EXPLORING NATURAL COMPOUNDS AS SAFER ALTERNATIVES OF CONVENTIONAL DRUGS FOR NON-SMALL CELL LUNG CANCER

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CANDIDATE'S DECLARATION

I, Bhavukta Virmani hereby certify that the work which is being presented in the thesis entitled "Exploring natural compounds as safer alternatives of conventional drugs for non-small cell lung cancer" in partial fulfillment of the requirements for the award of the Degree of Master of Technology, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from January, 2025 to May, 2025 under the supervision of Dr. Asmita Das.

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

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ABSTRACT

Cancer has become a major global challenge for the healthcare sector and requires extensive research to understand its causes and treatment. Various therapeutic approaches have been developed to regulate cancer cell growth and proliferation, including targeted therapies. Lung cancer is globally the most detected cancer. It is the major reason for cancer-related deaths. Tobacco smoking is considered to be one of the main causes associated with lung cancer, though non-tobacco contributors such as chronic lung diseases, occupational and environmental exposures, and behavioral patterns also play a role. Molecular alterations like EGFR mutations, ALK and ROS1 rearrangements, KRAS mutations, and RET rearrangements are crucial for understanding lung cancer biology. These genetic alterations provide insights into targeted treatment options and personalized therapy approaches. Most therapies primarily focus on inhibiting kinases associated with the activation of these molecular targets. Different tyrosine kinase inhibitors targeting these targets have been approved by FDA and are widely used for treatment of lung cancer. But some of these drugs bind to the off targets and cause side effects. So, the aim of this study is to find the off targets of the drug and find effective natural alternatives of the drug which does not cause any such side effects so that safe and effective treatments can be discovered.

TABLE OF CONTENTS

Title	Page No.
List of Tables	**vii**
List of Figures	**viii**
List of Symbols and Abbreviations	**ix**
CHAPTER 1: INTRODUCTION	**1-2**
CHAPTER 2: LUNG CANCER	**3-9**
2.1 Etiology of lung cancer	5
2.2 Epidemiology of lung cancer	6
2.3 Targets of lung cancer	7
2.3.1 EGFR	
2.3.2 ALK	
2.3.3 ROS1	
2.3.4 KRAS	
2.4 Targeted Therapies	9
CHAPTER 3: ANAPLASTIC LYMPHOMA KINASE	**10-11**
3.1 Mechanism	11
CHAPTER 4: TKI AS DRUGS	**12-17**
4.1 FDA approved drugs	14
4.2 Side effects of FDA approved drugs targeting ALK	14
4.3 Alectinib and its side effects	15
4.3.1 Liver toxicity	
CHAPTER 5: MATERIALS AND METHODS	**18-22**
5.1 Materials	18
5.2 Methodology	19
CHAPTER 6: RESULTS	**23-27**
CHAPTER 7: CONCLUSION, FUTURE SCOPE AND SOCIAL IMPACT	**28-31**
7.1 Future Scope	30
References	**32-35**
Appendix I: Natural Compounds	**36-40**
Plagiarism Report	

LIST OF TABLES

Table 4.1: FDA approved drugs for different targets for NSCLC

Table 6.1: Binding Energy of natural compounds with ALK

Table 6.2: Binding energy of natural compounds with ALK and BSEP

Table I.1: Natural compounds

LIST OF FIGURES

Fig 2.1: Classification of lung cancer

Fig 3.1: Mechanism of ALK rearrangement leading to uncontrolled cell growth

Fig 5.1: Methodology

Fig 6.1: Alectinib with ALK

Fig 6.2: Alectinib with BSEP

Fig 6.3: Alectinib (green) and lanuginosine (red) with BSEP

Fig 6.4: Alectinib (green) and lanuginosine (blue) with ALK

Fig 6.5: Alectinib (green) and grandisine A (red) with BSEP

Fig 6.6: Alectinib (green) and grandisine A (red) with ALK

LIST OF ABBREVIATIONS

1. NSCLC – Non-small cell lung cancer
2. SCLC – Small Cell Lung Cancer
3. EGFR – Epidermal Growth Factor Receptor
4. ALK – Anaplastic Lymphoma Kinase
5. ROS 1 – Receptor tyrosine kinase proto-oncogene 1
6. MET - Mesenchymal-Epithelial Transition factor
7. PAHs – Polycyclic Aromatic Hydrocarbons
8. FDA – Food and Drug Administration
9. EML4 – Echinoderma microtubule-associated protein like 4
10. TKIs – Tyrosine kinase inhibitors
11. RTK – receptor tyrosine kinase
12. DILI – Drug induced liver injury
13. BSEP – Bile salt exporter pump
14. RCSB PDB – Research Collaboratory for Structural Bioinformatics- Protein Data Bank
15. IARC – International Agency for Research on Cancer

CHAPTER 1

INTRODUCTION

Cancer is abnormal increase in number of cells in the body. It poses a worldwide health concern and its increasing number of cases demands for novel and alternatives therapies. Cancer is the second most cause of death across the globe. There are approximately hundred types of cancer. Lung, breast, prostate, blood and colorectal cancer are the most frequently diagnosed cancers. Although breast cancer is the more frequently diagnosed cancer worldwide but the mortality rate of lung cancer is higher. According to the recent global cancer statistics, each year, more than two crore people around the world are diagnosed with cancer, and around one crore people die from the cancer. The incidences of cancer are increasing, particularly in developing countries, due to various reasons including unhealthy lifestyle and changes in the environment combined with the aging global population.

Cancer is a genetic disorder caused due to mutation in DNA which leads to uncontrolled division and growth of cells. Although it is a genetic disorder it might not be always inherited. Cancer can be caused by external factors like physical, chemical and biological carcinogens. Key factors include alcohol consumption, tobacco use, physical inactivity and diet.

In the human body, cancer can start anywhere in our body. The human cells multiply under a controlled manner by a process known as cell division to generate

new cells and replace the old ones. The process of cell division sometimes breaks and leads to abnormal multiplication. This leads to formation of tumor which could be either benign or malignant. Malignant tumors are cancerous and can spread or invade to nearby tissues (metastasis) which can also be a major cause of cancer deaths. Cancer cases and related deaths continue to rise annually, thus there is an urgent need of new and improved prevention, early detection, and treatment strategies for cancer. In this study we will discuss about conventional therapeutic drugs and their safer alternatives for lung cancer.

CHAPTER 2

LUNG CANCER

Lung cancer is a major cause of medical concern around the globe and is the leading cause of deaths due to cancer worldwide. Around 350 people die because of lung cancer daily. Tobacco smoking is major causes of lung cancer, though non-tobacco contributors such as chronic lung diseases, occupational and environmental exposures, and behavioral patterns also play a role [1]. In 2023, it was predicted by the American Cancer Society that lung cancer will have the maximum death rate out of all types of cancers amongst both men and women. According to the recent data, 81% of the deaths caused by lung cancer are due to cigarette smoking and an another 3% is caused because of passive smoking [2]. The most prevalent symptoms for lung cancer are cough, chest pain, weight loss, fatigue, dyspnea, and hemoptysis.

Based on histological features, lung cancer can broadly be grouped into two categories (i) non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) [3]. It is a complex disease with a range of pathological and clinical symptoms.

