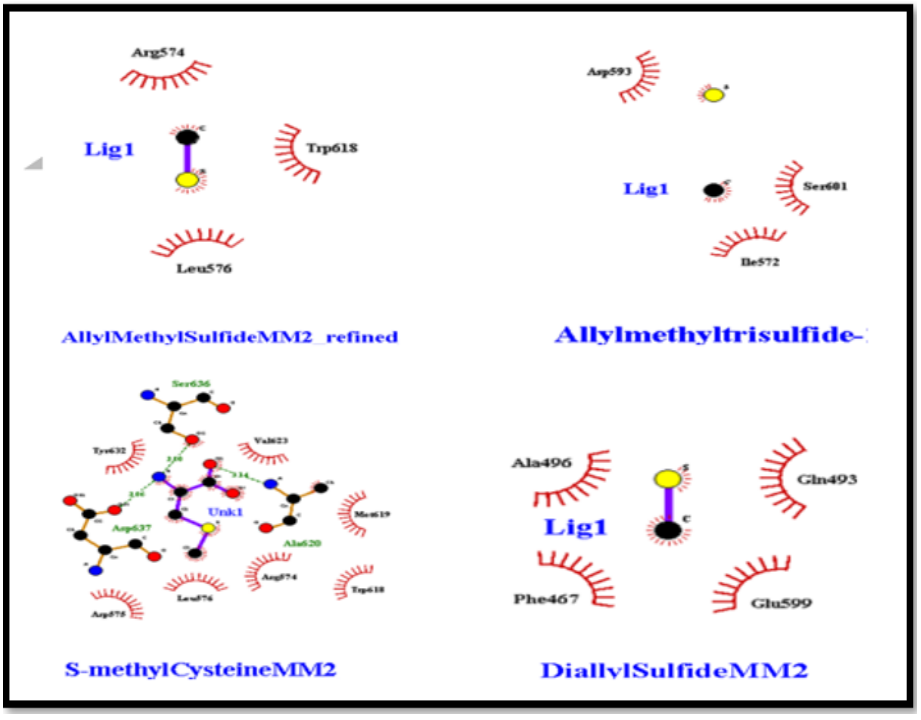


FIGURE 4.13: Interaction of allyl methyl sulfide, allyl methyl trisulfide, S-methyl cysteine and Diallyl sulfide with B-raaf protease.

Interaction of allyl methyl sulfide, allyl methyl trisulfide, S-methyl cysteine and Diallyl sulfide with B-raaf protease

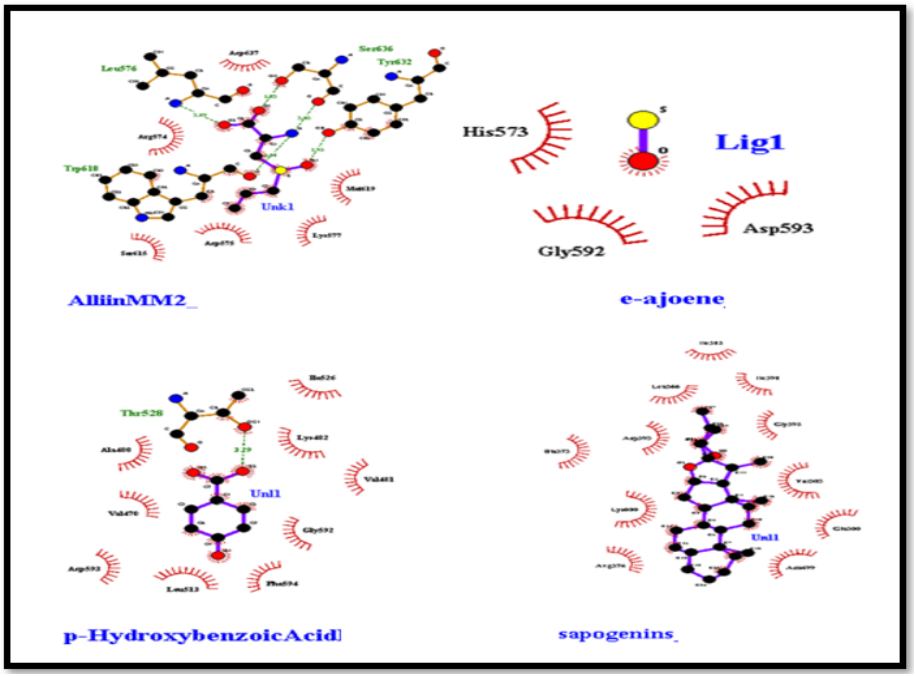


FIGURE 4.14: Interaction of Alliin, e-Ajoene, p-hydroxybenzoic Acid and sapogenins with B-raaf protease

