MULTI-TARGET PHYTOCHEMICAL APPROACH AGAINST AURKB AND KRAS G12C: COMPUTATIONAL SCREENING FOR PRECISION CANCER THERAPY

Dissertation submitted in partial fulfilment of the requirement for the degree of

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in

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by

Divya Sharma
(2K23/MSCBIO/78)

Under the supervision of:

Dr. ASMITA DAS
Associate Professor
Department of Biotechnology
Delhi Technological University

Department Of Biotechnology

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-110042, India

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Divya Sharma

(2K23/MSCBIO/78)

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-42

CANDIDATE'S DECLARATION

I, Divya Sharma, Roll No., 2K23/MSCBIO/78, student of M.Sc. Biotechnology, hereby declare that the project Dissertation titled **"Multi-Target Phytochemical Approach Against Aurkb And Kras G12C: Computational Screening For Precision Cancer Therapy"** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology/Bachelor of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

PLACE: Delhi **DIVYA SHARMA**

DATE: **2K23/MSCBIO/78**

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-42

CERTIFICATE

Certified that Divya Sharma (2k23/MSCBIO/78) has carried out their search work presented in this thesis entitled **"Multi-Target Phytochemical Approach Against AURKB And KRAS G12C: Computational Screening For Precision Cancer Therapy"** for the award of Master in Biotechnology from Department of Biotechnology, Delhi Technological University, Delhi, under my supervision.

The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/ Institution.

DR. ASMITA DAS
ASSOCIATE PROFESSOR
SUPERVISOR
Department of Biotechnology
Delhi Technological University

PROF. YASHA HASIJA
HEAD OF DEPARTMENT
Department of Biotechnology
Delhi Technological University

PLACE: Delhi

DATE:

ABSTRACT

Cancer continues to be a major contributor to deaths worldwide, with drug resistance significantly compromising treatment efficacy and patient prognosis. This study investigates the therapeutic potential of multi-target AURKB (Aurora Kinase B) and KRAS pathways using phytochemicals as an innovative approach to overcome limitations of conventional single-target therapies.

This study evaluated AURKB expression patterns across multiple cancer types through web tools like TIMER and UALCAN. This research established a critical mechanistic link between KRAS mutations and Aurora kinase activation, where KRAS-driven pathways enhance AURKA and AURKB expression through direct mechanisms and MYC activation, creating a dependency loop in KRAS-mutant cancer cells.

To identifying potential inhibitors, a library of 300 phytochemicals was curated from the NPACT database and downloaded from the PubChem database. These compounds were subjected to molecular docking using PyRx (v0.8). Five compounds Cycloartobiloxanthone, Guggulsterone, 6-Hydroxy-6a,12a-dehydro-alpha-toxicarol, Withaferin A, and Withaphysacarpin—demonstrated superior binding affinity to AURKB compared to reference drugs VX-680 and Danusertib. For KRAS G12C targeting, four phytochemicals Subtrifloralactone E, Artoindonesianin P, 24 Epibrassinolide and Orientanol C exhibited effective binding interactions with Cys12, comparable to the benchmark drug Sotorasib. The selected phytochemicals demonstrated favorable pharmacokinetic properties, fulfilling Lipinski's Rule of Five with high gastrointestinal absorption (bioavailability score 0.55) and moderate aqueous solubility. Toxicity assessments revealed non-mutagenic profiles (AMES negative) for all compounds, with most exhibiting non-hepatotoxic characteristics, presenting superior safety profiles compared to synthetic alternatives. Notably, these natural compounds avoided major CYP enzyme inhibition, reducing drug-drug interaction risks.

These findings highlight the promising therapeutic potential of phytochemicals as multi-target inhibitors capable of simultaneously disrupting oncogenic growth signals and cell division

machinery. The multi-target capabilities, reduced toxicity burden, and improved patient tolerance profiles position these natural compounds as valuable candidates for precision oncology applications. This multi-target approach addresses the critical limitation of single-target therapies that often lead to resistance through alternative survival mechanisms.

Future directions should focus on structure-activity relationship studies, molecular dynamics simulations, and integration of AI-driven screening tools to accelerate the discovery of plant-based targeted cancer therapeutics in precision oncology.

Keywords: Aurora Kinase B (AURKB), KRAS G12C, phytochemicals, multi-target therapy, cancer drug resistance, precision oncology, phytochemicals, in-silico analysis

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LIST OF ABBREVIATIONS

AURKB	Aurora Kinase B
LUSC	Lung squamous cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
PDAC	Pancreatic ductal adenocarcinoma
TGCT	Testicual germ cell tumors
OV	Ovarian Serous Cystadenocarcinoma
PAAD	Pancreatic ductal adenocarcinoma
LUAD	Lung adenocarcinoma
STAD	Stomach adenocarcinoma
KIRC	Kidney renal clear cell carcinoma
HNSC	Head and neck squamous cell carcinoma
LIHC	Liver hepatocellular carcinoma
BRCA	Breast invasive carcinoma
ESCA	Esophageal Squamous Cell Carcinoma
EAC	Esophageal Adenocarcinoma (EAC)
RTKs	Receptor tyrosine kinase
NPACT	Naturally occurring Plant-based Anti-Cancer Compound-Activity-Target database
MYC	Myelocytomatosis Oncogene
PDB	Protein Data Bank
PAINS	Pan-Assay INterference compoundS
ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
PASS	Prediction of Activity Spectra for Substances
GI	Gastrointestinal Absorption
PI3K	Phosphoinositide 3-Kinase
AKT	Protein Kinase B (PKB)
mTOR	Mechanistic Target of Rapamycin

CHAPTER-1

INTRODUCTION

The hallmarks of cancer include unchecked cell growth and proliferation, which is mostly caused by genomic mutations and genomic instability that interfere with normal cell cycle regulation and apoptosis mechanisms[1], [2]. According to the most recent GLOBOCAN data, the age-standardized rate (ASR) of cancer is 196.9 per 100,000 people, and the global incidence is estimated to be 19.98 million cases. 9.74 million registered deaths, or an ASR of 91.7 per 100,000 population, indicate an equally concerning mortality burden. Research demonstrates that chemical compounds play a significant role in cancer formation and genetic modifications. Environmental toxins with cancer-causing properties impact cellular components including the cytoplasm and nucleus through both direct and indirect mechanisms, leading to hereditary disorders and genetic changes[3], [4]. Additional cancer-causing factors, representing approximately 7% of all cancer cases, encompass viral infections, bacteria, and radiation [5]. Usually, cancer leads to the malfunction of essential genes and disturbs cellular relationships. Abnormal proliferation is the result of this cell cycle disruption. In healthy conditions, proto-oncogenes regulate cellular division and development; but when these genes experience genetic alterations, they become oncogenes, posing the greatest risk to cell viability [6]. Gene overexpression and mutations are two key mechanisms that contribute to oncogenesis. Gene overexpression in cancer is often driven by genomic amplification, transcriptional deregulation, or epigenetic changes. Studies have shown that elevated AURKs expression in cancer leads to chromosomal abnormalities and genetic instability [7]. Inhibition of Aurora kinase B (AURKB) not only disrupts mitosis but also affects several key cellular communication networks related to tumor development. AURKB inhibition restores p53 activity by preventing its phosphorylation and degradation, resulting in halted cell division and programmed cell death in p53-functional tumors [8]. Additionally, AURKB inhibition has been linked to downregulation in the PI3K/AKT/mTOR signaling cascade, contributing to decreased tumor growth and increased autophagy [9]. These results demonstrate the wider treatment potential of inhibiting the broader therapeutic impact of targeting AURKB beyond its mitotic role. Mutations, on the other hand, can activate oncogenes or inactivate tumor suppressor genes, such as KRAS, which is frequently mutated in various cancers [10]. Gene mutations in cancer can be broadly classified

into "driver" mutations, which confer a selective advantage to cancer cells, and "passenger" mutations, which are neutral. Driver mutations often occur in genes involved in key cellular processes, such as cell cycle regulation (e.g., TP53, CDKN2A), signaling pathways (e.g., RTK-RAS, PI3K/Akt), and DNA repair mechanisms (e.g., BRCA1/2)[11] [12] .

1.1 OVEREXPRESSION OF AURORA KINASE B

Serine/threonine kinases govern mitosis, the cell cycle's most strictly regulated step. These enzymes control the process by which cellular division processes that result in two genetically identical daughter cells", each of which has the same chromosomes and cytoplasm [13], [14]. (AURKs) family of **serine/threonine protein kinases** comprises **three primary members: AURKA, AURKB, and AURKC** [15]. AURKC participates in gametogenesis, which aids in the development of reproductive cells, whereas AURKA and AURKB mainly control mitosis, guaranteeing appropriate cell division [16]. Among these, AURKB plays a crucial part in the formation of tumors and resistance to treatment in cancer. Numerous cancers, such as non-small cell lung carcinoma [17], thyroid cancer[18], breast [19], prostate[20] , and colorectal cancers[21], have been linked to elevated AURKB expression. Furthermore, in various cancer types, resistance to anticancer treatments has been associated with Aurora B expression. Moreover, numerous in vitro investigations have shown a connection between drug resistance and mutations in the catalytic domain of AURKB[22], [23] .

1.2 MUTATION IN KRAS PROTEIN

The RAS gene family, which includes NRAS, HRAS, and KRAS, is commonly linked to genetic changes in a variety of tumor types seen in human populations, such as thyroid, colon, lung, pancreatic, and myeloid leukemia[2]. The G12C version of the KRAS mutation is present in about 2% of other solid tumors, 3% of colon cancers, and nearly 13% of instances of lung adenocarcinoma[24]. As a little GTPase, KRAS alternates between two states: one that is GDP-bound and the other that is GTP-bound. Two different protein types strictly govern this transition: guanine nucleotide exchange factors, which contribute to the activation of KRAS, and GTPase-activating proteins, which inactivate KRAS[25]. Thus, there is an increasing need of potential inhibitors for targeting and blocking of oncogenic KRAS across various cancers.

1.3 CONTROL DRUGS AND PHYTOCHEMICALS USED FOR AURKB AND KRAS.

Even though AURKB plays a crucial part in the development of cancer, the currently approved pan-AURK inhibitors Danusertib [26] and VX-680 (co-crystallized) have serious drawbacks. The well-known Aurora kinase inhibitor VX-680 was taken out of clinical studies because it was linked to the dangerous cardiac side effect of QTc prolongation [27] . Likewise, another pan-Aurora kinase inhibitor, Danusertib, has a short half-life and has hematological adverse effects such neutropenia, requiring regular administration [28].

Sotorasib was recently approved as a KRAS G12C inhibitor [29]. KRAS inhibitors halt the advancement of the cell cycle by interacting with cysteine residues in KRAS-G12C [30]. Although sotorasib is a major breakthrough in targeted cancer treatment, its drawbacks, including possible toxicity and hepatotoxicity, highlight the necessity of investigating substitute treatment choices with better safety records and more extensive benefits [31].

Phytochemicals, naturally occurring compounds found in plant-based foods such as whole grains, vegetables, and fruits, have shown great potential in cancer prevention and therapy. These compounds have been found to inhibit or stop the growth and progression of various cancers, as well as by lowering oxidative stress, blocking the angiogenesis process, inhibiting cell proliferation, inducing programmed cell death, and causing cell cycle arrest. About 35% of cancer cases can be avoided by adopting a healthy diet that primarily consists of plant-based foods, such as whole grains, vegetables, and fruits which contain carotenoids, flavonoids, and phenolics [9]. According to studies, there are now at least 60 phytochemicals being developed as potential anticancer drugs[32].

CHAPTER-2

LITERATURE REVIEW

2.1 AURORA KINASE B

A class of serine/threonine protein kinases known as aurora kinases plays a critical role in multiple cell division processes [33]. AURKB is a crucial component of the chromosomal passenger complex (CPC), which also consists primarily borealin, INCENP, and survivin molecules. These partners help Aurora B: Stay in the right place, stable and Choose the right targets (substrate specificity) [34]. In order to provide appropriate chromosome separation, cytoplasmic constriction, spindle organization and stabilization, nuclear envelope reformation, and cytokinesis completion, CPC actively migrates at various stages of mitosis and cytokinesis. Numerous processes, such as phosphorylation and protein–protein interactions, strictly regulate Aurora B activity [35],[36] . Chromosome 17 contains the AURKB gene which encodes the protein aurora kinase B [37].