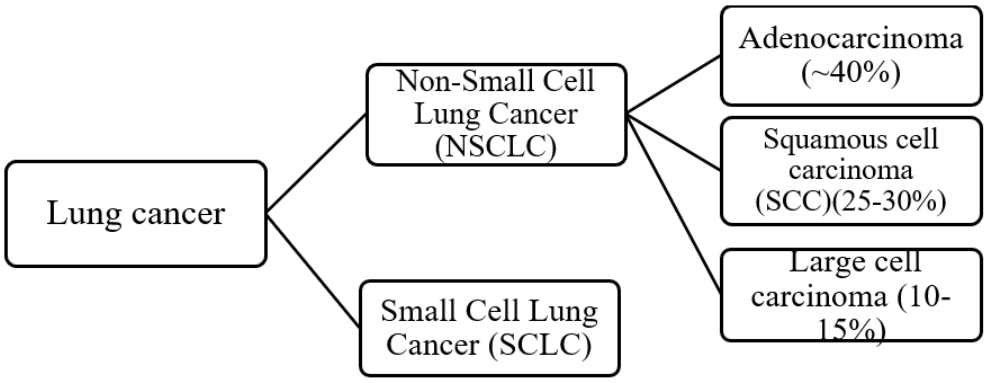

Fig 2.1: Classification of lung cancer

NSCLC is the most prevalent type of lung cancer and it accounts for more than 80% of all new diagnoses. It is characterized by the appearance and size of their cells under a microscope. In contrast to small cell lung cancer (SCLC), NSCLC cells are large and exhibit distinct biological behaviors. There are several subtypes of NSCLC, the most common being adenocarcinoma and squamous cell carcinoma. Less frequently, NSCLC may present as adenosquamous carcinoma or sarcomatoid carcinoma. Adenocarcinoma is the most prevalent subtype of lung cancer, essentially among non-smokers and females. It typically originates in the peripheral area of the lung. Histologically, it is marked by glandular formation, mucin production, and immunopositivity for markers. Squamous cell carcinoma has a strong association with smoking and generally arises centrally within the lungs. It is characterized by features such as keratin pearl formation, intercellular bridges, and expression of markers like p40, p63, and CK5/6.

Even after many developments in treatment of lung cancer, the prognosis is poor and merely 15% of the people with lung cancer survive after five-years of diagnosis. In developing countries, lung cancer is a significant public health concern as there are fewer medical advancements and less treatment options available.

In NSCLC, there are numerous tumor driver genes that have been identified over time, including epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), and receptor tyrosine kinase ROS proto-oncogene 1 mutants. These genetic alterations offer insights on targeted treatment options and personalized therapy strategies [4]. Targeted therapies like antibodies and small-

molecule inhibitors that particularly target the signaling pathways regulated by genes, have exhibited encouraging results in increasing survival rates and patient well-being. Combined with traditional chemotherapy, these targeted therapies have led to notable improvements in patient outcomes and prognosis. The kinase inhibitors are widely used in medical practice [6].

Detection of lung cancer involves clinical evaluation, imaging studies, laboratory tests, and tissue biopsy for histological confirmation. During the clinical assessment, patients are checked for symptoms. After clinical assessment, imaging studies of chest like X-ray and CT scan are carried out. They provide more detailed information about the size, location, and spread of the tumor. To confirm the diagnosis, tissue sampling (biopsy) is important. After this, finally, histopathological exam is done to determine the type of lung cancer.

2.1 Etiology of lung cancer:

The development of lung cancer involves a balance between the activation and elimination of carcinogens. Polycyclic aromatic hydrocarbons (PAHs) and N-nitrosamines found in smoke of tobacco, are metabolically activated by the body's enzymes to cause cancer. And there are other enzymes also present which work to eliminate these harmful substances from human body. Thus, the risk of developing cancer depends on the interplay between these two processes (activation and elimination of carcinogenic compounds).

Carcinogens can damage DNA through the formation of DNA adducts, which result from direct reactions with DNA, and by generating reactive oxygen species (free radicals), which cause oxidative damage. Specific carcinogens, such as 4-(methylnitrosamino)-1(3-pyridyl)-1-butanone and tobacco-specific N-nitrosamines, are highly potent inducers of lung cancer. Additionally, radioactive substances like radon, bismuth, and polonium in tobacco smoke further contribute to its carcinogenic potential.

2.2 Epidemiology of lung cancer:

Lung cancer's epidemiology reflects both widespread environmental exposures and lifestyle factors, particularly tobacco use. The lung cancer has been linked to genetic factors and some patients might have a hereditary component. According to a study, people with a family history of lung cancer exhibit twice the risk of developing cancer [5]. Females are more susceptible to lung cancer due to greater susceptibility to the carcinogenic factors of cigarette smoke [1].

Black Americans and Native Hawaiians have high chances of having lung cancer as compared to white individuals. This might be due to different genetic, socioeconomic, and behavioral factors. Environmental exposures might also increase the risk to some particular racial and ethnic groups. Genetic variations between racial and ethnic groups may also contribute to differences in susceptibility to the disease [5].

Lung cancer is very common among individuals over the age of 65, primarily due to the heavy smoking histories. Mortality from lung cancer generally increases with age, peaking around 80 to 85 years. While lung cancer is most commonly found in males of age 40 and older and females aged 60 and older [1]. Antioxidants, such as vitamins A, C, and E is considered to have protective effects against lung cancer.

2.3 Targets of lung cancer:

The Food and Drug Administration (FDA) has approved multiple drugs for treating NSCLC. These drugs primarily focus on inhibiting kinases associated with the activation of specific molecular targets like EGFR, ALK, ROS1, BRAF, MET, and RET.

2.3.1 EGFR (Epidermal Growth Factor Receptor):

It is transmembrane protein within the receptor tyrosine kinase family and has a function in cellular communication. EGFR gene is present on chromosome 7 and encodes the protein receptor. EGFR regulates many cellular processes like proliferation, survival, differentiation, and migration.

In lung cancer, EGFR mutations are the most commonly targeted driver mutations. Exon 21 Leu858Arg point mutations and Exon 19 deletions accounts for approx. 85% of EGFR alterations. EGFR is expressed in over 60% of the NSCLCs, making it a pivotal therapeutic target [4].

2.3.2 ALK (Anaplastic lymphoma kinase):

It is the part of the insulin receptor superfamily, it has a significant function in regulating normal cellular development, particularly in the nervous system. ALK can become oncogenic through chromosomal rearrangements, gene amplification, or mutations.

Around 3–5% of NSCLC cases have ALK gene rearrangement. This rearrangement results in assembly of ALK fusion proteins. The fusion protein plays a important role in lung cancer as it promotes uncontrolled cell growth and survival [6]

2.3.3 ROS 1

The proto onco gene is part of the insulin receptor subfamily. It resides on chromosome 6q22.1. It belongs to the receptor tyrosine kinase family, which plays essential roles in cell signaling, growth, and differentiation.

Genomic alterations of ROS1 frequently result in gene fusions with various partner genes, producing fusion proteins that act as potent oncogenic drivers. These fusion proteins lead to unregulated activation of ROS1 kinase activity, driving tumor development by enhancing cell proliferation, survival, and migration.

2.3.4 KRAS (Kirsten Rat Sarcoma Viral Oncogene Homolog)

The GTPase KRas (KRAS) is a key regulator of cellular signaling pathways involved in cell growth and differentiation. In cancer cells, KRAS mutations disrupt the hydrolysis of GTP, locking the protein in its active, GTP-bound state, which drives unregulated signaling. A notable example is the KRASG12C mutation, where a glycine residue is replaced by cysteine and this is commonly found in lung cancer [10].