Interaction of Alliin, e-Ajoene, p-hydroxybenzoic Acid and sapogenins with B-raaf protease.

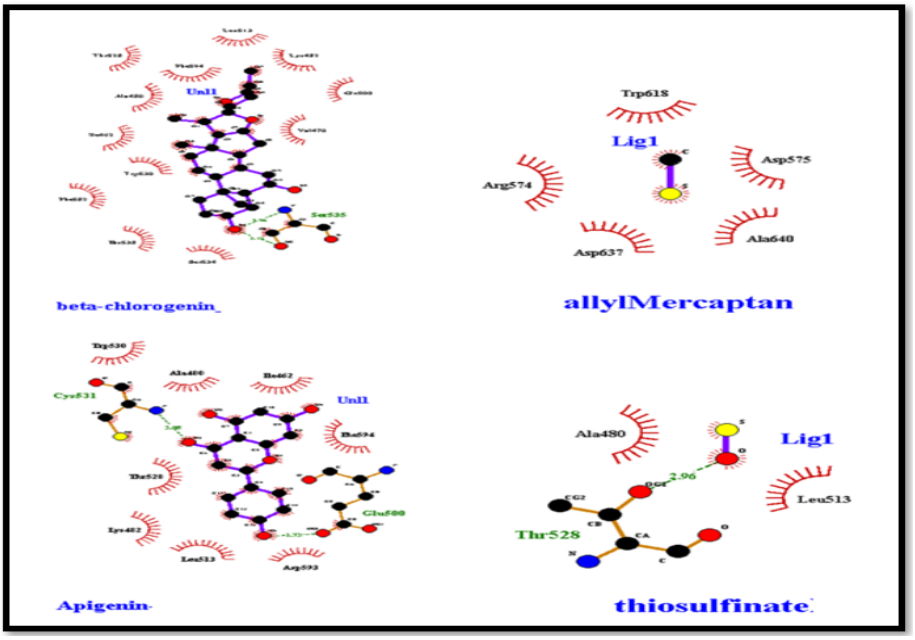


FIGURE 4.15: Interaction of beta-chlorogenin, allyl mercaptan, apigenin and thiosulfinate with B-raaf protease.

Interaction of beta-chlorogenin, allyl mercaptan, apigenin and thiosulfinate with B-raaf protease.