2.1.1 Structure and Function of AURKB

AURKB consists of three structural regions: (i) an N-terminal region, (ii) a catalytic kinase region, and (iii) a C-terminal region [37]. The N-terminal region exhibits variable sequence homology that confers specificity for protein-protein binding interactions. The catalytic region (central portion) of AURKB contains two structural lobes. This region features a β-sheet structure on the N-terminal portion and an α-helical structure on the C-terminal portion. A flexible hinge connects these two lobes, which, helps AURKB change its shape so it can become active and do it job [38] . The catalytic T-loop, which is located in the kinase domain's C-terminal lobe, is auto-phosphorylated at Thr232 to activate AURKB. AURKB has special tag called degrons that mark it for removal i.e. KEN sequence, D-box and DAD/A. The catalytic (central) region contains D-boxes, the N-terminal region possesses both the KEN sequence and DAD/A motif, and the C-terminal region contains another D-box [39] .

2.1.2 What AURKB Does During Cell Division

1. **Before Mitosis**: AURKB sits on the DNA (**chromatin**) and helps it get ready to divide by changing proteins like **histone H3** and **CENP-A**.

2. **In Pro-metaphase**: AURKB moves to structures called **kinetochores**. It helps fix any bad connections between the chromosomes and the spindle.
3. **During Metaphase to Anaphase**: AURKB moves to the **microtubules**, to makes sure all chromosomes are lined up right and split evenly [21]. It also controls a protein called **Kif-2A**. AURKB tells Kif-2A not to break down the microtubules too much, so everything stays stable.
4. **In Telophase**: AURKB shows up at the **mid-body**, helping the final separation. This step is called **cytokinesis**. It adds chemical tags called **phosphates** to important proteins that are found in the **cleavage furrow**. Some of these proteins are: **Vimentin**, **Desmin**, **Glial fibrillary acidic protein (GFAP)**. To cut the final bridge between the two cells, the cell uses a special team called **ESCRT (Endosomal Sorting Complex Required for Transport)**. They need to connect with a helper called **Vps4**, which powers ESCRT using energy (ATP). Under normal conditions, Aurora B delays abscission by inhibiting the activity of Vps4. This checkpoint ensures that cells do not prematurely separate while lagging chromosomes persist in the intercellular bridge. Once Aurora B detects that chromosome segregation has been properly completed and no chromatin bridges remain, its kinase activity decreases. This reduction lifts the inhibition on Vps4, allowing it to mediate membrane fission and complete the abscission process[40], [41].

2.1.3 Regulation of AURKB in Cancer

Aurora kinase B (AURKB) is frequently overexpressed across various malignancies, contributing to enhanced proliferation, survival, and tumor progression. Upstream activators like Myc control AURKB, and it also controls the actions of specific proteins like p53 and c-Myc. The expression of AURKB is significantly influenced by the Myc family of transcription factors, which includes c-MYC, MYCN, and MYCL. AURKA and AURKB levels are indirectly raised in B-cell lymphoma by c-MYC. On the other hand, MYCN exhibits a transcriptional regulation mechanism in retinoblastoma and neuroblastoma by directly binding to the AURKB promoter region. Notably, AURKB phosphorylates c-MYC at Ser67 in T-cell actue lymphoblastic leukemia(T-ALL), increasing the transcriptional function and protein stability. This creates a positive feedback loop by further amplifying AURKB expression [26] . The oncogenic tyrosine kinase Bcr-Abl, characteristic of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL), upregulates both AURKA and AURKB via the Akt signaling pathway. This activation promotes unchecked proliferation and resistance to

apoptosis [42]. AURKB maintains a bidirectional relationship with BRCA1 and BRCA2, both of which are central to DNA repair and genomic stability. Disruption of AURKB impairs mitosis and slows cell growth, whereas loss of BRCA1/2 leads to aberrant cell division, potentially via p53 and cyclin A–dependent pathways. This interaction underscores the mitotic checkpoint's dependence on tumor suppressor networks. RASSF7, frequently overexpressed in ovarian, pancreatic, and uterine cancers, facilitates mitotic spindle organization and cell division. It is essential for AURKB activation, and its silencing impairs proper cytokinesis and cell cycle progression, indicating a supportive role in tumorigenesis. By targeting certain oncoproteins, such as AURKA and AURKB, for ubiquitin-mediated degradation, FBXW7 suppresses tumors. It is p53-dependent, and when p53 is lost, miR-25 expression rises and downregulates FBXW7, which causes AURKA/B to accumulate [43]. Through the CDK1 axis, MDM2, a p53 negative regulator, also regulates AURKB-driven mitotic processes separately from p53. Additionally, via phosphorylating p53 at Ser269 and Thr284, AURKB collaborates with NIR to suppress p53. Through the EBV protein EBNA3C, AURKB downregulates p73, a p53 homolog, in virus-related malignancies, including those associated with Epstein-Barr virus (EBV), underscoring its function in viral-mediated oncogenesis. Cells with functional p53 undergo growth arrest upon Aurora B expression, whereas p53-deficient cells tolerate it and form aggressive, metastatic tumors in vivo. Aurora B alone does not initiate cancer but enhances transformation when combined with oncogenes like Ras, indicating a cooperative role in tumor progression [44].

2.1.4 How Do Inhibitors Work?

These inhibitors are like off-switches for Aurora B. They stop Aurora B from working by preventing it Activating itself (called autophosphorylation) and changing another protein called histone H3, which is needed for cell division. When Aurora B is blocked cancer cells get stuck in the middle of division (in the G2/M phase)Then, the cancer cells die (apoptosis) because they can’t divide [42] .

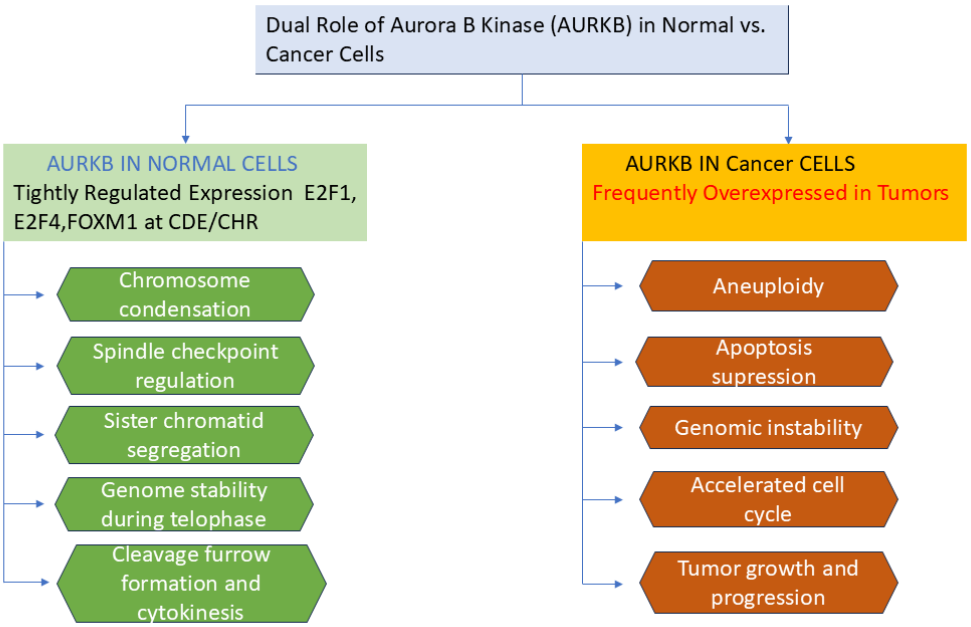

Fig.2.1: AURKB function in normal cells (regulated mitotic processes) versus cancer cells (overexpression leading to tumorigenesis).

2.2 KRAS

Inside body, there are special proteins called **RAS proteins**. There are four kinds: HRAS, NRAS, KRAS4A and KRAS4B variants. Of these, **KRAS** represents the most frequently altered gene in various malignancies. KRAS mutations, especially those involving codons 12 and 13, cause the KRAS protein to remain activated over time, which in turn triggers carcinogenic signaling pathways [43]. The RAS family's KRAS GTPase is essential for controlling a number of cellular functions, including cellular proliferation, maturation and viability, as outlined in subsequent sections. KRAS works like a switch When it holds GDP, it's OFF, when it holds GTP, it's ON. To turn ON, KRAS gets help from GEFs (Guanine Exchange factor). To turn OFF, it has an in-built timer called GTPase, which changes GTP back to GDP. KRAS has a built-in ability to break down GTP into GDP. This breaking process is called GTP hydrolysis, and it works like a timer—eventually turning KRAS OFF. When KRAS mutates, Its GTPase timer breaks. KRAS stays ON all the time and initiates multiple

downstream signaling pathways, MAPK and PI3K-AKT signaling networks, promoting cellular growth. These pathways facilitate the growth and spread of tumor , suppress apoptosis, and encourage unchecked cell division[44] .

2.2.1 Structure of KRAS

Structurally, KRAS consists of two principal regions: a conserved G-region, comprising roughly 170 amino acids, plus a hypervariable region (HVR) of around 25 amino acids at C-terminus. Numerous functionally important motifs that are involved in nucleotide binding and hydrolysis are found in the G-domain. Among these, the phosphate-binding loop(P-loop), encompassing residues 10 to 16, is crucial for the maintenance of the phosphate groups of GTP or GDP. Upon GTP binding, two more domain —referred to as Switch I (residues 32–38) and Switch II (residues 59–67)—experience conformational modifications. These structural alterations are critical for the interaction of KRAS with downstream target proteins, such as RAF, and play a pivotal role in transmitting proliferative cascade [45] .

According to genetic study, three amino acidd-G12, G13, and Q61-account for 98% of the RAS mutations that result in cancer. Alterations at these positions are thought to maintain the GTP-bound state, leading to persistent signaling activity. All these residues are positioned near the GTP phosphate region, with G12 and G13 located within the P-loop and Q61 situated in the Switch II domain [46].

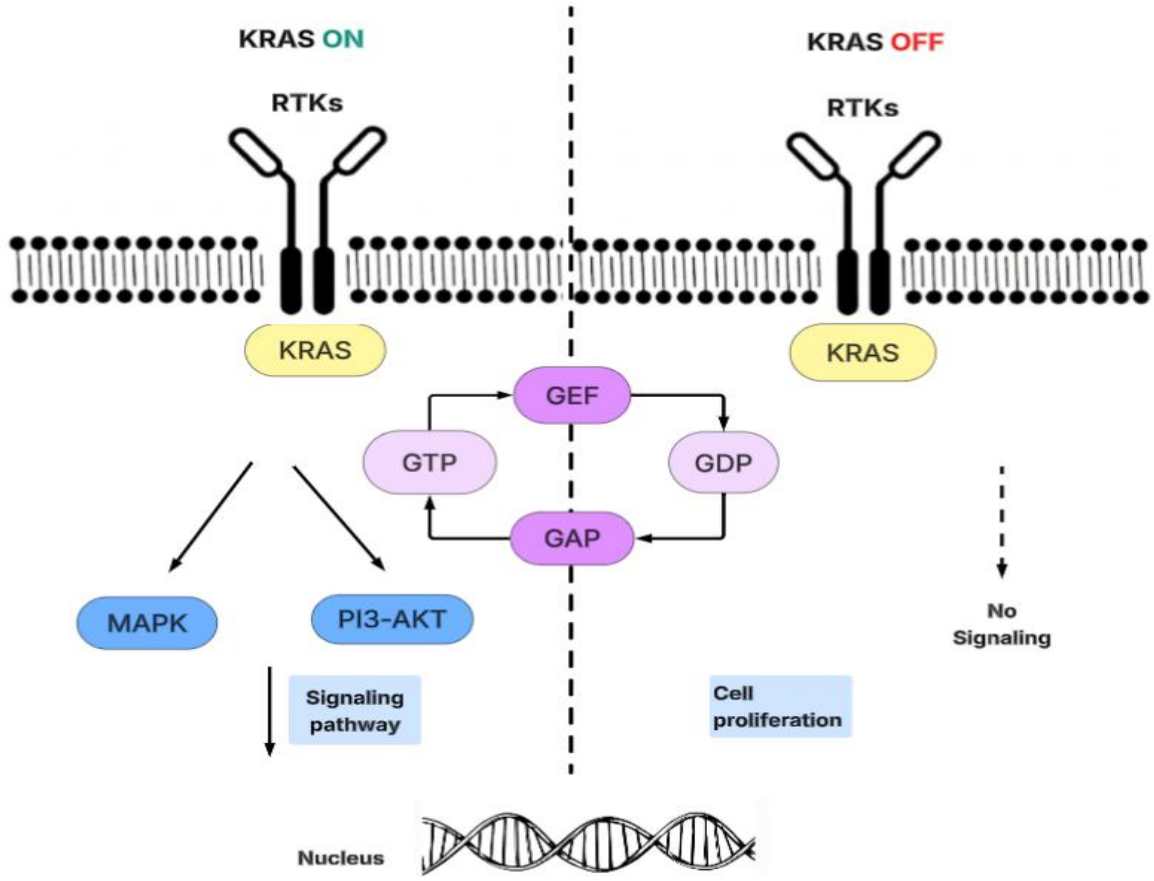

Fig.2.2: KRAS signaling pathway showing GTP-bound active state (ON) promoting cell proliferation through MAPK and PI3-AKT pathways versus GDP-bound inactive state (OFF).