2.4 Targeted Therapies

Targeted therapy has become a revolutionary approach in cancer treatment. As our underlying knowledge about the molecular mechanisms of cancer development and progression has deepened, these therapies have gained more attention. This has led to adoption of personalized medicine, where therapies are adjusted according to the specific genetic and molecular characteristics of an person’s tumor. Targeted treatments aim to increase therapeutic effects while decreasing damage to healthy tissues by focusing on unique vulnerabilities within cancer cells and causing fewer adverse effects and better patient outcomes [2].

In the case of lung cancer, the identification of driver mutations in genes such as EGFR and ALK has significantly advanced treatment to target these mutations [2]. Several targeted therapies authorized by FDA exist that inhibit EGFR and ALK signaling, providing critical insights into their mechanisms of action and their potential to improve clinical outcomes in lung cancer patients.

CHAPTER 3

ANAPLASTIC LYMPHOMA KINASE (ALK)

ALK is a protein kinase and gene found on chromosome 2p23. It can become oncogenic through chromosomal rearrangements, gene amplification, or mutations. Around 3–5% of NSCLC cases have ALK gene rearrangement. These rearrangements, caused by translocations or inversions on chromosome 2, involve the fusion of the ALK gene's exon 20 with variable regions from partner genes. Echinoderm microtubule-associated protein-like 4 (EML4) is the mostly found partner gene in NSCLC. The fusion protein plays a crucial role in lung cancer as it promotes uncontrolled cell growth and survival [6].

ALK rearrangements are more commonly reported in never or light smokers, individuals of non-Asian ethnicity, and males. Sometimes, patients with ALK-positive NSCLC are typically younger and often do not have any history of smoking, thus, highlighting a distinct demographic and molecular profile for this subtype of lung cancer. They mostly have adenocarcinoma subtype. ALK+ NSCLC are usually present with centrally located tumors, large pleural effusions, and the absence of a pleural tail. These tumors have a higher tendency for nodal metastasis and lymphangitic carcinomatosis [8].

Since ALK-positive (ALK+) NSCLC is driven by gene fusions, tyrosine kinase inhibitors (TKIs) have been specifically designed to target this. ALK alterations

in NSCLC can be efficiently managed using a range of ALK-targeted therapies. Till now there are six ALK inhibitors which have been authorised by FDA for their use in treatment of advanced ALK+ NSCLC [8]. These include crizotinib, alectinib, ceritinib, ensartinib, brigatinib, and lorlatinib. These drugs have demonstrated the ability to produce durable responses and significantly improve survival outcomes. As a result, treatment with ALK inhibitors is now considered as benchmark treatment for people having advanced ALK+ NSCLC.

3.1 Mechanism:

The rearrangement of the gene results in the production of the EML4-ALK fusion protein. The partner gene (EML4) drives ALK activity by altering gene expression and facilitates multimerization of the ALK kinase domain. This gene rearrangement and expression is believed to enhance biological processes such as cell differentiation, proliferation, and resistance to apoptosis. ALK fusions activate various signaling pathways, including MAPK, PI3K/AKT, MEK/ERK, MEKK2/3, CRKL/C3G, JAK/STAT, and MEK5-ERK5, which contribute to tumor progression [8].

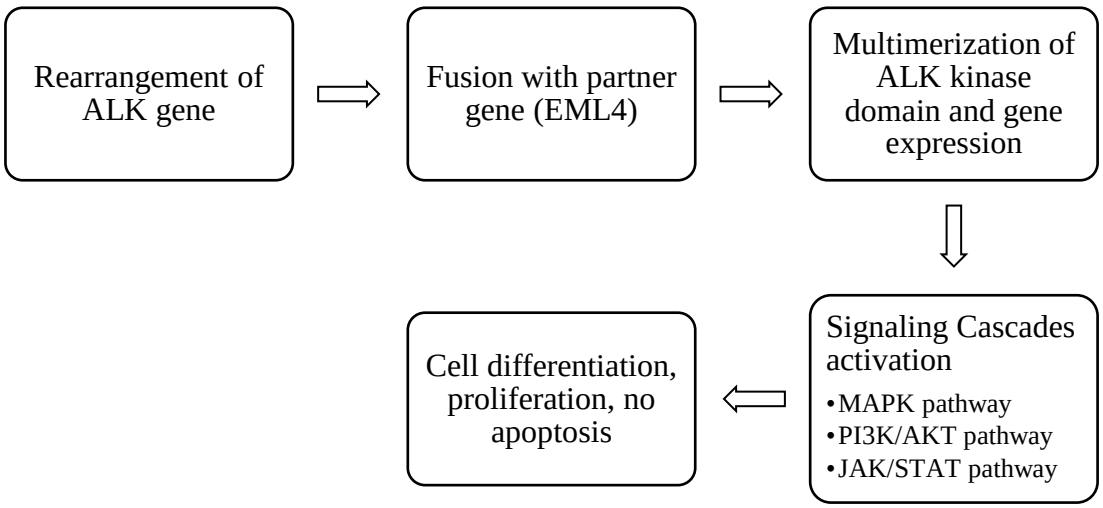

Fig 3.1: Mechanism of ALK rearrangement leading to uncontrolled cell growth

CHAPTER 4

TYROSINE KINASE INHIBITORS (TKIs) AS DRUGS

Protein tyrosine kinases (PTKs) are enzymes that regulate protein function by adding phosphate groups to specific amino acid residues. This phosphorylation induces conformational changes, switching proteins from inactive to active states, and plays a critical role in controlling the cell cycle and transmitting external signals. Dysregulated PTK activity is often linked to cancer development and progression and is therefore considered as key targets for anticancer drug development.

Tyrosine kinases (TKs) phosphorylate tyrosine residues using ATP as a phosphate donor. In response to various stimuli, tyrosine kinases play a important role in controlling the cellular functions like proliferation, growth, differentiation, survival, migration, metabolism, and apoptosis. There are mainly two types of TKs: receptor tyrosine kinases (RTKs), which are membrane-bound and activated by extracellular signals, and non-receptor tyrosine kinases (NRTKs), which operate within the cell. Due to their association with various types of cancers, many tyrosine kinase inhibitors (TKIs) are under clinical investigation as potential cancer therapies [10,11].

Tyrosine Kinase Inhibitors are used as targeted therapy. They inhibit the function of tyrosine kinases enzymes which are essential for signaling pathways controlling the cellular processes. This type of therapy is widely used in treating

cancers where tyrosine kinases become dysregulated due to genetic alterations like mutations, overexpression, or chromosomal rearrangements.

There are several TKIs present as drug for cancers which have been authorised by FDA. The Food and Drug Administration (FDA) is an organization that oversees the approval process for drugs. FDA ensures that the drugs meet stringent standards for safety, effectiveness, and quality before being made available. The process of drug development and approval involves multiple steps, including preclinical studies and extensive clinical trials, to thoroughly evaluate the drug's performance and risks.