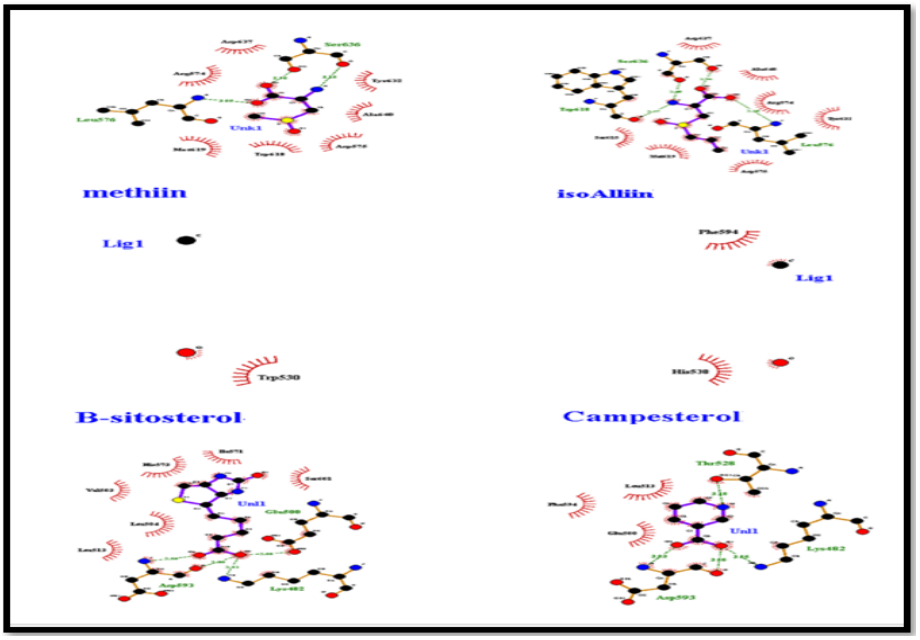


FIGURE 4.16: Interaction of methiin, isoAlliin, B-sitosterol and campesterol with B-raaf protease

Interaction of methiin, isoAlliin, B-sitosterol and campesterol with B-raaf protease.

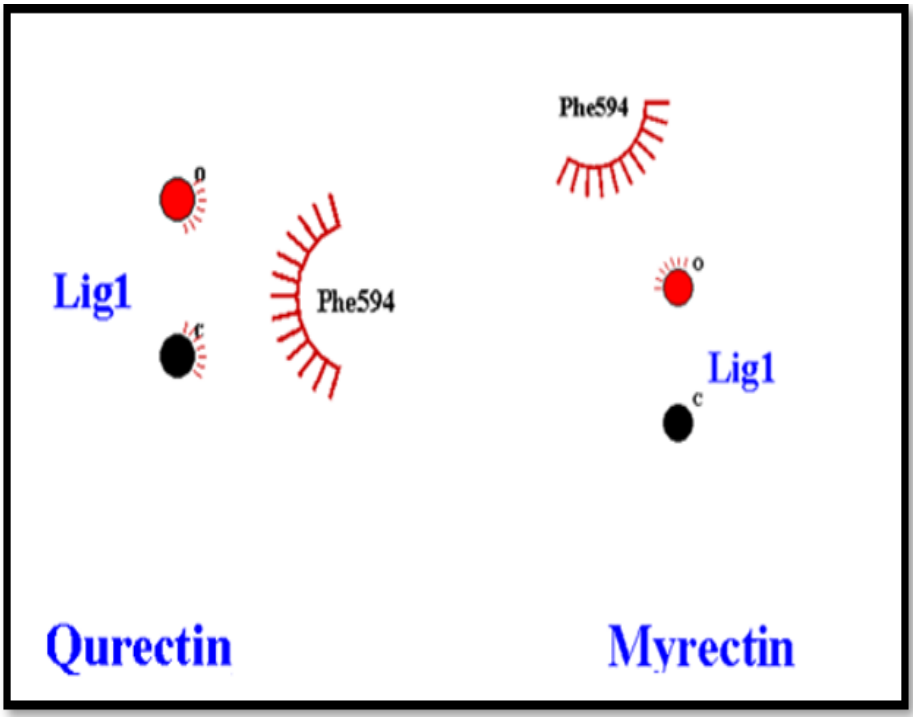


FIGURE 4.17: Interaction of biotin, Nicotinic acid, qurectin and Myrectin with B-raaf protease.

Interaction of biotin, Nicotinic acid, qurectin and Myrectin with B-raaf protease.

4.10 Lead Compound Identification

The physiochemical and pharmacokinetic properties of the ligands determine the destiny as drug or non-drug like compound. The first filter for this identification is Lipinski rule of Five and second screening is done through pharmacokinetic properties. Those compounds which do not follow more than 2 rules are not considered as drug like.

4.11 Selection of Antiviral Drug

The selection of most efficient anti-inflammatory drug is based on the physiochemical, ADMET properties along mechanism of action with side effects. For physiochemical properties PubChem online database is used and for ADMET properties of drugs pkCSM online tool is used. Mechanism of action is identified through Drug Bank and KEGG databases.

4.12 Abraxane Drug

Abraxane (Paclitaxel), a taxoid chemotherapeutic drug, is used as initial therapy and maintenance therapy for the treatment of advanced ovarian carcinoma as well as other cancers like breast and lung cancer. Abraxane is specifically indicated for the treatment of locally advanced or metastatic non-small cell lung cancer and metastatic breast cancer. In individuals whose disease cannot be treated by surgery or radiation therapy, it is used in combination with cisplatin as first-line therapy. As shown in the table 4.15.