2.3 DUAL INHIBITION OF KRAS AND AURKB: A SYNERGISTIC THERAPEUTIC STRATEGY IN KRAS-MUTANT CANCERS

KRAS gene mutations are known to cause cancer by keeping growth signals switched on all the time, which makes cells grow out of control and live longer than they should. Recent evidence indicates that KRAS mutations lead to upregulation of Aurora kinases A and B (AURKA and AURKB), either by enhancing the transcription of their mRNA or by increasing mRNA stability. Moreover, KRAS can induce the oncogenic transcription factor MYC, which in turn further upregulates AURKA and AURKB expression, amplifying their oncogenic influence.

Functionally, AURKA has been shown to facilitate KRAS activity through the promotion of its farnesylation, a post-translational modification that is crucial for proper membrane localization and signal transduction. AURKB, on the other hand, is primarily involved in mitotic

progression and cytokinesis, thereby supporting the proliferative capacity of KRAS-transformed cells.

Experimental data underscore the dependency of KRAS-mutant cells on Aurora kinases. Pharmacological inhibition of AURKA and AURKB has been shown to impair cancer cell viability by inducing mitotic arrest and promoting apoptosis. This reveals a critical vulnerability in KRAS-driven tumors, where Aurora kinases act as indispensable downstream effectors [47]. Notably, in cancers lacking KRAS or BRAF mutations, AURKB is often stabilized by AKT—a key node in the PI3K/PTEN pathway. In such cases, AKT inhibition effectively destabilizes AURKB and disrupts cell division. However, in KRAS-mutant tumors, this mechanism appears bypassed. KRAS-driven signaling maintains AURKB activity independently of AKT, rendering AKT inhibitors less effective. Thus, direct targeting of AURKB becomes essential in this context [48] [49]. Importantly, targeting AURKA or AURKB alone can impair tumor cell proliferation, but compensatory survival pathways may limit therapeutic efficacy. Similarly, KRAS inhibition alone may reduce oncogenic signaling, but residual mitotic drivers like AURKB can sustain cancer cell division. Therefore, a dual-inhibition strategy—simultaneously targeting KRAS and AURKB—offers a more comprehensive approach. This combination suppresses both oncogenic signaling and mitotic machinery, leading to enhanced apoptosis and reduced potential for resistance development. Collectively, these findings support a model in which KRAS mutations drive a dual dependency on oncogenic signaling and mitotic regulation, with AURKB playing a pivotal role in sustaining proliferation. Therapeutic regimens co-targeting KRAS and AURKB have the potential to induce robust tumor regression by simultaneously disabling growth signals and cell division processes, thereby offering a promising strategy for overcoming resistance and improving outcomes in KRAS-mutant cancers.

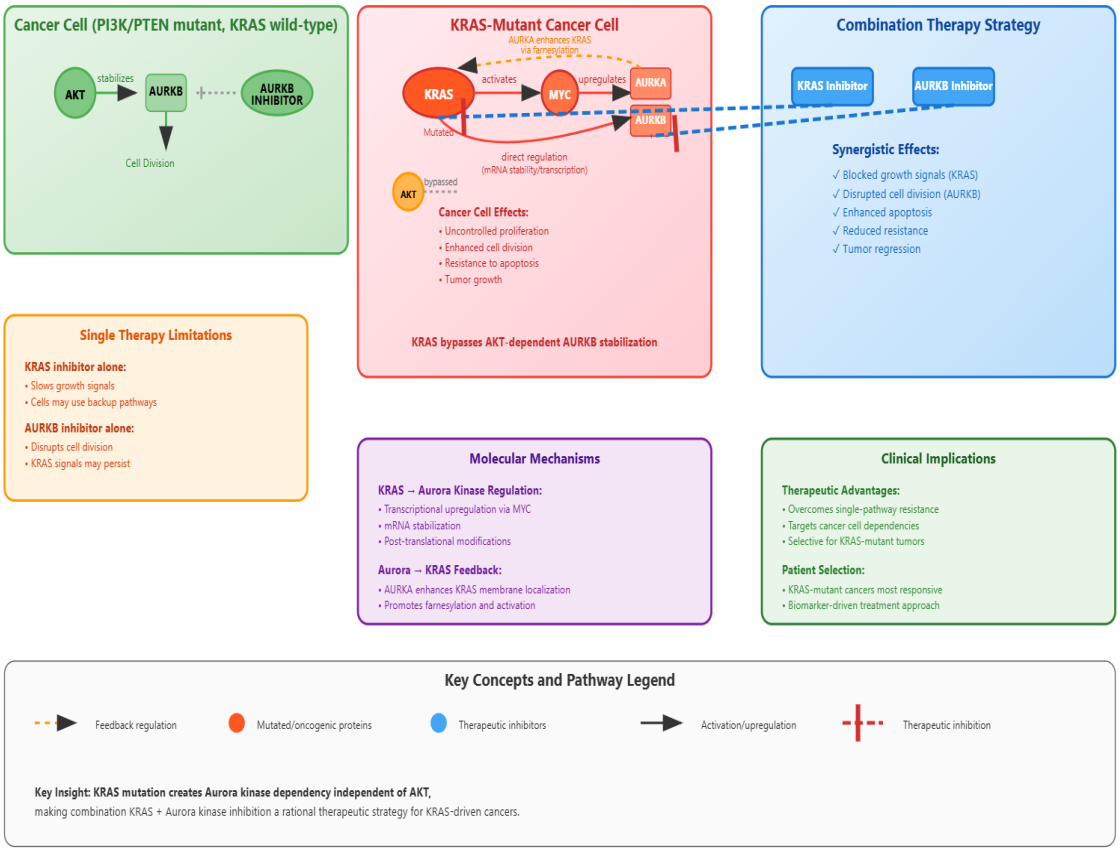

Fig.2.3: KRAS-Aurora Kinase Pathway in Cancer: Mechanism and Therapeutic Targeting

2.4 PHYTOCHEMICALS IN CANCER THERAPY AND THE NPACT DATABASE

Phytochemicals, sometimes referred to as phytonutrients, are bioactive, non-nutritive substances that are found naturally in plants and work as a defensive mechanism against diseases, herbivores, and environmental stressors. Approximately 62% of current anticancer drugs are plant-derived, including paclitaxel, vincristine, and camptothecin [50]. Unlike synthetic drugs that target single pathways, phytochemicals simultaneously modulate multiple cellular targets, reducing drug resistance and enhancing therapeutic efficacy. Phytochemicals exert their anticancer effects through various mechanisms like Cell Cycle Regulation, Apoptosis Induction, Angiogenesis Inhibition, and Metastasis prevention [51]. The use of plant-derived compounds in medicine dates back thousands of years, with traditional healing systems like Ayurveda, Traditional Chinese Medicine, and indigenous practices forming the foundation of modern phytopharmacology[52].

The NPACT (Naturally Occurring Plant-Based Anti-cancerous Compound-Activity-Target)

The database aggregates natural compound information from 762 scientific publications, featuring 1,574 plant-derived molecules with demonstrated anticancer properties across 353 tumor cell types. Researchers can access 5,214 experimental records containing inhibitory concentration data (IC_{50}, ED_{50}, EC_{50}, GI_{50}) alongside in vivo study results. The repository further catalogs 1,980 confirmed molecular target interactions, revealing how these natural substances interfere with cancer cell processes. The database provides structural information, physicochemical properties, biological activity data, and ADMET profiles for each compound[53].

2.5 COMPUTER AIDED DRUG DESIGN INCLUDES

2.5.1 Computational Molecule Model Construction

Both living and non-living systems contain chemical bonds that hold two or more homo/hetero atoms together to form molecules. Drugs are essentially tiny molecules with the ability to interact, bind, and regulate receptor function, which can be used to treat and cure any illness. Proteins known as receptors interact with ligands, chemicals, and substances to sustain cellular activity in living systems. Similarly, cell signaling receptors, enzymes, hormone receptors, and neurotransmitter receptors are some of the main receptors in our bodies. The process of structurally creating tiny compounds that can bind and inhibit the behavior of certain protein receptors that cause disease is known as drug design [54].

2.5.2 Relationship between the drug and receptor

The process of developing a therapeutic molecule that can attach to and interact with a target is known as drug design. The drug's molecule has the ability to bind to DNA cross-links and stop DNA replication. Malignant tumors that progress to more dangerous stages after becoming cancerous can benefit from treatment. There are receptors for hormones, neurotransmitters, growth factors, cytokines, and other endogenous regulating ligands. Thus, the role of these receptors is to identify ligands and start a response.
Molecular modeling is the term for computational methods used to simulate a molecule. Computer-aided drug design refers to the process of using these modeling tools to develop pharmaceuticals.

The computer-assisted drug design approach is useful, automated, quick, inexpensive, and virtual. A process known as docking is used to predict the interactions between two molecules

that have been linked to form a stable complex. The "Lock and Key" concept can be used to explain molecular docking. In the system, the protein acts as the lock and the ligand as the key. It indicates which ligand orientation is best for attaching to a particular protein. Docking requires the utilization of protein molecules. The ligands and protein structure are the inputs for docking[55].

2.5.3 Function of Scoring

To find interactions between proteins and between proteins and DNA, scoring methods have been devised. Mathematical methods for forecasting the intensity of a two-molecule interaction are called scoring procedures.

The steps in the docking process are:

I) Choose a molecule: Take ligand and receptor molecules into account. The ligand molecule is flexible, while the receptor molecule is stiff.

II) Dock the molecules: Insert the ligand molecule into the receptor's binding pocket. Make as many distinct orientations as you are able to.

III) Model evaluation: Assess the model/docking outcomes in light of the resulting energy.

2.6 PHARMACOKINETICS: THE ANALYSIS OF DRUG ADMET PROPERTIES:

Absorption, distribution, metabolism, elimination/excretion, and toxicity (ADMET) assays are among the many that successful medicinal compounds must pass. The purpose of an absorption test is to measure the amount of medication that has been efficiently absorbed. After absorption, it must be appropriately delivered to the target organ within the body through the lymphatic and circulatory systems. It's important to examine how well drug molecules may reach their intended target, which is accomplished by metabolism and excretion processes. The process by which the body uses enzymes in the liver to break down smaller molecules is called metabolism. Elimination, which takes place through the kidneys or feces, is the alternative way that medications are eliminated. Pharmacokinetic characteristics are therefore essential to the synthesis of therapeutic compounds. Pharmacokinetics is the study of how the body responds to medications. The rates of GI absorption, BBB penetration, OCT substrate, and

excretion/elimination are all measured by pharmacokinetics [56].

2.6.1 Absorption

Through absorption, a chemical is moved from the delivery point to its final location. Some drugs must be transported by a carrier, although the majority are absorbed passively. The pH level of drugs may change depending on where they are absorbed because basic medications are better absorbed in the intestine and acidic pharmaceuticals are better absorbed in the stomach. Given that its surface area is far larger than the stomach's, it is likely that the small intestine absorbs drugs primarily there. Amphipathic drugs are absorbed without any problems. Bile salts emulsify certain drugs that float as globules in the colon due to their intractable nature in water into smaller, finer molecules. After being injected into a muscle or the subcutaneous layer, medications can enter the circulatory system through tiny gaps in the capillary walls. For oral drugs to be absorbed, they must be soluble in the gastrointestinal (GI) tract's aqueous content, which is ultimately mediated via GI tract barriers to reach blood, dnrug physicochemical characteristics are among the factors influencing drug absorption.
, the method of administration, and physiological or pathological conditions like blood flow, pH, and interactions with food or other drugs. Intravenous drugs have 100% bioavailability, while oral drugs may have reduced absorption[57].

2.6.2 Distribution

How medication spread throughout the body depends on variables such as circulation pattern, and the ability for medications to penetrate physiological barriers. Following uptake from the gastrointestinal system, numerous compounds pass through the liver's portal circulation, where they can undergo metabolism prior to entering general blood flow—this phenomenon is called first-pass metabolism. Some drugs bind to plasma proteins, remaining in equilibrium between bound and unbound forms; only the free (unbound) form is pharmacologically active. Additionally, for drugs to the brain and spinal cord (CNS), they need to penetrate the blood-brain barrier (BBB), which only allows small, highly lipophilic molecules to pass. This barrier protects the CNS from potentially harmful substances but also limits drug delivery to the brain [58].

2.6.3 Molecule toxicity prediction

ADME and toxicity testing have lately been established by researchers and pharmaceutical businesses using in silico-based approaches. Even before a pharmaceutical molecule is discovered, its toxicity can be predicted using these techniques. Even if in-silico methods are easier, there are still certain obstacles to go beyond.