Table 4.1: FDA approved drugs for different targets for NSCLC

Target	FDA approved drugs
EGRF	Gefitinib Erlotinib Afatinib Dacomitinib
ALK	Crizotinib Alectinib Ceritinib Brigatinib Ensartinib Lorlatinib
ROS1	Entrectinib Lorlatinib Crizotinib
KRAS	Sotorasib Adagrasib

4.1 FDA approved drugs against ALK

It was observed that the earliest ALK-TKI, Crizotinib, demonstrated better efficacy in both first-line and second-line settings during phase 3 trials as compared to the cytotoxic chemotherapy. As a first-line treatment, it achieved a remarkable median overall survival exceeding four years.

Second-generation ALK-TKIs were developed to address resistance and have improved central nervous system (CNS) penetration and greater potency than crizotinib. The second generation ALK-TKIs include ceritinib, alectinib, brigatinib, and ensartinib, which are effective in people whose cancer progressed even after treatment with crizotinib.

4.2 Side effects of FDA approved drugs targeting ALK:

Although these drugs have been approved by FDA but sometimes, they still show side effects/ adverse effects in few individuals. These side effects are caused due to their impact on both cancer cells and normal cells that rely on tyrosine kinase signaling. Some general side effects include skin reactions like rash, gastrointestinal issues – nausea and vomiting, hepatotoxicity (liver injury), fatigue, loss of appetite and weight changes. Some of these might even turn severe.

Crizotinib is commonly associated with a few adverse effects including vision disorders, gastrointestinal disturbances, electrocardiographic abnormalities, hypogonadism and hepatotoxicity.

Some common side effects of alectinib includes liver injury, shortness of breath, bradycardia, myalgia and hemolytic anemia. ALT and bilirubin elevations are observed in patients. Brigatinib also shows similar symptoms including high blood pressure, vision problem, pancreatitis, hepatotoxicity and high blood sugar. It may cause levels of bilirubin in your blood and enzymes called aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in your blood.

These drugs along with some other oral tyrosine kinase inhibitors (TKIs) for lung cancer can also induce peripheral neuropathy through several mechanisms like vitamin deficiencies as it interferes with the absorption of vitamins, such as vitamin B12, which are essential for nerve health. The TKIs can affect the metabolism of vitamins, reducing their bioavailability or increasing their breakdown. They can directly inhibit enzymes involved in vitamin metabolism, leading to decreased production or activation of vitamins. For example, methionine synthase is involved in the metabolism of vitamin B12 and folate, so, some TKIs can inhibit methionine synthase, leading to vitamin B12 and folate deficiencies.

The drugs can also cause heart problems like heart failure and irregular heart rhythms by directly damaging the heart muscle cells. Some drugs can indirectly affect the heart by causing changes in blood pressure, electrolyte imbalances, or other conditions that can strain the heart [25].

4.3 Alectinib and its side effects

Alectinib is a small molecule. It is a tyrosine kinase receptor inhibitor and show strong activity in inhibiting anaplastic lymphoma kinase (ALK) which is rearranged and mutated. This mutated ALK promotes the uncontrolled growth and proliferation of cell and is over expressed in cancer cell. Alectinib comes in 150 mg capsules and is sold under the brand name Alecensa [21].

Alectinib is a highly selective. It is a next-generation ALK inhibitor that has demonstrated the ability to block mutated ALK activity in cell models and has shown objective clinical responses in people with advanced, ALK-positive NSCLC, and refractory to prior treatments.

The ALEX phase III study compared alectinib to crizotinib as a first-line therapy for ALK+ NSCLC patients. The results demonstrated that alectinib provided enhanced CNS efficacy and substantially slowed CNS progression, establishing it as a more effective first-line option. Alectinib's showed enhanced CNS penetration as it lacks the ability to interact with P-glycoprotein, allowing it to cross the blood–brain barrier more effectively than crizotinib [2]. Thus, unlike crizotinib, a first-generation

ALK-TKI, second-generation inhibitors such as alectinib, ceritinib, and brigatinib offer improved central nervous system (CNS) penetration, a critical factor in managing brain metastases.

Alectinib received FDA approval in 2015 as a second-line treatment for ALK+ NSCLC and later in 2017 it was approved for first-line use, based on findings from the ALEX trial, where patients were administered 600 mg twice daily [8].

Alectinib shows a few side effects which includes liver injury, shortness of breath, bradycardia, myalgia and hemolytic anemia. Studies have reported that alectinib can cause liver injury that can be medically relevant. A few cases were reported and at least 2% of patients who were being treated with alectinib suffered severe abnormalities in liver and had to stopped their therapy early. Alectinib therapy was also linked to regular spike in alkaline phosphatase (47%) and bilirubin (39%) levels. These elevation in levels of alkaline phosphatase and bilirubin after taking the drug is caused due to drug induced cholestatic liver disease which is a subclassification of liver injury [26].

4.3.1 Liver toxicity/injury

Drug-induced liver injury (DILI) contributes to nearly 7% of all adverse drug reactions recorded in pharmacovigilance registries. Liver injury may result from direct damage to hepatocytes, vascular structures or bile ducts, or through disruption of normal bile flow. Mostly all medications carry the potential to cause liver toxicity. Drug-induced cholestatic liver disease represents a specific type of liver injury that is marked by elevated levels of alkaline phosphatase and bilirubin caused mainly due to interaction with the drug. It can be caused through various mechanism like direct inhibition of transport proteins and inhibition of nuclear receptor causing change in gene expression [22]. The primary mechanism underlying cholestasis is the selective disruption of bile excretion caused by a drug. According to the studies, some drugs can directly inhibit the function of transport proteins like BSEP, MRP2, or MDR3.

Hepatobiliary transporters, especially the canalicular ATP-dependent bile salt export pump (BSEP) has main function in the biliary excretion of various organic

compounds, like bile acids. When BSEP function is impaired, cytotoxic bile acids accumulate in hepatocytes, triggering oxidative stress and cell death through FAS-mediated apoptosis and necrosis pathways. Morgan et al. [40] demonstrated in their study that drugs or their metabolites that inhibit BSEP in vitro can contribute to drug-induced liver injury (DILI) because inhibition of the bile salt export pump (BSEP) has can cause accumulation of toxic bile acids in the liver. Through this several drugs were identified as strong BSEP inhibitors—such as troglitazone, which was discontinued from the market, and imatinib, which received warnings for hepatotoxicity [23]. To overcome these side effects, natural compounds not causing these similar side effects were studies to find a safer alternative

CHAPTER 5

MATERIALS AND METHODS

5.1 Materials

The protein chosen as target and off target were ALK (PDB ID: 2XB7) and BSEP (PDB ID: 8PM6) respectively were taken from the RCSB PBD. The PDB is a library of 3D structural data of large biomolecules like proteins. It is an open access digital data bank in all of biology and medicine. The ligand and natural compounds were obtained from PubChem. PubChem is an accessible chemistry database. It gathers data on a variety of topics, including physical and chemical properties, chemistry, identification, biological activities, safety, toxicity, patents and many more topics. Around 400 natural compounds (Table I.1) were downloaded from PubChem and docked with the target and off target to obtain the results.

AutoDock Vina was used to perform molecular docking. AutoDock is used to predict the binding energies of molecules/drug to the target molecules using a semiempirical free energy force field. AutoDock Vina is the modification of AutoDock. It provides improved accuracy and speed because it uses advanced scoring function and a hybrid global-local optimization algorithm. It is considered suitable for virtual screening and lead optimization as it supports flexible docking and has a reliable scoring function which offers better correlation with experimental data. It is significantly faster especially when working with large datasets or multiple docking

simulations [31,32]. It explores all the ligand orientations and conformations within a receptor's binding site and based on that generates multiple potential binding poses. These binding poses are then evaluated based on factors like hydrogen bonding, van der Waals interactions, and electrostatic forces. Finally, Vina refines these poses using a local search method and select the pose with the lowest binding energy as the most favorable interaction [31,32].