TABLE 4.15: Physiochemical properties of Abraxane

S. No	Properties	Abraxane
1.	Chemical Formula	$C_{47}H_{51}NO_{14}$
2.	Molecular weight	853.918

TABLE 4.15: Physiochemical properties of Abraxane

S. No	Properties	Abraxane
3.	Log P	3.7357
4.	HBD	4
5.	HBA	14
6.	Rotatable Bond	10

This table above shows the physiochemical properties and Lipinski rule of five of Abraxane.

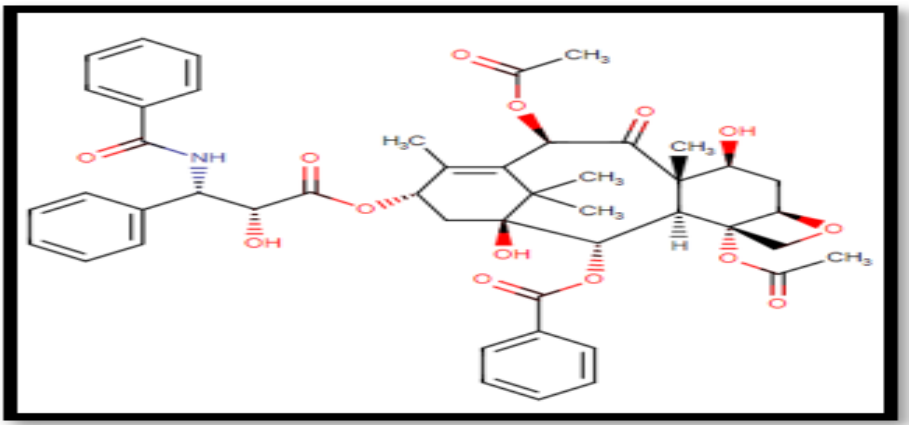


FIGURE 4.18: Shows molecular structure of Abraxane.

4.13 ADMET Properties of Selected Drug

The ADMET properties of selected drug Abraxane were identified by using online tool pkCSM online tool by putting input (Abraxane) as SMILES. ADMET properties describes the influence of drug level, kinetics and pharmacological activity of a compound that would be used as drug [142]. ADMET properties of selected compounds are shown in tables below.

TABLE 4.16: ADMET properties of Abraxane

ADMET Property	Model Name	Predicted
Absorption	Water solubility	-2.92

Table 4.16: ADMET properties of Abraxane

ADMET Property	**Model Name**	**Predicted**
Absorption	Caco2 permeability	1.14
Absorption	Intestinal absorption	66.621
Absorption	Skin permeability	-2.735
Absorption	P-glycoprotein substrate	Yes
Absorption	P-glycoprotein I inhibitor	Yes
Absorption	P-glycoprotein II inhibitor	Yes
Distribution	VDss	0.918
Distribution	Fraction unbound	0.129
Distribution	BBB permeability	-1.546
Distribution	CNS permeability	-3.263
Metabolism	CYP2D6 substrate	No
Metabolism	CYP3A4 substrate	Yes
Metabolism	CYP1A2 inhibitor	No
Metabolism	CYP2C19 inhibitor	No
Metabolism	CYP2C9 inhibitor	No
Metabolism	CYP2D6 inhibitor	No
Metabolism	CYP3A4 inhibitor	No
Excretion	Total clearance	-0.264
Excretion	Renal OCT2 substrate	No
Toxicity	AMES toxicity	No
Toxicity	Max.tolerated dose	0.708
Toxicity	hERG I inhibitor	No
Toxicity	hERG II inhibitor	Yes
Toxicity	Oral Rat Acute Toxicity (LD50)	2.933
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	4.678
Toxicity	Hepatotoxicity	Yes
Toxicity	Skin Sensitisation	No
Toxicity	T. Pyriformis toxicity	0.285
Toxicity	Minnow toxicity	2.79

4.14 Mechanism of Action of Abraxane

Paclitaxel is a microtubule-stabilizing anticancer drug that kills cells by interfering with mitosis in dividing cells. - and -tubulin heterodimers are stacked head to tail and put together to form a cylinder to form microtubules. Microtubule dynamics may be suppressed, which could slow or stop the transition from metaphase to anaphase by preventing chromosomes from migrating from the spindle poles to the metaphase plate. A stage of mitotic arrest is reached by cells, from which they can proceed to one of several outcomes. Through a variety of multidrug resistance-associated proteins, paclitaxel is sensitive to cellular drug resistance brought on by drug efflux [150].