- **i.)** Toxicity can involve different harmful effects, such as causing cancer (carcinogenicity), damaging or killing cells (cytotoxicity), and other dangerous impacts. It's important to define which type of toxicity is being studied.
- **ii.)** There isn't enough data available, especially when it comes to studies on humans, which makes it hard to accurately predict toxicity.
- **iii.)** In-silico (computer-based) methods can be limited to specific types of molecules, and sometimes it's difficult to tell clearly whether a compound is toxic or not making the results less reliable.

There are numerous ways that drug molecules might cause toxicity. For instance, toxicity might result not only from the drug itself but also from its metabolites. The cytotoxic and mutagenic properties of some medication compounds are designed to destroy diseased or malignant cells, but it is very likely that they will also affect healthy cells. The most fundamental kind of toxicity is hepatotoxicity, in which the drug or one of its metabolites damages the liver [59].

CHAPTER-3

METHODOLOGY

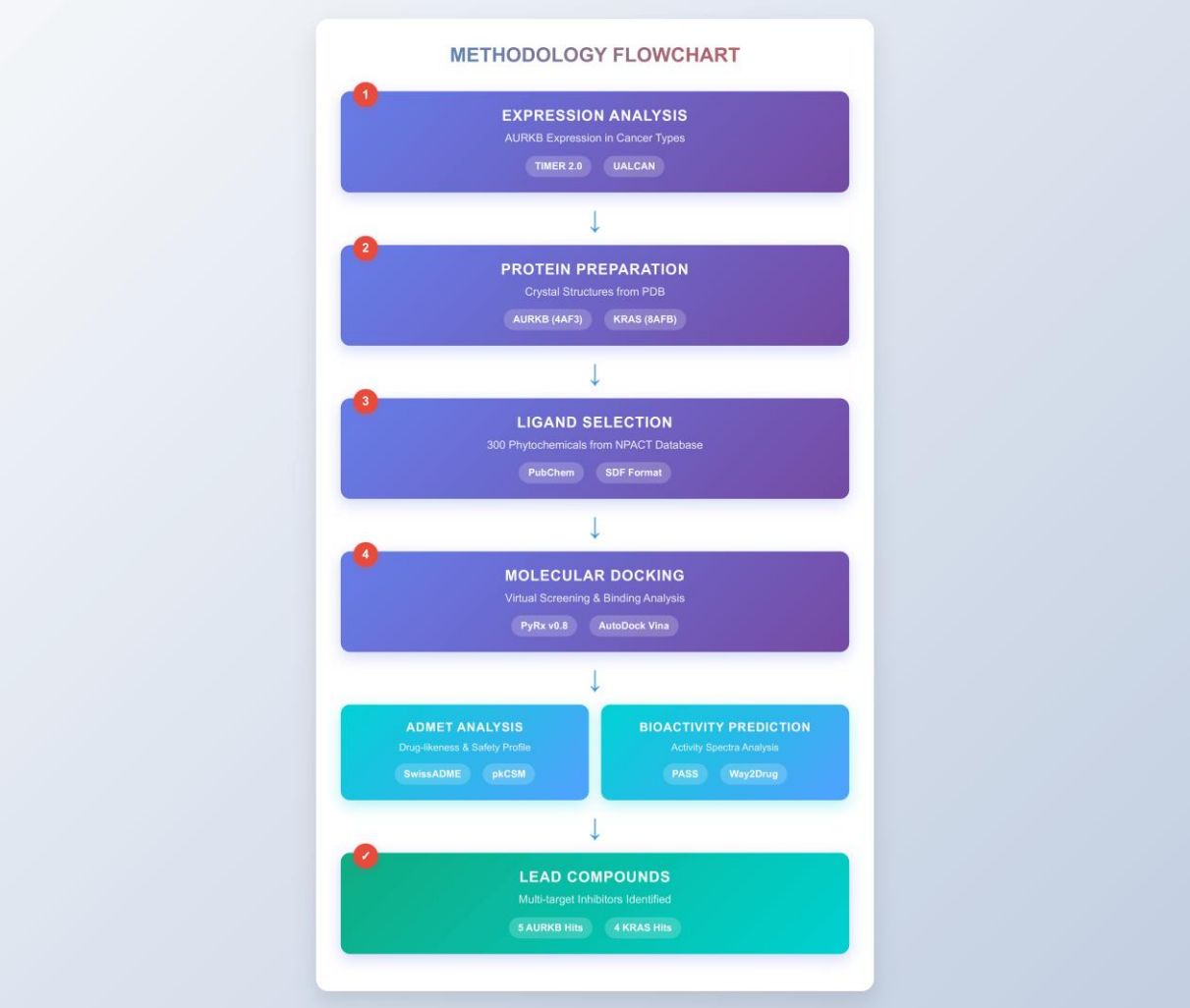

Fig 3.1 Methodology Flowchart

3.1 ANALYSIS OF EXPRESSION OF AURKB

The UALCAN web site and TIMER 2.0 were used to analyze the levels of AURKB expression in different malignancies. A heatmap representing the differential AURKB level of expression across all TCGA tumors was created using TIMER 2.0 (http://timer.cistrome.org). The web-based tool TIMER 2.0 systematically examines gene expression profiles and immune infiltrates

in a variety of cancer types. To determine the amounts of AURKB protein, CPTAC samples that were accessible via the UALCAN web portal were used.

3.2 PROTEIN PREPARATION

The crystal structure of the Human AURKB complex with VX-680 (PDB: 4AF3) at 2.75 Å resolution [60], consisting of chain A, The crystal structure of KRAS-G12C in complex with compound 23 (BI-0474) (PDB:8AFB) at 1.12 Å resolution [61], consisting of chain A, was obtained from the PDB (www.rcsb.org) database. Protein structure preparation was undertaken utilising BIOVIA Discovery Studio (Dassault Systèmes, San Diego, USA) [62] by deleting various unrequired ligands and water molecules and adding polar hydrogen atoms. The PDB structure of selected target was converted to PDBQT within the PyRx (v0.8) software.

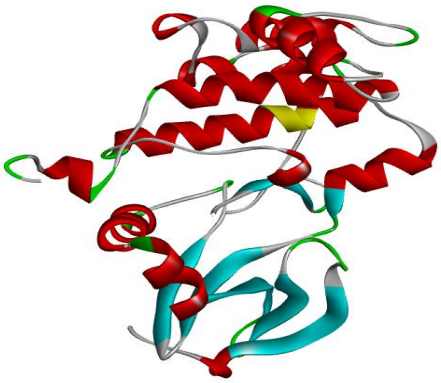

Fig 3.2 - PDB structure of 4AF3 Protein

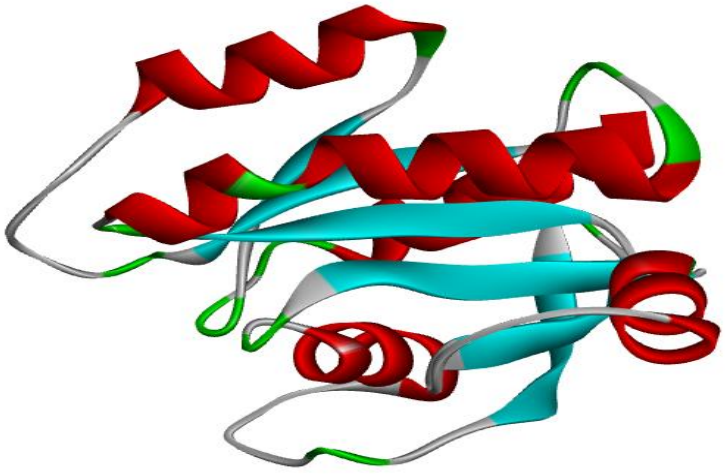

Fig 3.3: PDB structure of 8AFB Protein

3.3 LIGAND SELECTION AND PREPARATION

The NPACT (Naturally Occurring Plant-Based Anti-cancerous Compound Activity-Target) database (http://crdd.osdd.net/raghava/npact/) was utilized to identify natural compounds with established anticancer activity. database was employed to screen natural compounds known for their anti-cancer properties. This resource provides detailed information such as SMILES and SMART representation, molecular scaffolds, drug-likeness, and documented in vitro and in vivo activities of the compound [53]. A total of 300 selected phytochemicals were obtained in three-dimensional SDF format from the PubChem repository [63]. These molecular structures were then optimized through energy minimization using Open Babel [64], and converted into PDBQT format, preparing them as ligands for molecular docking studies conducted in PyRx (v0.8).

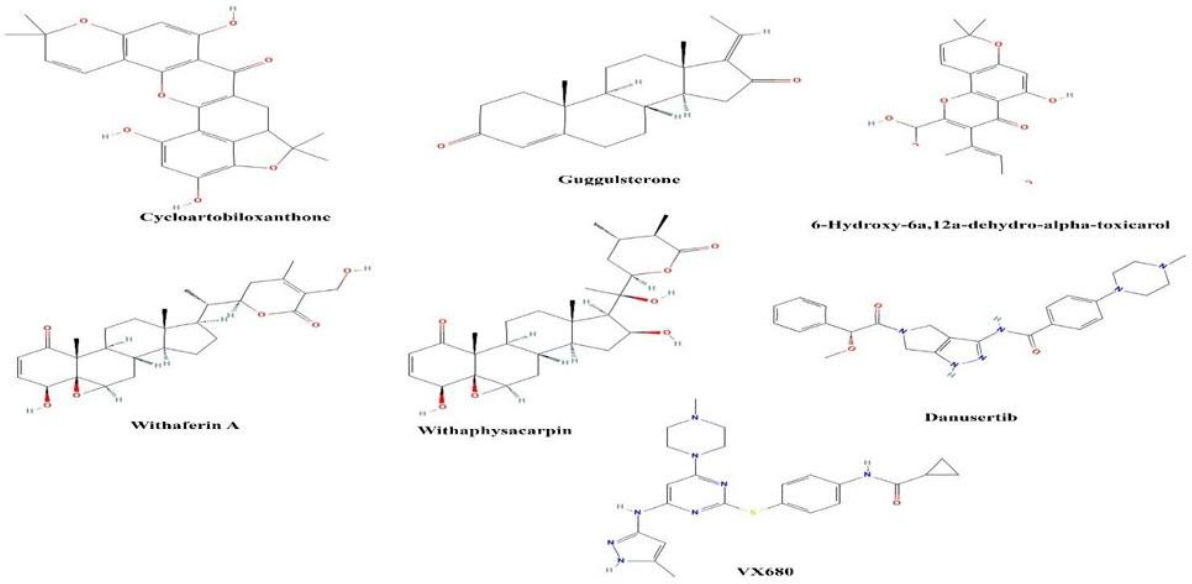

Fig.3.4: Chemical structures of AURKB-targeting phytochemicals: (1) Cycloartobiloxanthone, (2) Gugglusterone, (3) 6-Hydroxy-6a,12a-dehydro-alpha-toxicarol, (4) Withaferin A, (5) Withaphysacarpin and reference inhibitors - Danusertib and VX680, downloaded from PubChem