PyMol [33] was used for visualization. It is powerful and open source molecular visualization system. It is primarily used for visualizing, analyzing and manipulating protein structures in 3D.

5.2 Methodology

Lung cancer can be caused due to different molecular alterations. There are many FDA approved drugs targeting these molecules. The molecular targets of cancer include EGFR, ALK, ROS1, BRAF, MET, and RET. In this study, we have selected ALK as our target protein and we studied all the FDA approved drugs targeting ALK like Crizotinib, Alectinib, Brigatinib, Ensartinib, etc. Despite being approved from the FDA, these drugs cause side effects in some cases. There are multiple anti-cancerous drugs that are available, but they do show some adverse effects that limit their use. Along with drug resistance, severe adverse effects is still a main obstacle for the successful treatment. Due to these severe side effects of conventional drugs, as well as the high mortality rates in cancer patients, there is a critical need to develop alternative therapies that offer reduced toxicity and enhanced therapeutic efficacy [41].

So, we investigated the side effects caused by these ALK-TKIs and identified the underlying molecular pathways responsible for triggering the adverse effects. From the literature review, we found that alectinib was showing liver injury as one of the side effects and on further studying the literature, it was found that on molecular level, one of the reasons for liver injury was related to the direct inhibition of BSEP, a transporter protein of bile acids. So, using molecular docking, the binding energy of the drug to BSEP was evaluated to computationally confirm its association with the side effect.

Computational approaches have become a potent tool for drug research in recent years. Molecular docking is an effective method for determining how well a ligand will attach to a target protein and in what orientation it forms stable complex. Molecular docking enables us to characterize how small molecules interact with the binding sites of target proteins and to explain the fundamental biological processes at the atomic level. A ligand's most efficient method of binding to a macromolecular partner is to be predicted using molecular docking algorithms.

In comparison to conventional drug discovery techniques, it has a number of benefits, including speed, cost-effectiveness, and the capacity to screen sizable libraries of compounds. Docking uses scoring functions to assess the binding affinity and activity of small molecules to their protein targets. This makes it a vital tool in the rational design of pharmaceuticals. The accuracy of docking predictions is highly sensitive to the geometry of the input ligand. Even a minor alteration in ligand conformation can lead to substantial changes in both the docking poses and their corresponding scores.

BSEP (PDB ID: 8PM6) was taken as the off target and docking was carried out using AutoDock Vina [31,32]. For performing molecular docking, first the protein and ligand files were prepared in .pdbqt format using AutoDock Tools. Following this, molecular docking was performed usingAutoDock Vina. The output was also obtained in the form of a pdbqt file which was visualized using PyMol and the binding energies were obtained in text (.txt) file and the results were analyzed.

At present, numerous clinical trials are examining the safety and effectiveness of natural compounds in cancer prevention and treatment. Studies suggests that natural agents hold strong potential for cancer prevention with minimal or no adverse effects. At present times, a significant proportion of modern pharmaceutical drugs—especially antibiotics and cancer therapies—are derived from natural sources. Almost 60% to 80% drugs are now originating from natural products. Additionally, nearly one-third of the world’s top-selling drugs are either natural compounds or derived from natural compounds.

So, following the molecular docking of drug with off target, we explored approximately 400 natural compounds given in Table I.1. The natural compounds already used as therapeutic drugs were collected from various published studies. These natural compounds were typically derived from plants, animals, fungi, or microorganisms and have biological activity, especially as medicine. The natural compounds were screened using molecular docking to assess their binding affinity with the primary cancer-related protein target, ALK (PDB ID: 2XB7). Anaplastic lymphoma kinase (ALK), a protein kinase [29], was taken as cancer related target protein because it has been identified as promising target [30]. Once few promising natural compounds having binding energy similar to the drug were identified, they were further screened to ensure that they do not bind to off-target molecules that could lead to side effects. This was done by comparing the binding energy of drug with the off target to the binding energy of natural compounds to the off target, BSEP.