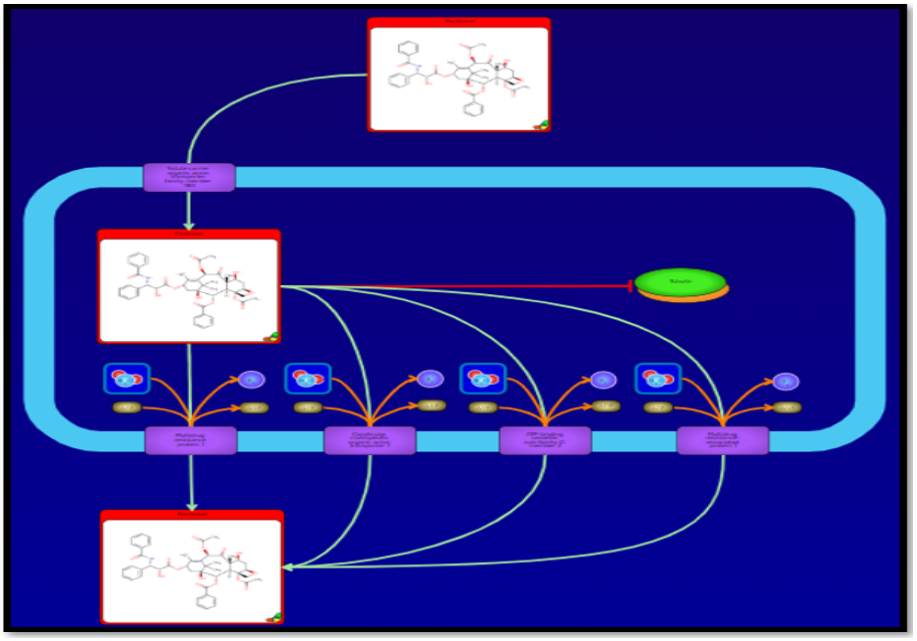


FIGURE 4.19: Mechanism of action of Abraxane.

4.15 Abraxane Effects on Body

It contains irritant compounds which may cause serious eye injury, cause skin and respiratory sensitization, may damage liver, injure kidney and results in bone depression. It has a suspected mutagen and being toxic to fertility or the developing child.