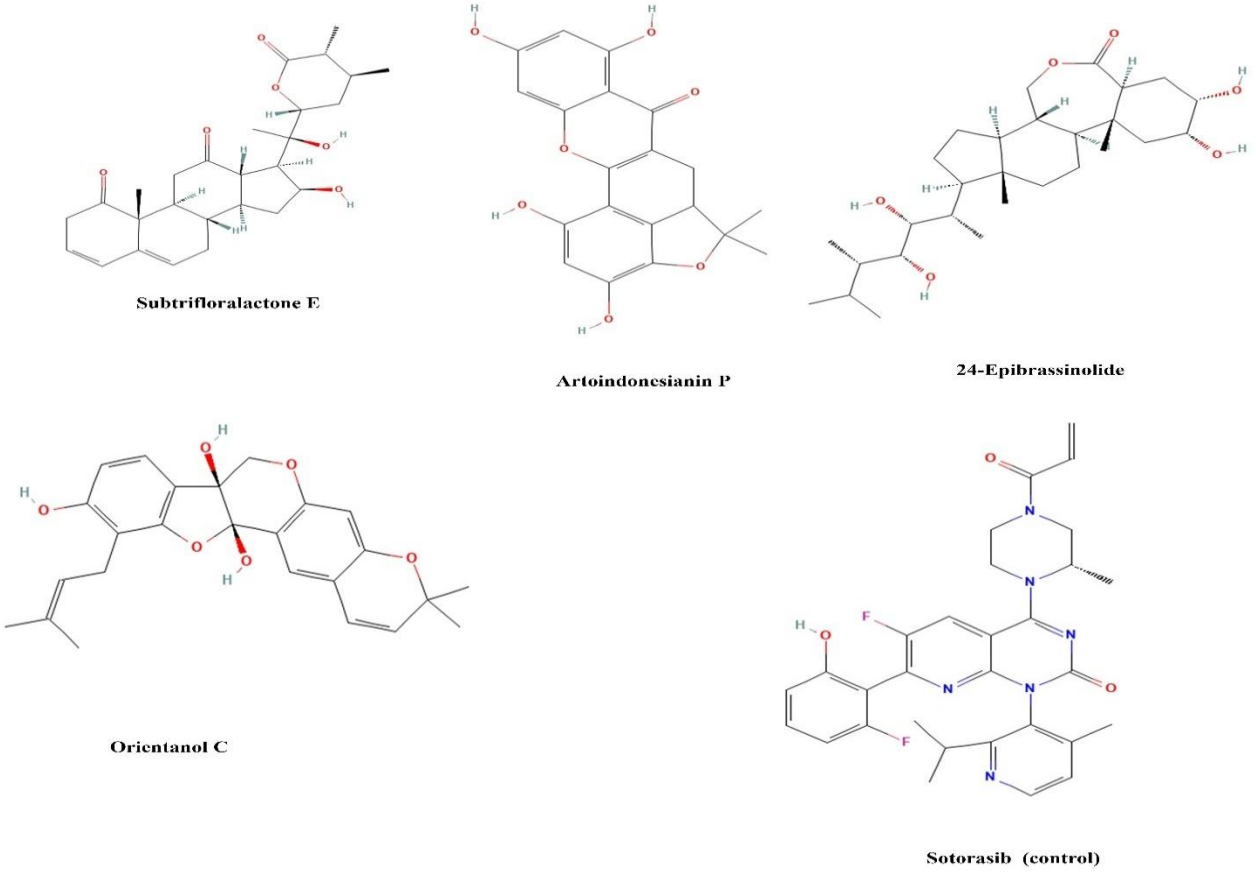

Fig.3.5 Chemical structures of KRAS-targeting phytochemicals: (1) Subtrifloralactone (2) E, Artoindonesianin P, (3) 24 Epibrassinolide , (4) Orientanol C reference inhibitors Sotorasib, downloaded from PubChem

3.4 DOCKING-BASED VIRTUAL SCREENING

PyRx (v0.8) [65] was used to carry out molecular docking simulations, which utilizes AutoDock Vina as the docking engine. The protein structure contained a co-crystallized inhibitor, which was used to identify the active binding site. Biovia Discovery Studio was employed to visualize the protein-ligand complex and determine the binding site coordinates. The docking grid was adjusted to envelope the region where the co-crystallized inhibitor was bound. For AURKB to envelope the enzyme's binding site with coordinates set at (X = 20, Y = 22, Z = -10) Å and for KRAS coordinates set at (X = 14.90, Y = -10.28, Z = 21.95), a grid box of size of 25 × 25 × 25 Å was defined to encompass the active site region. To ensure a thorough exploration for optimal ligand binding conformations, the exhaustiveness parameter was set to 8.

3.5 IN SILICO ADMET ANALYSIS

SwissADME [66] and pkCSM [67] were used to further evaluate the physicochemical descriptors, drug likelihood, and pharmacokinetic properties of the chosen phytochemicals. To assess drug-likeness properties, Lipinski's Rule of Five was applied, which helped screen compounds with acceptable oral bioavailability by removing phytochemicals with undesirable physicochemical characteristics. Additionally, the list of phytochemicals for possible lead compounds was refined by further removing non-specific binders with the aid of the PAINS (Pan-Assay Interference Compounds) filter.

3.6 ASSESSMENT OF COMPOUND BIOACTIVITY THROUGH PASS PREDICTION

The biological activity concerning the screened phytochemicals was analysed by utilising PASS (Prediction of Activity Spectra for Substances) tool available on Way2Drug platform (https://way2drug.com/PASSonline/) [68]. The compounds were submitted in SMILES format, and the tool provided probable activity spectra with Pa (Probability of Activity) and Pi (Probability of Inactivity) scores. Only activities with Pa > 0.6 were considered for further analysis, indicating a high likelihood of biological relevance.

CHAPTER-4

RESULT

4.1 UPREGULATION OF AURKB ACROSS MULTIPLE CANCERS

AURKB levels was obtained to be notably elevated in tumor tissues across TCGA datasets of TIMER 2.0 as seen in the heatmap (Fig.4.1). The box plot illustrates the variable expression of AURKB in the tumor tissues (red) and control tissues (blue) pan cancer. Several malignancies exhibited elevated levels of AURKB such as BRCA, LUAD, LIHC, LUSC, COAD, HNSC, UCEC and STAD, among others ($p < 0.001$). The CPTAC samples from the UALCAN online portal were subsequently used for determining protein levels of AURKB, which corroborated the findings of AURKB mRNA upregulation. Many forms of cancers, including liver cancer, head and neck, PAAD, lung cancer, ovarian cancer and UCEC showed increased protein levels of AURKB as compared to normal levels (Fig.4.2).

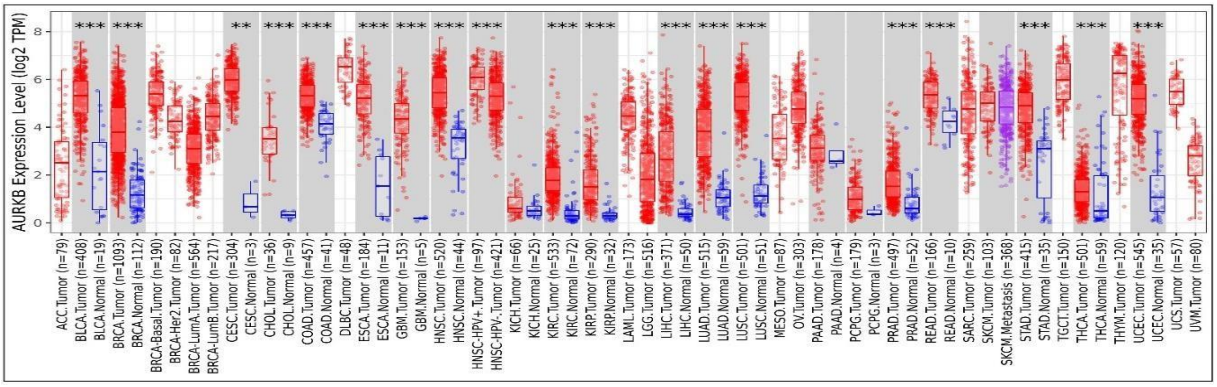

Figure 4.1 AURKB differential mRNA expression pan cancer. Differential expression of AURKB in normal and tumor tissues across TCGA tumors utilizing TIMER 2.0 database. Significant higher expression of AURKB across various cancer shown in box plots.

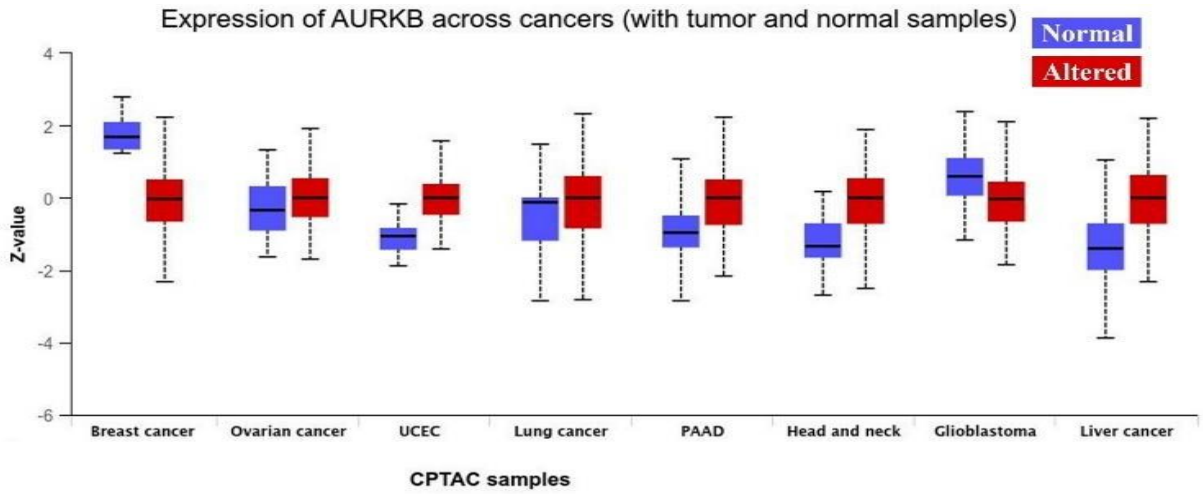

Figure 4.2 Differential expression of AURKB across various cancer using the UALCAN tool. AURKB protein levels are seen to be upregulated in tumors samples as opposed to the paired normal samples, p-value <0.01.

4.2 DOCKING RESULTS OF IN SILICO STUDY

4.2.1 Docking Results of Aurkb

PyRx was used to virtually screen the 300 chemicals that were obtained from the PubChem database. Based on their lowest binding energy values—a key feature of potential inhibitors in this study—the top five phytochemicals were determined. According to Table 4.1, these phytochemicals include Cycloartobiloxanthone (-11.7 kcal/mol), Guggulsterone (-11.4 kcal/mol), 6-Hydroxy-6a,12a-dehydro-alpha-toxicarol (10.9 kcal/mol), Withaferin A (-10.7 kcal/mol) and Withaphysacarpin (-10.6 kcal/mol). The following phytochemicals were then examined using Visualization in Discovery Studio to determine where they were located in the binding pocket. It was discovered that the necessary amino acid residues located within the binding site completely encased the docked phytochemicals. Consistent binding strength was confirmed by additional analysis of the different interactions between the drugs and amino acids, indicating the site's potential as a therapeutic target. Figure 4.3 and 4.4 shows the interaction between the five phytochemicals and the important amino acid residues located within the binding site. These interactions include hydrophobic, electrostatic, Van der Waals, Pi-Pi, Pi-Sulfur, Pi-alkyl, and H-bonding interactions. The same molecular docking procedure was used to compare the target protein AURKB (PDB: 4AF3) with two common Aurora Kinase

inhibitors, VX680 and Danusertib, within PyRx(v0.8). VX-680 and Danusertib were shown to have binding energies of -10.5 kcal/mol and -9.3 kcal/mol, respectively. They also exhibited various interactions such as Hydrogen-bonding, electrostatic interactions, Pi-Pi interactions, Pi-sulfur interactions, Pi-alkyl interactions, hydrophobic interactions and Van der Waals interaction, interacting with the essential amino acid residues situated within the binding site (Figure 4.3 and 4.4). The molecular docking clearly shows the phytochemicals exhibiting higher binding affinity and lower free energy of binding as opposed to the co crystallised ligand VX-680. Detailed analysis revealed that both the ligand and VX-680 formed similar bonds along the protein with common amino acids ALA157, LEU207 and LEU83. These results confirmed that the phytochemicals interacted with the binding pocket with high affinity and low binding free energy.

TABLE 4.1 Top Four Phytochemicals Along with PubChem Identifier, Docking Score, Hydrogen Bond Count And Interacting Amino Acid Residues.