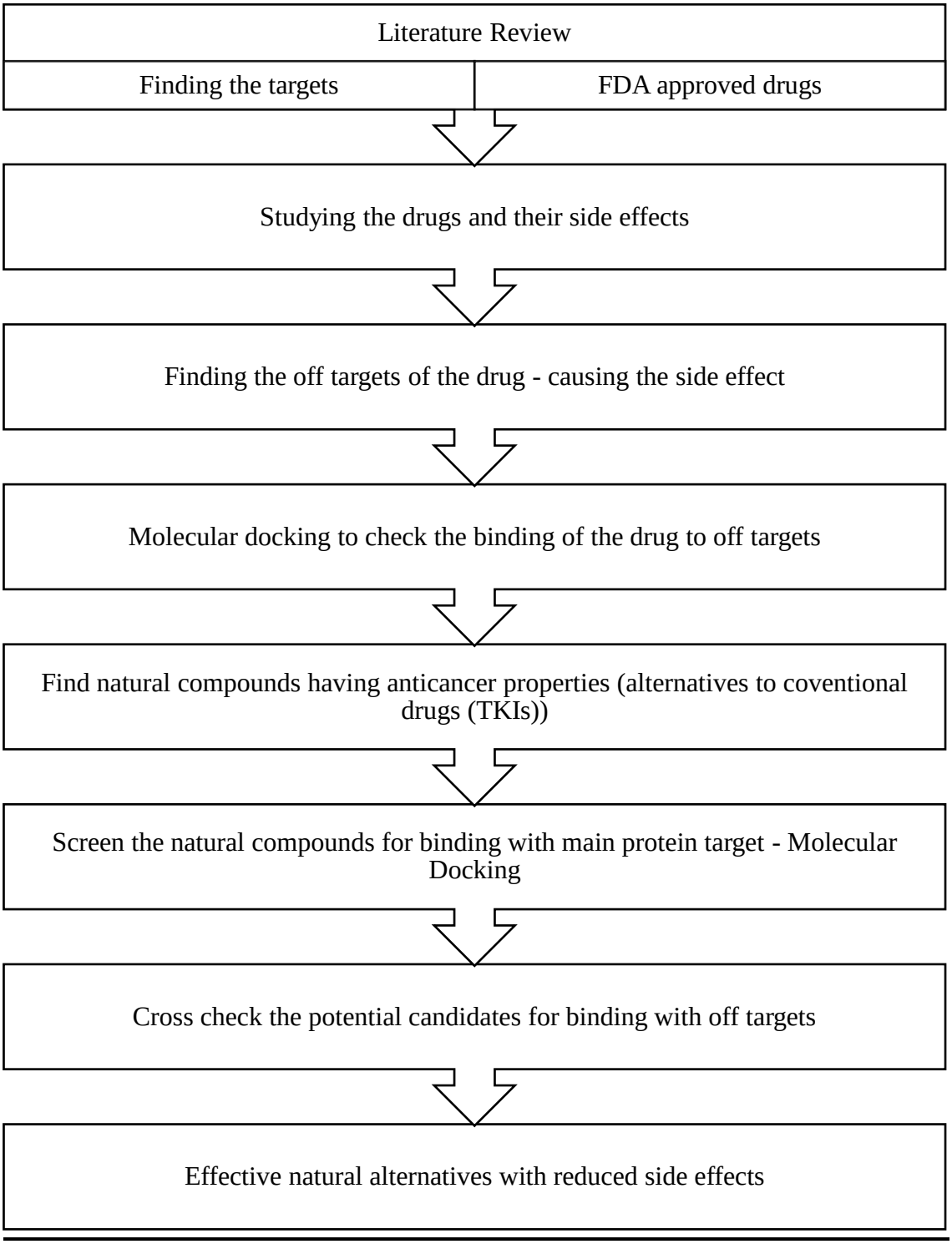

Fig 5.1: Methodology

CHAPTER 6

RESULT

Lung cancer is the most frequently detected cancer worldwide, with approximately two crore new cases and around one crore deaths every year. These numbers highlight the immediate need for effective treatments focused on combating the disease, enhancing patient survival and quality of life and decreasing side effects. Some FDA approved drugs that used for treatment cause side effects. So, in this study we have aimed to find the off targets of the drug which is causing side effects and will also discover natural compounds that are effective and do not bind to off targets and cause side effects.

Firstly, molecular docking was performed for alectinib [27,28] with anaplastic lymphoma kinase (ALK) and bile salt export pump (BSEP) using AutoDock Vina. AutoDock Vina uses free binding energy between the target protein and ligand. It is expressed in kcal/mol. This energy indicates how strongly the ligand binds to the protein. A more negative binding affinity suggest a stronger interaction, indicating the ligand may be potentially more effective [31,32]. Thus, alectinib was chosen among all the other FDA approved drugs because it had low binding energy with ALK as compared to other drugs. Binding energy obtained for alectinib with ALK was -9.7 kcal/mol and with BSEP -9.2 kcal/mol. The best binding poses were visualized using PyMol [33].

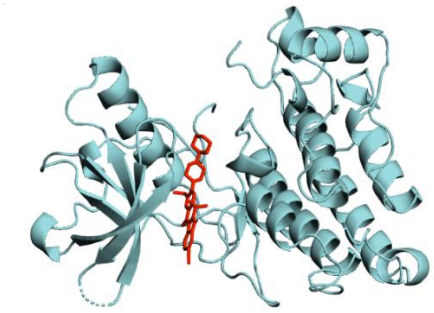

Fig 6.1: Alectinib with ALK

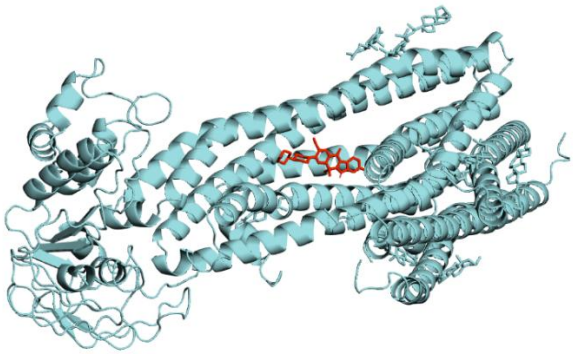

Fig 6.2: Alectinib with BSEP

Since alectinib showed similar binding energy to BSEP as ALK, thus, we looked for natural alternative to the conventional drug with reduced adverse effects for lung cancer treatment. We then docked the natural compounds with ALK, the target protein. Approximately 400 natural compounds were docked to get the compounds showing similar binding energy to the target protein as the drug, alectinib. Out of 400, 29 natural compounds including lanuginosine, theaflavin, daunorubicin, doxorubicin, tanshinone, munjistin, grandisine A, asimilobine, mangiferin, and magnolialide exhibited similar binding energy as alectinib. The binding scores for these 29 compounds as given in Table 6.1 were between -9.7 kcal/mol to -8.3 kcal/mol. We took the similar and nearest values of binding energies.

Table 6.1: Binding Energy of natural compounds with ALK

S No.	Natural Compounds	BE with ALK (kcal/mol)
1.	Lanuginosine	-9.7
2.	Anonaine	-9.7
3.	Sanguinarine	-9.7
4.	Theaflavin	-9.6
5.	Daunorubicin	-9.6

Continued on page no. 25

Table 6.1 continued

S No.	Natural Compounds	BE with ALK (kcal/mol)
6.	Gartanin	-9.5
7.	Tanshinone IIA	-9.5
8.	Aristolactams	-9.5
9.	Liriodenine	-9.5
10.	Diosmin	-9.5
11.	Doxorubicin	-9.4
12.	8-Desoxygartanin	-9.4
13.	Tanshinone	-9.4
14.	Baicalin	-9.4
15.	Naphthoflavon	-9.3
16.	Neoponcirin	-9.2
17.	Chlorogenin	-9
18.	Lupinifolin	-9
19.	Munjistin	-8.9
20.	Dinoxin B	-8.9
21.	Jolkinolide A	-8.9
22.	Grandisine A	-8.9
23.	Piperolactam A	-8.8
24.	Physcion	-8.8
25.	Rutin	-8.8
26.	Asimilobine	-8.7
27.	Mangiferin	-8.5
28.	Cephaeline	-8.3
29.	Magnolialide	-8.3

These 29 compounds were further docked with BSEP, to check whether they are or not binding to the off-target which is causing the side effect, liver injury/toxicity. On docking these 29 compounds, it was observed from Table 6.2 that lanuginosine [34], grandisine A [35], asimilobine [36] and cephaeline [37] showed high binding energy as compared to the drug, alectinib.

Table 6.2: Binding energy of natural compounds with ALK and BSEP

S No.	Natural Compounds	BE with ALK (kcal/mol)	BE with BSEP (kcal/mol)
1.	Lanuginosine	-9.7	-6.8
2.	Grandisine A	-8.9	-6.5
3.	Asimilobine	-8.7	-6.5
4.	Cephaeline	-8.3	-6.9