4.16 Abraxane Docking

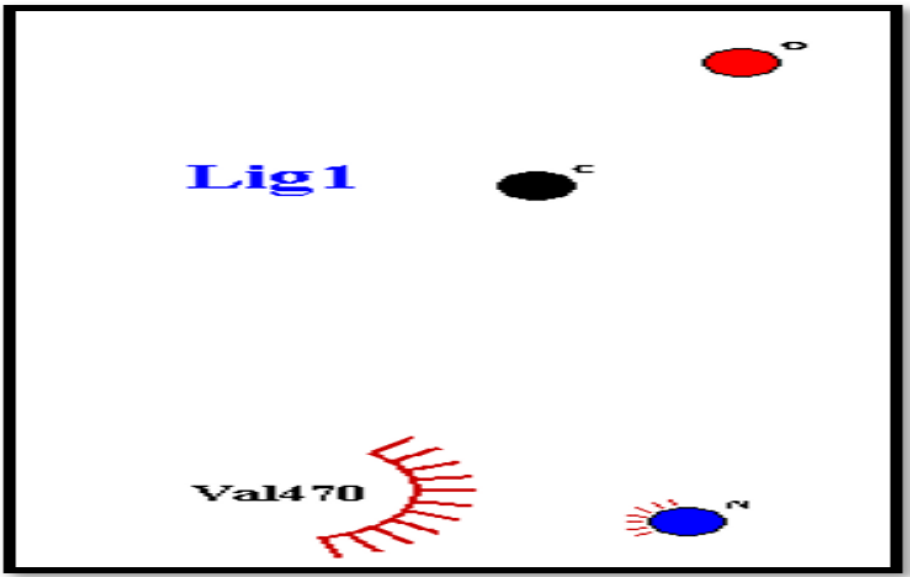


FIGURE 4.20: Docked structure of Abraxane and B-Raaf protease

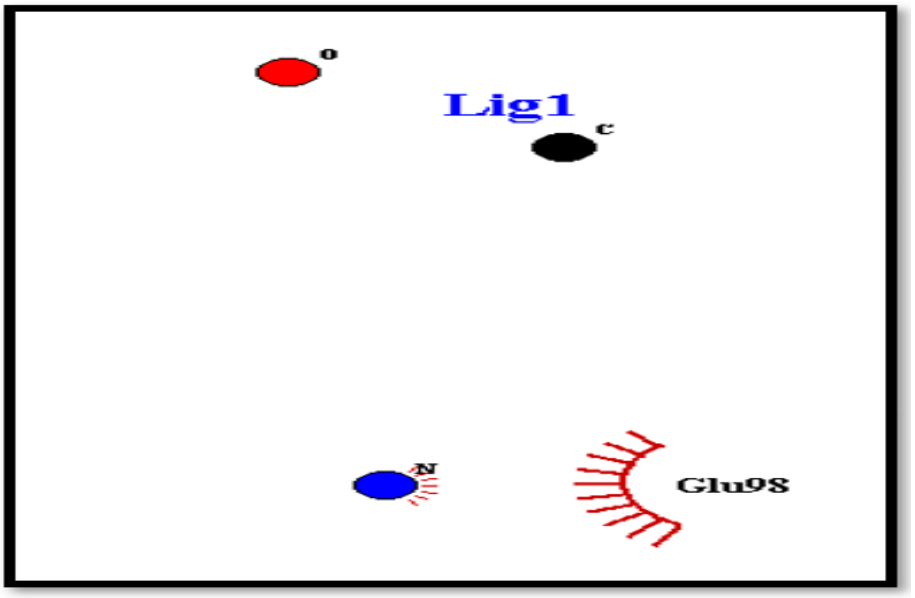


FIGURE 4.21: Docked structure of Abraxane and K-Ras protein.

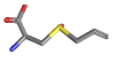
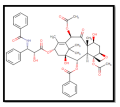
4.17 Comparison of Abraxane and Best Ligand

This comparison helps us to identify the better treatment for Lung's cancer. It is based on following parameters like; ADMET properties and physiochemical properties of Abraxane and selected ligand.

4.17.1 Comparison of Physiochemical Properties

Physiochemical properties of Abraxane and best ligands are compared as shown in Table 4.17 This comparison helps us to identify the better treatment for lungs cancer.

TABLE 4.17: Comparison of physiochemical properties of Best Ligand of *Allium sativum* and Abraxane.

Properties	**Alliin**	**Abraxane**
Molecular formula	$C_6H_{11}NO_3S$	$C_{47}H_{51}NO_{14}$
Structure		

4.17.2 Comparison of Docking Results

Comparison of docking values help to find best binding affinity of selected ligand. Comparison of docking results of Abraxane and best ligands (Alliin) from Allium sativum has been shown in Tables 4.18 and 4.19.

TABLE 4.18: Comparison of Docking Results of Best Ligand of *Allium sativum* and Abraxane.

Sr. No	**Properties**	**Alliin**	**Abraxane**
1.	Binding score with K-Ras	-5.1	-8.3
2.	Cavity size with K-Ras	4973	1382

TABLE 4.19: Comparison of Docking Results of Best Ligand of *Allium sativum* and Abraxane.

Sr. No	**Properties**	**Alliin**	**Abraxane**
1.	Binding score with B-Raaf	-5.2	-8.7
2.	Cavity size with B-Raaf	3919	412

The binding score and cavity size of Abraxane is less than Alliin.