Phytochemical Name	**Pubchem Identifier**	**Estimated Binding Energy (Kcal/mol)**	**Hydrogen Bond form**	**Key interacting Amino Acid Residues (H-bond, Pi-Pi, Hydrophobic,etc)**
Cycloartobiloxanth one	10342859	-11.7	1	Ala157, Leu207, Leu83, Ala104
Guggulsterone	6439929	-11.4	1	Phe219, Ala217, Leu207, Val91
6-Hydroxy-6a,12a-dehydro-alpha-toxicarol	44257423	-10.9	2	Glu161, Lys106, Val91, Leu83, Leu207, Phe219, Leu154, Phe88, Ala157, Gly84,
Withaferin A	265237	-10.7	2	Tyr156, Pro158, Leu207, Leu83, Lys164
Withaphysacarpin	4456700 5	-10.6	1	Pro158, Glu161
Danusertib (Control)	1144289 1	-9.3	1	Arg159, Phe88, Leu207, Val91, Ala217, leu83, Glu161

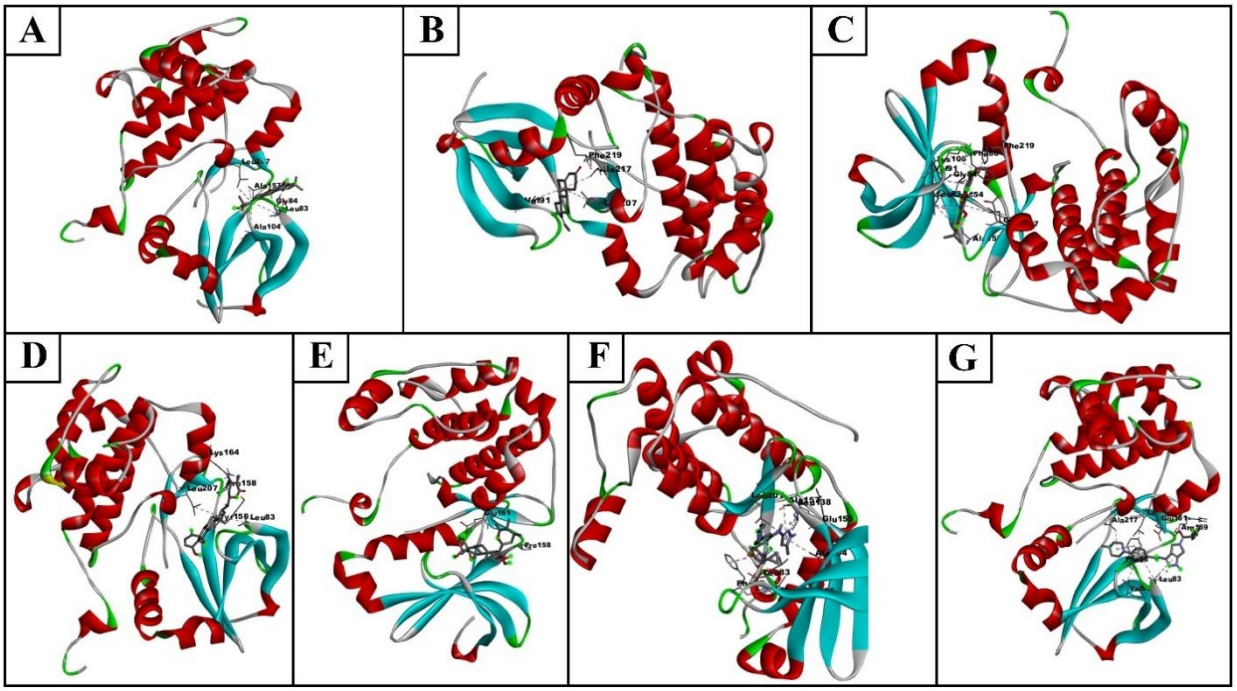

Fig. 4.3 3D interaction visuals highlighting the amino acid contacts formed between AURKB and each ligand: Cycloartobiloxanthone(A), Guggulsterone(B), 6-Hydroxy-6a,12a-dehydro-alpha-toxicarol(C), Withaferin A (D), Withaphysacarpin (E) Danusertib (F) and VX-680 (G)

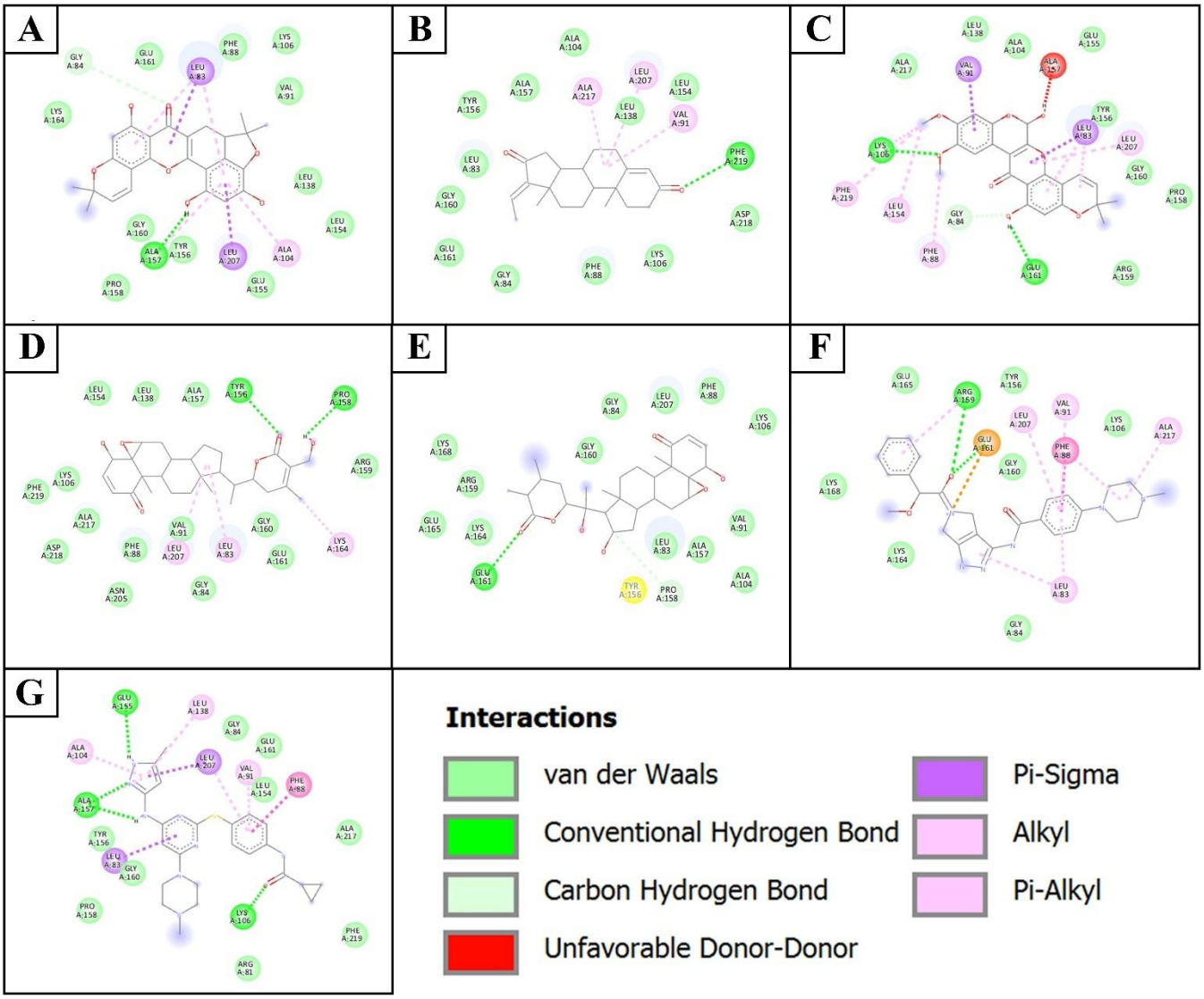

Fig. 4.4 2D interaction visuals highlighting the amino acid contacts formed between AURKB and each ligand: Cycloartobiloxanthone(A), Guggulsterone(B), 6-Hydroxy-6a,12a-dehydro-alpha-toxicarol(C), Withaferin A (D), Withaphysacarpin (E), Danusertib (F) and VX-680 (G).

4.2.2 Docking Results of Kras

In this work, 300 anti-cancerous phytochemicals from diverse categories were chosen for docking with the KRAS G12C protein (PDB ID: 8AFB). Several possible compounds with better binding affinity than the positive control sotorasib were identified by virtual screening (Table 4.2). Following detailed analysis and visualization of the molecular docking interactions, this study identifies four phytochemicals that demonstrated stronger binding affinity through interactions with key residues of the KRAS protein. The docking score obtained for the standard drug Sotorasib, in interaction with the KRAS G12C receptor, was −8.8 kcal/mol. Sotorasib

interacted with Val9, Met72, Tyr64, Phe78, Ile100, Gln99, Tyr96, Gly60, Arg68, His95, Asp92, Glu63 Ala59, Ala11 and Lys88 Notably, Glu62 formed hydrogen bonds, while Cys12 and Gln61 were involved in Pi-Alkyl interactions (Figure 4.6 3E). The four phytochemicals displayed even higher docking scores. Subtrifloralactone E scored −11.6 kcal/mol and interacted with Asp92, Lys88, His95, Glu62, Tyr64, Asp69, Arg102, Met72, Val103, Gln99, Ile100, Val9, Thr58, Gly60, Tyr96, Ala11, and Cys12, hydrogen bonds formed at Gln61, Arg68, and Ala59 residues (Figure 4.6 3A). Artoindonesianin P, showing a binding energy of −10.9 kcal/mol, interacted with Gly 60, Ala11, Lys16, Thr58, Val9, Phe78, Ile100, Gln99, Val103, Tyr64 and showed hydrogen bonds with Gly10, Asp69, Arg102, and Glu63, along with Pi-sulfur interactions at Cys12, Pi-cation interactions at Arg68, and Pi-Alkyl interactions at Met72 (Figure 3B). 24 Epibrassinolide, yielding a docking energy of −10.3 kcal/mol, and forming interactions with Met72, Thr58, Val9, Ala11, Gln61, Cys12, Arg68, Glu62, Tyr96, and Gln99, forming hydrogen bonds with Arg102 and Gly60 residues (Figure4.6 3C). Orientanol C, with a calculated binding affinity of −10.1 kcal/mol, engaged with residues Val103, Glu62, Gln61, Gly60, Ala11, Gly10, Tyr96, Thr58, Glu63, Ile100, Asp69, Arg102, Gln99, Phe78, and Glu37. Additional interactions included hydrogen bonds with Lys16, Ala59, and Arg68, as well as Pi-Alkyl bonds formed with Cys12, Tyr64, Val9, and Met72 (Figure 4.6 3D).

Table 4.2: Top Four Phytochemicals Along With PubChem Identifier, Docking Score, Hydrogen Bond Count And Interacting Amino Acid Residues.

Phytochemical Name	**Pubchem Identifier**	**Estimated Binding Energy (Kcal/mol)**	**Hydrogen Bond form**	**Key interacting Amino Acid Residues (H-bond, Pi-Pi, Hydrophobic,etc)**
Subtrifloralactone E	21600009	-11.6	4	GLN 61, ARG 68, ALA 59, GLU63
Artoindonesianin P	10316935	-10.9	5	GLY 10, ASP 69, ARG 102, GLU 63, MET 72, ARG 68, CYS 12
24-Epibrassinolide	115196	-10.3	2	ARG 102, GLY 60

Orientanol C	42607512	-10.1	3	LYS 16, ARG 68, ALA59, CYS 12, TYR 96, VAL9, MET 72, TYR64, Thr58
Sotorasib (control)	137278711	-8.8	1	GLU 62, GLN 61, CYS 12, ASP 92, HIS 95

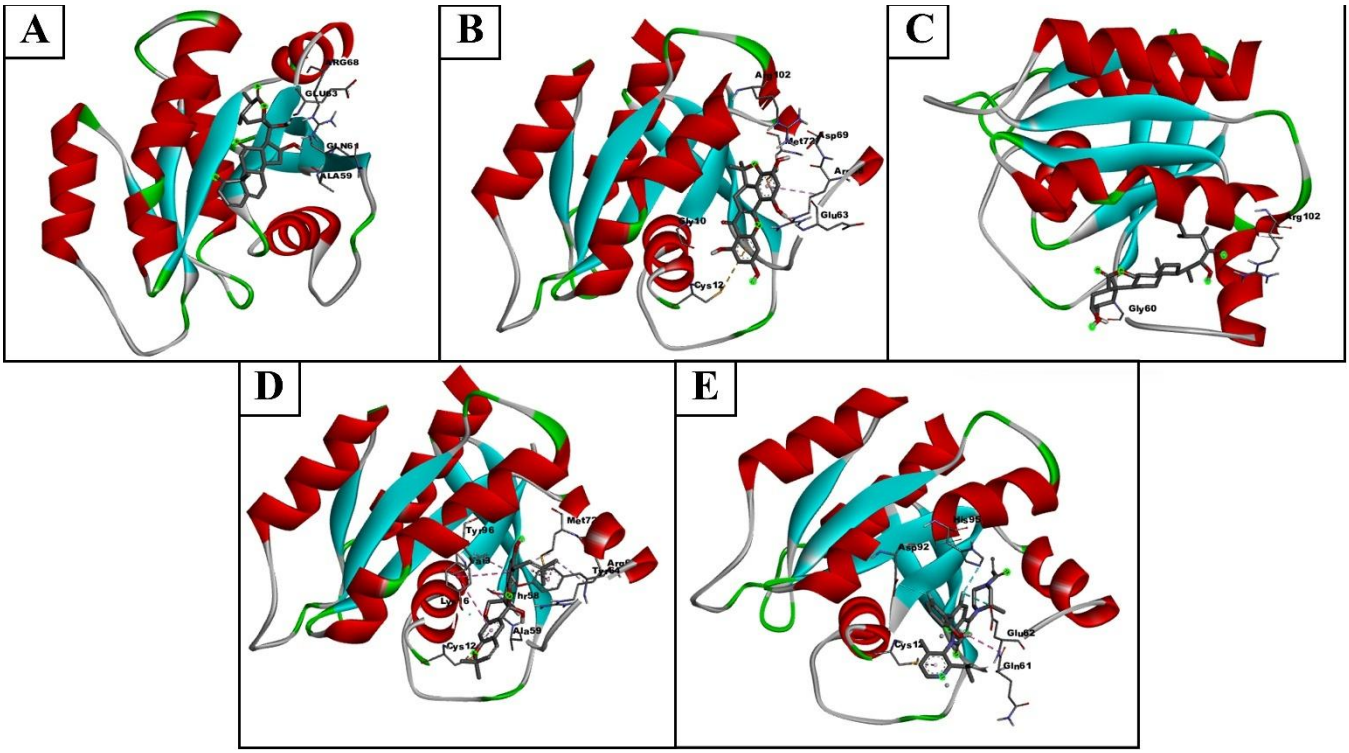

FIG. 4.5 3D interaction visuals highlighting the amino acid contacts formed between KRAS and each ligand: Subtrifloralactone E (A), Artoindonesianin P (B), 24-Epibrassinolide (C), Orientanol C (D), and Sotorasib (E)