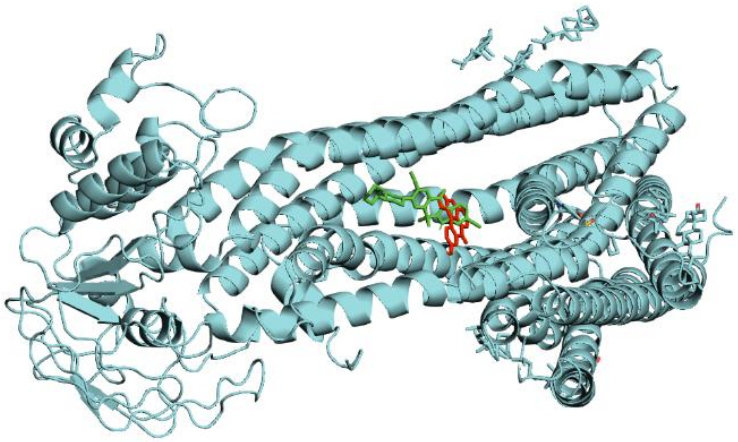

Fig 6.3: Alectinib (green) and lanuginosine (red) with BSEP

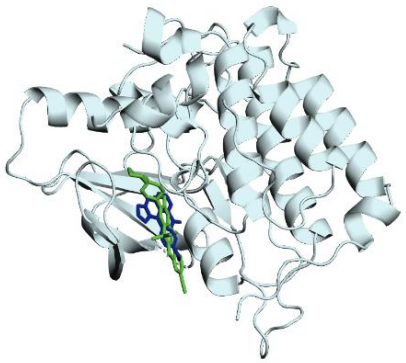

Fig 6.4: Alectinib (green) and lanuginosine (blue) with ALK

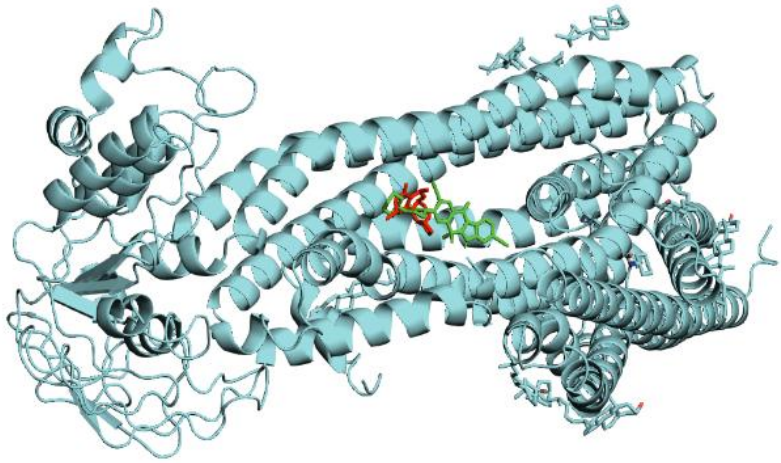

Fig 6.5: Alectinib (green) and grandisine A (red) with BSEP

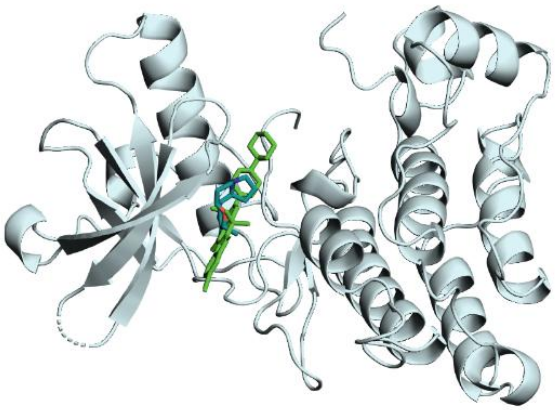

Fig 6.6: Alectinib (green) and grandisine A (blue) with ALK

Natural compounds offer a sustainable and highly effective source for treating a wide range of disorders, including life-threatening diseases such as cancer. Recently, bioactive compounds derived from natural sources have gained significant attention as promising anticancer agents. Lanuginosine [34], grandisine A [35], asimilobine [36] and cephaeline [37] showed promising results as alternatives for conventional drugs.

CHAPTER 7

CONCLUSION, FUTURE SCOPE AND SOCIAL IMPACT

Millions of deaths are caused every year due to the four most common types of cancers, including breast, lung, prostate, and rectum/colon cancer. Lung cancer is a major causes of cancer deaths. Therefore, targeted therapy development and marketing are most extensive for lung cancer right now. The most prevalent form of lung cancer is NSCLC and one of the most common therapeutic targets for it is ALK. ALK is a tyrosine kinase, whose mutation causes uncontrolled cell proliferation and growth. Thus, FDA approved drugs against ALK were studied and it was found that alectinib has the least binding energy. On studying about alectinib, it was found that it caused liver injury in patients having this medicine. Liver injury was caused due to direct inhibition of BSEP, transport molecule of bile acids whose accumulation caused liver injury.

From the docking results it was observed, alectinib was having similar binding to BSEP as to ALK. As the drug was causing side effects, natural compounds were tested and docked against ALK to find a suitable compound. After docking, 29 compounds were selected based on their binding score and these were further docked with BSEP to check whether they are having same binding energy or not. After docking these 29 compounds it was concluded that grandisine A [35], asimilobine [36], lanuginosine [34] and cephaeline showed high binding energy as compared to the drug

alectinib, thus not binding that easily to the off target. It can also be observed that all these natural compounds bind in the same binding site as alectinib.

The natural compounds are found in different natural sources. Lanuginosine, an aporphine alkaloid, has shown therapeutic potential, especially in treatment of cancer and in research related to Alzheimer's disease. It exerts strong anticancer effect on multiple cancer types. It can be found in *Magnolia compressa*, *Fissistigma oldhamii* and other organisms. It can also be extracted from plants like *Annona squamosa* (sugar apple). Grandisine A is an alkaloid and its natural source is *Elaeocarpus angustifolius* and *Elaeocarpus grandis*. These are Australian rainforest tree.

Asimilobine is an aporphine alkaloid and primarily sourced from plant species within the Magnoliaceae family. It has been isolated from the stems and leaves of *Fissistigma oldhamii* and the bark of *Beilschmiedia alloiophylla*, and can also be extracted from *Magnolia officinalis*, *Phoebe formosana*, among other plant sources.

Cephaeline, an alkaloid extracted from the ipecac plant, has potential in therapeutic applications, particularly in cancer treatment and antiviral therapy. Cephaeline can be found in *Alangium salviifolium*, *Dorstenia contrajerva*, and other organisms.

In conclusion, development of targeted therapies has marked a major advancement in cancer treatment as it offers more precise and personalized approaches, particularly for cancers such as lung, colorectal, and prostate cancer. The discovery of specific molecular targets has led to the development of drugs that specifically inhibit critical pathways for tumor growth and progression. While these therapies have transformed cancer care, there are still some challenges including drug side effects, biomarker identification, and determining optimal drug combinations which must be addressed to further improve their effectiveness and clinical outcomes.

Given their accessibility, reduced cytotoxicity, and broad-spectrum biological activity, natural products stand out as one of the most promising alternatives to conventional cancer therapies. Natural compounds continue to represent a powerful

and virtually inexhaustible source of potential chemotherapeutic agents. Their structural diversity, biological activity, and lower toxicity profiles make them particularly attractive for the development of novel anticancer drugs. Many currently approved treatments, including alkaloids, taxanes, and flavonoids, are derived from natural sources, underscoring their relevance and therapeutic potential.

7.1 **Future Scope:**

Around 20 million new cancer cases were recorded worldwide in 2022, highlighting the growing prevalence of cancer due to factors such as population growth, aging, and lifestyle-related risks like tobacco use, obesity, and environmental pollutants. Projections by WHO and the IARC suggest that by 2050, annual cancer diagnoses could exceed 35 million, representing a 77% increase from current levels.

There is need to find effective treatment for cancer. The future of cancer therapy lies use of natural compounds as alternatives for conventional drug. Natural compounds can be combined advanced drug delivery systems or given as combination therapies to increase the precision, efficacy and safety of cancer treatment. Thus, the results obtained through computational analysis can be further verified through in vitro and in vivo experiments to confirm their accuracy and biological relevance. Additionally, the selection of appropriate in vitro and in vivo models is a critical step in preclinical evaluations. Employing models that accurately mimic human cancer biology is important to minimize the gap between computational research and clinical application.

To assess the efficacy of natural compounds against lung cancer, clinical research can be carried out by adapting a prime methodology, standard preparations, large diverse sample, and long-term follow-ups. This will provide us with natural alternative as a viable treatment option, providing a safer and effective approach to managing lung cancer.