4.17.3 Comparison of ADMET Properties

ADMET properties comprises of values regarding to drug absorption, distribution, metabolism, excretion and toxicity. These values help us to determine the activity and efficiency of drugs.

Table 4.20: Comparison of ADMET properties

ADMET Property	**Model Name**	**Properties of Alliin**	**Predicted Values of Abraxane**
Absorption	Water solubility	-2.888	-2.92
Absorption	CaCo2 permeability	0.619	1.14
Absorption	Intestinal absorption	76.495	66.621
Absorption	Skin permeability	-2.735	-2.735
Absorption	P-glycoprotein substrate	No	Yes
Absorption	P-glycoprotein I inhibitor	No	Yes
Absorption	P-glycoprotein II inhibitor	No	Yes
Distribution	VDss	-0.553	0.918
Distribution	Fraction unbound	0.462	0.129
Distribution	BBB permeability	-0.271	-1.546
Distribution	CNS permeability	-3.472	-3.263
Metabolism	CYP2D6 substrate	No	No
Metabolism	CYP3A4 substrate	No	Yes
Metabolism	CYP1A2 inhibitor	No	No
Metabolism	CYP2C19 inhibitor	No	No
Metabolism	CYP2C9 inhibitor	No	No
Metabolism	CYP2D6 inhibitor	No	No
Metabolism	CYP3A4 inhibitor	No	No
Excretion	Total clearance	0.365	-0.264
Excretion	Renal OCT2 substrate	No	No

TABLE 4.20: Comparison of ADMET properties

ADMET Property	**Model Name**	**Properties of Alliin**	**Predicted Values of Abraxane**
Toxicity	AMES toxicity	No	No
Toxicity	Max. tolerated dose	1.164	0.708
Toxicity	hERG I inhibitor	No	No
Toxicity	hERG II inhibitor	No	Yes
Toxicity	Oral Rat Acute Toxicity (LD50)	2.051	2.933
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	1.9	4.678
Toxicity	Hepato- toxicity	No	Yes
Toxicity	Skin Sensitisation	No	No
Toxicity	T. Pyriformis toxicity	0.268	0.285
Toxicity	Minnow toxicity	2.598	2.79

So, it is determined that Alliin shows better results than Abraxane according to ADMET properties.

4.17.4 Comparison of Lipinski Rule of Five

Comparison of Lipinski Rule of Five between Abraxane and best ligands of *Allium sativum* (Alliin) is shown in table 4.21, indicates that active compounds have

follow better Lipinski rule of five.

Table 4.21: Comparison of Lipinski Rule of Five of Best Ligand of *Allium sativum* and Abraxane.

Sr. No.	**Properties**	**Alliin**	**Abraxane**
1.	Log P Value	-0.667	3.7357
2.	Molecular weight	177.225	853.918
3.	H- Bond Acceptor	3	14
4.	H- Bond Donor	2	4
5.	Rotatable Bonds	5	10

So, it is determined that Alliin shows better results than rAbraxane according to Lipinski rule of Five.

Chapter 5

Conclusions and Recommendations

The aim of this research is to identify a compound using computational method for the treatment of specialized tumors like Cancer that could be used in near future as an efficient drug. After performing data mining studies on literature databases twenty ligands were selected for the current research work.

The proteins used for virtual screening were K-ras GTPase and B-raaf protease. CB Dock automated version of Auto Dock vina is used for the docking studies. Protein ligand interactions of these ligands were analyzed using Ligplot plus version v.1.4.5. After the detailed analysis of their binding score, physiochemical properties and ADMET properties, Alliin is identified as a potent inhibitor for inflammation.

From the above mentioned physiochemical and ADMET values it is concluded that the Alliin activity in comparison to Abraxane is better in activity. In drug designing, structure-based drug designing and lead discovery have been used efficiently. All the software's and tools used in the current research study are reliable and authenticate.

Alliin follows the Lipinski rule of 5s and shows best ADMET properties than Abraxane. So, from the current research work we identified that near in future

Alliin can act as an efficient and a possible alternative drug for the treatment of Cancerous Diseases, with having less side effect as compare to synthetic drugs.

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