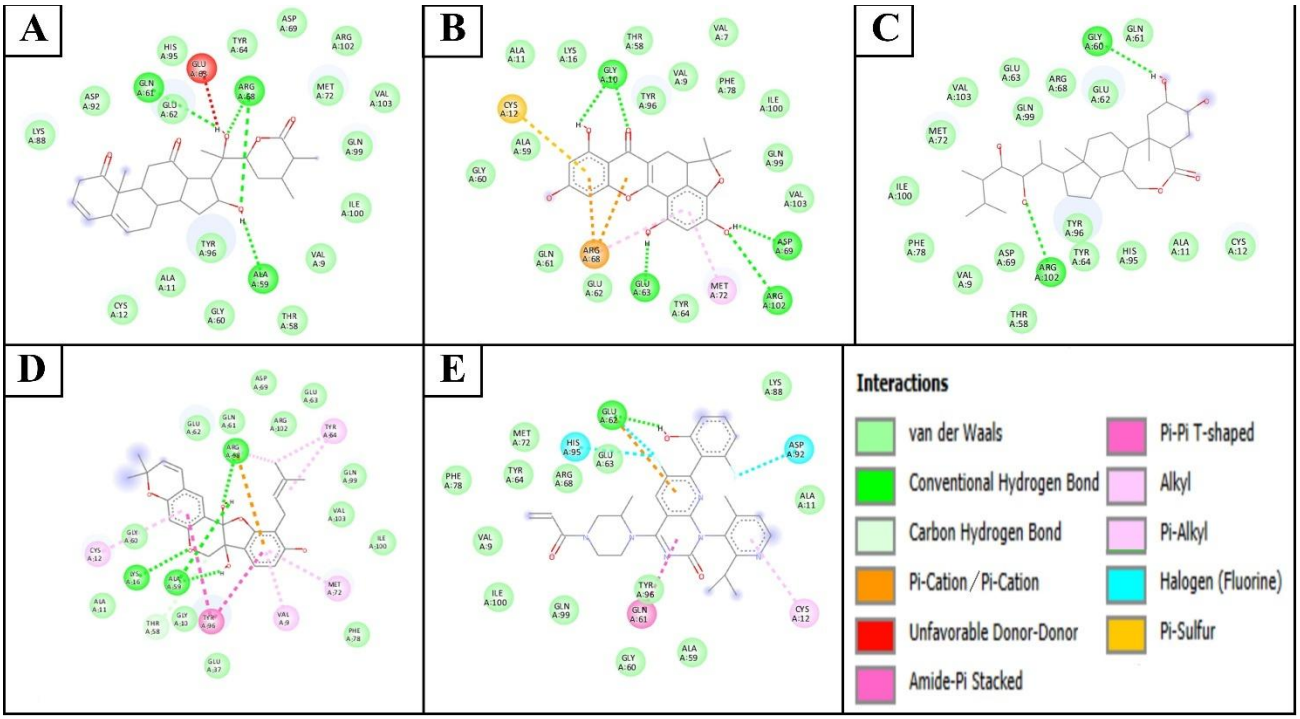

Fig 4.6: 2D visualizations highlighting amino acid contacts between KRAS and Subtrifloralactone E (A), Artoindonesianin P (B), 24-Epibrassinolide (C), Orientanol C (D), and Sotorasib (E)

4.3 PHARMACOKINETIC AND DRUG LIKENESS SCREENING OF PHYTOCHEMICALS

4.3.1 SCREENING OF PHYTOCHEMICAL INHIBITORS TARGETING AURKB

The pharmacokinetic and toxic effects of the five identified phytochemicals were assessed via computational ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) modeling. The pharmacokinetic characteristics were assessed using SwissADME, and the toxicity was predicted using pkCSM. Lipinski's Rule of Five criteria, which evaluates parameters such as molecular weight, hydrogen donor, hydrogen acceptor, and lipophilicity, was used to select the compounds that satisfied its requirements. These requirements were satisfactorily fulfilled by cycloartobiloxanthone, guggulsterone, 6-hydroxy-6a, 12a-dehydro-alpha-toxicarol, withaferin A, and withaphysacarpin, indicating their drug-likeness.

The screening of possible lead compounds was further refined by applying the PAINS (Pan Assay Interference Compounds) filter, which removed non-specific binders (Table 4.3).

Following ADMET analysis, as indicated in Table 4.4, the five phytochemicals' aqueous solubility was determined to be moderate, with an ESOL (logS) ranging from -5.46 to 4.68. This suggests that the compounds may face formulation and bioavailability issues. Subsequent investigation showed that all of the chosen compounds had a consistent bioavailability score of 0.55, which suggested a moderate systemic availability after treatment, and high gastrointestinal absorption (GIA), which indicated effective oral uptake. The five phytochemicals do not inhibit major CYP enzymes (e.g., CYP2D6 and CYP3A4), in contrast to the control drugs Danusertib and VX-680, which inhibit CYP3A4 thus pose a higher risk in relation to drug-drug interactions. Furthermore, none of the tested compounds act as OCT2 substrates, implying no active renal excretion via this transporter. Following with the Ames test to evaluate the mutagenic potential of the compounds, none of the phytochemicals, nor the controls VX-680 and Danusertib, exhibited mutagenicity, as indicated by negative Ames test results, suggesting that these compounds are unlikely to induce genetic mutations and may have a favorable safety profile in terms of genotoxicity. However, hepatotoxicity results reveal notable differences. While Cycloartobiloxanthone, 6-Hydroxy-6a,12a-dehydro-alpha toxicarol, Withaferin A, and Withaphysacarpin demonstrated no hepatotoxic potential, Guggulsterone, VX-680, and Danusertib were predicted to be hepatotoxic. The observed hepatotoxicity in Guggulsterone warrants further investigation into its metabolic impact and potential liver toxicity risks. With structural modifications or formulation advancements, there remains the possibility of mitigating its hepatotoxic effects, thereby enhancing its suitability as a therapeutic candidate for Aurora Kinase inhibition. These findings underscore the potential of Cycloartobiloxanthone, 6-Hydroxy-6a,12a-dehydro-alpha toxicarol, Withaferin A, and Withaphysacarpin as safer alternatives for further development in Aurora Kinase inhibition research.

TABLE 4.3. PAINS AND RO5 FILTER OF TOP 5 HITS OF PHYTOCHEMICALS (AURKB)

Phytochemical	**Mol. Weight**	**H-bond Donor**	**H-bond Acceptor**	**LOGP**	**TPSA**	**Lipinski Violation**	**PAINS alerts**
Cycloartobiloxanthone	434.44 g/mol	3	7	3.69	109.36 Å²	0	0
Guggulsterone	312.45 g/mol	0	2	4.03	34.14 Å²	0	0
6-Hydroxy-6a,12a-dehydro- alpha-toxicarol	424.40 g/mol	2	8	2.99	107.59 Å²	0	0
Withaferin A	470.60 g/mol	2	6	3.42	96.36 Å²	0	0
Withaphysacarpin	488.61 g/mol	3	7	2.65	116.59 Å²	0	0

TABLE 4.4 ADMET Properties of Top 5 Hits of Phytochemicals, Danusertib and VX680

Phytochemical	**GI absorption**	**Bioavailability**	**ESOL (Log S)**	**BBB permeation (log BB)**	**CYP2D6, CYP3A4 Inhibitor**	**OCT2 substrate**	**AMES/Hepatotoxicity**
Cycloartobiloxanthone	High	0.55	-5.46 (MS)	-0.987	No	No	Nil/Nil
Gugglsterone	High	0.55	-4.26 (MS)	0.188	No	No	Nil/Yes
Toxicarol	High	0.55	-4.76 (MS)	-0.826	No	No	Nil/Nil
Withaferin A	High	0.55	-4.97 (MS)	0.588	No	No	Nil/Nil
Withaphysacarpin	High	0.55	-4.68 (MS)	-0.628	No	No	Nil/Nil
Danusertib (control)	High	0.55	-4.72 (MS)	-1.157	Yes (CYP3A4)	NO	Nil/yes

VX680 (control)	high	0.55	-3.75 (S)	-1.518	Yes (CYP3A 4)	NO	Nil/Yes

4.3.2 SCREENING OF PHYTOCHEMICAL INHIBITORS TARGETING KRAS

The four phytochemicals so obtained were further evaluated to determine their pharmacokinetic and drug-likeness properties to further highlight their potential as drug candidates. For this, Lipinski's Rule of Five and PAINS (Pan-Assay Interference Compounds) analysis was conducted which confirmed favorability of the four phytochemicals as they exhibited no Lipinski violations and no PAINS alerts (Table 4.5). After conducting ADMET analysis (Table 4.6) further validated pharmacokinetics properties, revealing high gastrointestinal (GI) absorption in all the four phytochemicals, with a bioavailability score of 0.55, which was comparable to the control drug Sotorasib. The solubility (ESOL, logS) ranged from -4.28 to -5.82, in contrast to Sotorasib which showed the lowest solubility (-5.82). Enzyme inhibition predictions indicated that Orientanol C, and Sotorasib were CYP3A4 inhibitors, while Orientanol C was also a CYP2D6 inhibitor. Toxicity assessments revealed that Sotorasib (control drug) and Artoindonesianin P were hepatotoxic, whereas the other phytochemicals were non-hepatotoxic. To conclude, AMES test was undertaken whose predictions confirmed the absence of mutagenic potential of the four phytochemicals (AMES negative) (Table 4.6). Overall, the selected phytochemicals, demonstrated promising drug-like properties, favourable pharmacokinetics, which was comparable to, if not better than, Sotorasib. They also showed better toxicity profile than Sotorasib, except for Artoindonesianin P, which was predicted to be hepatotoxic. These findings highlight the potential of these phytochemicals as safer alternatives for further drug development.

TABLE 4.5 Pains and Ro5 Filter of Top 4 Hits of Phytochemicals (Kras)

Phytochemical	**Mol. Weight**	**H-bond Donor**	**H-bond Acceptor**	**LOGP**	**TPSA**	**Lipinski Violation**	**PAINS alerts**
Subtrifloralactone E	456.579 g/mol	2	6	3.0089	100.90 Å²	0	0
Artoindonesian	368.34	4	7	3.09	120.36 Å²	0	0

in P	g/mol			31			
24-epibrassinolide	480.68 g/mol	4	6	4.6436	107.22 Å²	0	0
Orientanol C	422.47 g/mol	3	6	3.9028	88.38 Å²	0	0

Table 4.6 Admet Properties of Top 4 Hits of Phytochemicals And Sotorasib

Phytochemical	**GI absorption**	**Bioavaila-bility**	**ESOL (LogS)**	**BBB permeation (log BB)**	**CYP2D6 Inhibitor**	**CYP3A4 inhibitor**	**OCT2 substrate**	**AMES/ Hepatotoxicity**
Subtrifloralactone E	High	0.55	-4.28	-0.336	No	No	No	No/No
Artoindonesian in P	High	0.55	-4.31	-1.083	No	No	No	No/Yes
24-epibrassinolide	High	0.55	-5.54	-0.682	No	No	No	No/No
Orientanol C	High	0.55	-4.91	-0.695	Yes	Yes	No	No/No
Sotorasib (control)	High	0.55	-5.82	-1.383	No	Yes	Yes	No/Yes

4.4 PREDICTION OF BIOLOGICAL ACTIVITIES USING PASS ANALYSIS

PASS analysis was conducted for prediction of biological activities of the selected phytochemicals as potential inhibitors of AURKB and KRAS. The analysis revealed that phytochemical, cycloartobiloxanthone, guggulsterone, 6-hydroxy-6a, 12a-dehydro-alpha-

toxicarol, withaferin A, and withaphysacarpin, for AURKB. Subtrifloralactone E, Artoindonesianin P, 24-epibrassinolide and orientanol C for KRAS, exhibited significant antineoplastic, apoptosis agonists, antileukemic and chemopreventive properties (Table 4.7), with notable affinity for AURKB and KRAS. These findings suggest their potential role as therapeutic candidates in targeting AURKB and KRAS for cancer treatment.