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Appendix-I

NATURAL COMPOUNDS

Approx. 400 natural compounds were collected from different literatures reviews and were docked to check for their binding with ALK. All the compounds were analyzed on the basis of their binding energy in comparison to the binding energy of drug with ALK. In the table given below are the molecules that were used in the study.

Table I.1: Natural compounds

Theaflavin	Pyranocoumarin
Anonaine	Hematoxylin
Lanuginosine	Triptolide
Gitogenin	Isogambogenic acid
Daunorubicin	Beta-Sitosterol
Aristolactams	Magnolialide
Camptothecin	Eucalyptin
Diosgenin	Barbaloin
Camptothecin	Betulinic Acid
Tanshinone IIA	Columbin
Arjunetin	Myricetin
Roemerine	Myrtillin
Withanolide D	Peonidin-3-glucoside
Artemisinin	Reynosin
Chlorogenin	Somniferine
Doxorubicin	Pseudolaric acid
Rutin	Astaxanthin
Doxorubicin	Campesterol
Asimilobine	Costunolide
Rubiadin	Integerrimine
Jolkinolide A	Senecionine
Quercetin-3–4-diglucoside	Sugiol
Munjistin	Afzelechin
Neoechinulin A	Calacorene
Piperolactam A	Ulocladol
Gambogic Acid	Parthenolide

Decursinol angelate	Aloesin
Ergosterol	Isocorydine
Liensinine	Lappalone
Neferine	Naringenin
Physcion	Mauritine A
Celecoxib	Oenin
Tanshinone	Palmatine
Stigmasterol	Podophyllotoxin
Salvicine	Isorhamnetin
Farnesiferol C	Lapachol
Celastrol	Procyanidins
Decursin	Alpha gurjunene
Alizarin	Bisdemethoxycurcumin
Purpurin	Glaucine
Stepharine	Marmesin
Psammaplin A	Panobinostat
Ferruginol	Chrysosplenetin
Nirtetralin	Trigonelline
Mitomycin C	Metformin
Anthroquinones	Pyrrolizidine
Beta eudesmol	Vinyldithiins
Valencene	Ajoene
Phyltetralin	Allicin
Plumbagin	Emodin
Isocalamendiol	Deguelin
Maytansine	Daurisoline
Honokiol	Curcumin
Caryophyllene	Chrysin
Skimmianine	Apigenin
Sylvatine	3,3-diindolylmethane
Pterostilbene	Piperine
Germacrene-D	Berberine
Noroxyhydrastinine	Matrine
Psoralen	Atractylodes
Zingiberene	Artesunate
Guineensine	Andrographolide
Squalene	Icariin
Beta elemene	Capsaicin
Epothilone	Paclitaxel
Bisabolene	Vanillin
Geranylgeraniol	Gedunin

Hypaphorine	Securinine
Scopoletin	Cytisine
Triacanthin	Aucubin
Pentostatin	Etoposide
Azulene	Cepharanthine
Carvacrol	Xanthohumol
Carvone	Silybin
Beta thujone	Parthenolide
Limonene	Kaempferol
Allixin	Icaritin
Indole-3-Carbinol	Formononetin
Alpha-asarone	Baicalin
Piperitone	Asiatic acid
Sabinene	Acovenoside A
Alpha pinene	Vit D3
Eugenol	Sanguinarine
Camphene	Resveratrol
Citral	Quercetin
Eucalyptol	Pristimerin
Protocatechuic acid	Piperlongumine
Ocimene	Luteolin
Geraniol	Licochalcone A
Linalool	Hispidulin
Hesperidin	Dalrubone
Ginsenoside Rh2	Damsin
Gingerol	Dehydroeburicoic acid
Genistein	Demethoxyyangonin
Erianin	Demethylcalabaxanthone
Epigallocatechin Gallate	Dihydrocucurbitacin B
1,2,4-trihydroxyheptadec-16-ene	Dihydrocucurbitacin F
1,3-diacetylvilasinin	Dinoxin B
2,3-dihydrowithaferin A	Diosmin
2-deoxycucurbitacin D	Duartin
3,3',4',5,6,7,8-heptamethoxyflavone	Elliptinol
3Beta-E-Feruloylbetulinic Acid	Emorydone
3-O-(E)-p-Coumaroylbetulin	Encecalin
5-desmethylsinensetin	Encelin
5-hydroxysophoranone	Enoxolone
6,8-Diprenyleriodictyol	Ergolide
6-methoxy-tremetone	Eriocitrin
6-oxopristimerol	Eriodictyol

8-Desoxygartanin	Erybraedin A
8-Epihelenalin	Erycristagallin
10-Epi-olguine	Erysubin F
12-Hydroxychiloscyphone	Euparin
28-deoxonimbolide	Galangin
28-oxoallobetulin	Galbacin
Acacetin	Galbelgin
Acteoside	Garcinialiptone A
Aculeatin A	Garcinialiptone B
Acuminatin	Garcinone C
Agathisflavone	Gartanin
Aglafolin	Genipin
Allobetulin	Harunganin
Altaicalarins A	Helenalin
Amygdalin	Hinokinin
Apigenin	Hiravanone
Arnidiol	Hispidulin
Betulin	Isoacteoside
Bigelovin	Isomartynoside
Bisabolone	Jacaranone
Cabralealactone	Kawain
Celastrol	Licarin A
Chamaejasmine	Liriodenine
Cnicin	Lupinifolin
Costunolide	Machilin A
Cucurbitacin A	Malacitanolide
Cucurbitacin Q1	Mangostanin
Cycloxanthochymol	Marcanines A
Dalparvinene	Martynoside
Melianin B	Triptolide
Meliavolkinin	Rosmarinic acid
Mucronulatol	Ursolic acid
Myricetin	Ethoxysanguinarine
Naphthoflavon	Melatonin
Naringenin	Noscapine
Narirutin	Adenanthin
Natsudaidain	Cryptotanshinone
Neodiosmin	Borneol
Neoponcirin	Dicoumarol
Nimbolide	Falcarindiol
Nobiletin	Heteronemin

Obovatifol	Prodigiosin
Odoroside H	Brusatol
Oleandrin	Bixin
Osthole	Crassin
Paucin	Eupatorin
Pinocembrin	Phloretin
Pinosylvin	Salvigenin
Ponciretin	Enterolactone
Pristimerin	Secoisolariciresinol
Psorothamnones B	Alyssin
Pteropodine	Embelin
Reissantins A	Nordihydroguaiaretic acid
Rhoifolin	Trans-nerolidol
Ridentin	Beta-Caryophyllene oxide
Sativanone	Forbesione
Savinin	Aaptamine
Schweinfurthin A	Demethyloxyaaptamine
Sesamin	Isoaaptamine
Shogaol	Sinapine
Sinensetin	Isoliquiritigenin
Tangeretin	Tectorigenin
Tubulosine	Wogonin
Withaphysacarpin	Clitocine
Wogonin	Cucurbitacin b
Xanthochymol	Cinchonine
Cephaeline	Glabridin
Emetine	Matairesinol
Uzarigenin	Isomorellin
Caffeine	Mangiferin
Colchamine	Salicin
Cytidine	Pilocarpine
(+)-epijasmonic acid	Imipenem
11-hydroxyvittatine	Aztreonam
Paxilline	Bevirimat
Silibinin	Torreyanic acid
Baccatin III	Spisulosine
Calanolide A	Cryptophycin
Prostratin	Spongouridine
Arteether	Spongothymidine
Grandisine A	Galphimine

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