TABLE 4.7 PASS Analysis

Phytochemical	**<Pa>**	**<Pi>**	**Activity**
Cycloartobiloxanthone	0,935	0,004	HIF1A expression inhibitor
	0,875	0,005	Antineoplastic
	0,864	0,005	Apoptosis agonist
	0,823	0,004	Antileukemic
	0,818	0,004	Chemopreventive
	0,818	0,004	Kinase inhibitor
	0,737	0,005	Antineoplastic (colorectal cancer)
Guggulsterone	0,888	0,005	Antineoplastic
	0,753	0,012	JAK2 expression inhibitor
6-Hydroxy-6a,12a-dehydro-alpha-toxicarol	0,929	0,004	HIF1A expression inhibitor
	0,790	0,013	Antineoplastic
	0,767	0,005	Chemopreventive
	0,744	0,009	Kinase inhibitor
Withaferin A	0,916	0,005	Antineoplastic
	0,722	0,005	Antineoplastic (breast cancer)
Withphysacarpin	0,892	0,005	Antineoplastic
	0,732	0,005	Antineoplastic
	0,560	0,009	Antimetastatic
Subtrifloralactone E	0,726	0,005	Antineoplastic (colon cancer)
	0,653	0,006	Antileukemic
Artoindonesianin P	0,901	0,005	HIF1A expression inhibitor
	0,839	0,013	Antineoplastic
	0,777	0,011	Antileukemic
24-epibrassinolide	0,813	0,013	Apoptosis agonist
	0,748	0,019	Antineoplastic
Orientanol	0,779	0,013	HIF1A expression inhibitor
	0,760	0,017	Antineoplastic
	0,732	0,005	Chemopreventive
	0,655	0,020	Apoptosis agonist

CHAPTER-5

DISCUSSION

Cancer will continue to rise as a worldwide health challenge and a leading contributor to mortality, with poor prognosis due to drug resistance. Novel therapeutic and diagnostic strategies are the need of the hour. Combination therapy has improved patient outcomes by reducing drug resistance, lowering drug dosage, and minimizing chemotherapy side effects.

Given AURKB's and KRAS involvement in regulating the cell cycle and oncogenic signaling, Targeting AURKBs and KRAS alongside conventional treatments is a promising strategy, necessitating the identification of selective inhibitors.

For this work, AURKB was explored as a possible therapeutic target using in-silico techniques, and the analysis demonstrated its considerable enhanced expression pan cancer. AURKB's upregulation in tumor tissues as opposed to normal tissues was confirmed by differential mRNA and protein expression analyses using the TIMER 2.0 and CPTAC datasets. The UALCAN analysis showed higher protein expression of AURKB in liver cancer, HNSC, PAAD, lung cancer, ovarian cancer, and UCEC, while the TIMER 2.0 analysis showed upregulation of AURKB in BRCA, LUAD, LIHC, LUSC, COAD, HNSC, UCEC, and STAD. A total of 300 anti-cancer phytochemicals were identified from the NPACT database and screened, among which the following five compounds—Cycloartobiloxanthone, Guggulsterone, 6-Hydroxy-6a,12a-dehydro- alpha-toxicarol, Withaferin A, and Withaphysacarpin—exhibited strong binding affinity toward AURKB, surpassing that of the reference drugs VX-680 and Danusertib. The selected phytochemicals demonstrated promising drug-like properties as they fulfilled Lipinski's Rule of Five. While the moderate aqueous solubility (ESOL values between -5.46 and -4.68), their high gastrointestinal absorption and consistent bioavailability score of 0.55 highlight their potential as effective orally administered therapies. None of the phytochemicals exhibited mutagenic risks based on the Ames test results, reinforcing their genotoxic safety. Although Guggulsterone showed predictions of hepatotoxicity, thus more research is required for evaluating the metabolic impact and potential risks to liver function of Guggulsterone. The other phytochemicals—Cycloartobiloxanthone, 6-Hydroxy-6a,12a-dehydro-alpha-toxicarol, Withaferin A, and Withaphysacarpin—demonstrated no hepatotoxicity, presenting a safer profile compared to the control drugs VX-680 and Danusertib, which were also predicted to be hepatotoxic. In addition to their safety profiles, the phytochemicals exhibit selective pharmacokinetic properties, avoiding major CYP enzyme

inhibition, which reduces the risk of drug-drug interactions—a key limitation of the control drugs. None of the selected compounds act as OCT2 substrates, further supporting their potential for systemic use without significant renal excretion concerns.

Another protein selected for this work KRAS G12C mutation represents a valuable therapeutic target owing to its potential for covalent binding with Cys12 and form hydrophobic interactions within the cryptic allosteric pocket. This interaction locks KRAS in an inactive state. [69]. In this study, phytochemicals such as Artoindonesianin P and Orientanol C exhibited binding interactions with Cys12, analogous to the benchmark drug Sotorasib. Sotorasib interacted with Val9, Met72, Tyr64, Phe78, Val9, Ile100, Gln99, Tyr96, Gly60, Arg68, His95, Asp92, Glu63, Ala59, Ala11, Lys88, Glu62, Cys12 and Gln61 (Figure 3E). Interestingly, Val9, Tyr64, Arg68, Ala11, Cys12, Tyr96, Gln61, Gly60, Met72, Gln99, Glu62 binding residues of the KRAS protein were obtained to be common between the top four phytochemical (Subtrifloralactone E, Artoindonesianin P, 24-epibrassinolide and Orientanol C) and the control Sotorasib (Figure 3A-3E). The selected phytochemicals exhibited favorable pharmacokinetic profiles, including no violations of Lipinski violations, high gastrointestinal absorption (bioavailability score of 0.55), and good solubility Orientanol C and Sotorasib were identified as CYP3A4 inhibitors, with Orientanol C additionally inhibiting CYP2D6. In toxicity assessments, Sotorasib and Artoindonesianin P were predicted to be hepatotoxic, whereas the remaining phytochemicals demonstrated non-hepatotoxic profiles. Furthermore, all selected compounds were AMES negative, indicating the absence of mutagenic potential. Thus, their favourable pharmacokinetics and lower toxicity suggest safer drug potential than Sotorasib.

KRAS mutations play a key role in driving cancer by continuously activating pathways that promote cell growth and survival. These mutations also increase the levels of Aurora kinases AURKA and AURKB, either directly or through activation of MYC, another cancer-promoting factor. AURKA enhances KRAS function by helping it localize to the cell membrane, while AURKB supports cell division. As a result, KRAS-mutant cancer cells become highly reliant on these kinases.

Studies show that blocking AURKA or AURKB can reduce tumor cell growth and trigger cell death. However, targeting only one may not be sufficient due to alternative survival mechanisms. Interestingly, while AURKB is typically stabilized by AKT in some cancers, KRAS-mutant cells bypass this control, making them less responsive to AKT inhibitors. This underscores the need for direct inhibition of AURKB in these cases.

Combining KRAS and AURKB inhibitors disrupts both growth signals and cell division

machinery, producing a stronger anti-cancer effect than targeting either alone. This dual-targeting approach may offer a more durable response and help overcome resistance in KRAS-driven tumors.

CHAPTER-6

CONCLUSION AND FUTURE IMPLICATION

This integrated study demonstrates the critical role of AURKB overexpression across various human cancer types and establishes its significance as both a prognostic biomarker and therapeutic target in precision oncology. The research reveals a strong mechanistic link between KRAS mutations and Aurora kinase activation, where KRAS-driven pathways enhance AURKA and AURKB expression through direct mechanisms and MYC activation. This creates a dependency loop where KRAS-mutant cancer cells become highly reliant on Aurora kinases for survival and proliferation.

Our findings highlight the promising therapeutic potential of phytochemicals as dual inhibitors targeting both KRAS and AURKB pathways. These natural compounds demonstrated superior binding affinities to critical protein residues while exhibiting favorable pharmacokinetic properties and improved toxicity profiles compared to synthetic alternatives. The phytochemicals showed effectiveness in disrupting both oncogenic growth signals and cell division machinery, addressing the limitations of single-target approaches that often lead to resistance through alternative survival mechanisms.

The significance of phytochemicals over synthetic drugs lies in their multi-target capabilities, reduced toxicity burden, and potential for combination therapies. Unlike synthetic inhibitors that may cause severe side effects or promote resistance, these natural compounds offer a more holistic approach to cancer treatment with better patient tolerance profiles. Their ability to simultaneously modulate multiple oncogenic pathways positions them as valuable candidates for precision oncology applications.

Future investigations should prioritize exploring structure-activity relationships (SAR), molecular dynamics simulations, and chemical optimization to enhance potency and selectivity. Integrating natural compound databases like NPACT with AI-driven screening tools could further accelerate the discovery of targeted cancer therapeutics, paving the way for plant-based interventions in precision oncology.

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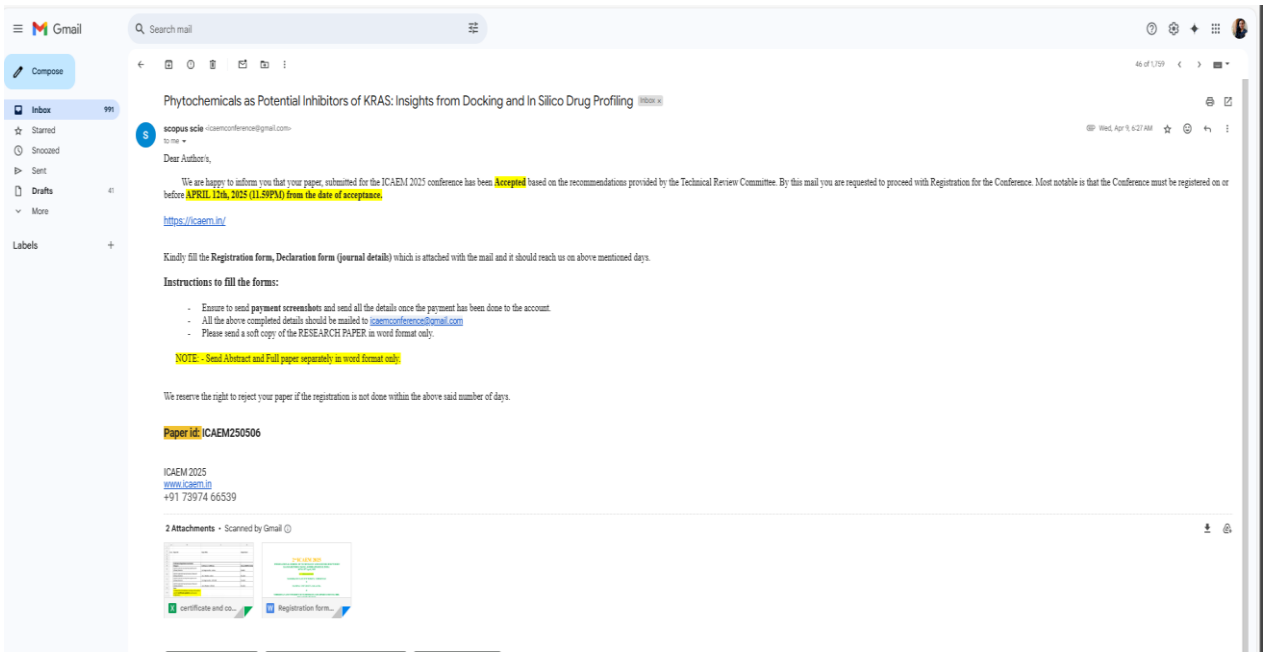
LIST OF PUBLICATIONS AND THEIR PROOFS

1. I Presented conference paper entitled “Phtochemicals as Potential Inhibitors of KRAS: Insights from docking and In Silico.” in scopus indexed conference at ICAEM 2025 organized by International school of technology and sciences for women in collaboration with Samarkand State University, Uzbekistan held on 14th of April 2025.



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Certificate of Registration

This is to Certify that the paper entitled

Phytochemicals as Potential Inhibitors of KRAS: Insights from Docking and In Silico Drug Profiling

Authored By

Divya Sharma ,Delhi Technological University, New Delhi

has been presented at

"2nd International Conference on "Advances In Engineering And Medical Sciences– 2025"

held on 14th & 15th April 2025 at

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2. Conference Paper entitled “” has cleared the review round and has been accepted at the International Conference on Emerging Technologies in Science and Engineering (ICETSE-2025), which will be held in the month of June, 2025.

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