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Exploring the Efficacy of Plant Extracts of *Tinospora cordifolia* and Doxorubicin in Cancer Treatment

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CANDIDATE'S DECLARATION

I, **Tanya Singh**, Roll No. **2K21/IBT/12** of M.Tech (Industrial Biotechnology), hereby declare that the Project Dissertation titled **"Exploring the Efficacy of Plant Extracts of *Tinospora cordifolia* and Doxorubicin in Cancer Treatment"** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the Degree of Master of Technology, is original and not copied from any source without proper citation. The work has not been previously formed the basis for award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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Date: 29-05-2023

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CERTIFICATE

I hereby certify that the Project Dissertation titled **"Exploring the Efficacy of Plant Extracts of *Tinospora cordifolia* and Doxorubicin in Cancer Treatment"** which is submitted by Tanya Shrivastava, Roll no. 2K21/IBT/12 [Department of Biotechnology], Delhi Technological University, Delhi in partial fulfilment of requirement for the award of Degree of Master of Technology, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part of full for any Degree or diploma to this University or elsewhere.

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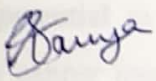
Signature: 

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ABSTRACT

Cancer continues to be a prominent factor in global mortality rates, and while conventional treatments such as chemotherapy have improved outcomes, their efficacy is often limited by toxicity and resistance. In recent years, implant-based immunotherapies have emerged as a promising alternative approach that can target cancer cells while minimizing off-target effects. Furthermore, Researchers have investigated natural botanical extracts as a possible reservoir of anti-cancer substances owing to their wide range of biologically active components. The primary objective of this dissertation is to assess the effectiveness of implant-based immunotherapies and plant extracts in the context of cancer treatment, with a focus on comparing the binding of doxorubicin, a commonly used chemotherapeutic drug, to cancer cells with the binding of bioactive compounds from plant extracts. In silico experiments will involve using molecular docking techniques to compare the binding of doxorubicin and plant extracts to cancer cells. In this computational docking analysis, we explored the efficacy of plant extracts from *Tinospora cordifolia*, specifically Tinocordiside, Palmatine, Dichloromethane, along with the chemotherapeutic drug Doxorubicin, in cancer treatment. Through molecular docking simulations, we examined the binding interactions between these compounds and key cancer-related proteins, including Haemoglobin, Bcl-2, p53, and Ras. The outcomes of the docking analysis unveiled the characteristics or properties of the compounds, exhibited varying affinities and hydrogen bonding interactions with the target proteins. These findings suggest that Tinocordiside, Palmatine, and Doxorubicin have the potential to interact with cancer-related proteins, indicating their possible efficacy in cancer treatment. The dissertation would contribute to novel and efficient cancer treatment approaches can be advanced, potentially mitigating the adverse effects commonly linked to conventional chemotherapy, while also exploring the potential of implant-based immunotherapies as a promising approach for cancer treatment.

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List of Abbreviations

Abbreviations	Full form
WHO	World Health Organization
PAMPs	Pathogen-associated molecular patterns
DAMPs	Damage-associated molecular patterns
IL	Interleukin
NK	Natural killer
TLR	Toll-like receptor
T. cordifolia	*Tinospora cordifolia*
ROS	Reactive oxygen species
ADMET	Absorption, distribution, metabolism, excretion, and toxicity
CDKs	Cyclin-dependent kinases
RTKs	Receptor tyrosine kinases
Bcl-2	B-cell lymphoma 2
PDB	Protein Data Bank
3D	Three-dimensional
NMR	Nuclear magnetic resonance
NCBI	National Centre for Biotechnology Information
HB	Haemoglobin
RMSD	Root mean square deviation
HIS	Histidine
GLY	Glycine
ARG	Arginine
GLU	Glutamic acid
ASP	Aspartic acid
LEU	Leucine
THR	Threonine
PHE	Phenylalanine
ASN	Asparagine
TYR	Tyrosine
SER	Serine
GLN	Gluta mine

CHAPTER 1

INTRODUCTION

As of 2021, cancer remains a significant global health issue, and it is one of the leading causes of death worldwide. According to the World Health Organization (WHO), cancer accounted for approximately 10 million deaths worldwide in 2020 [1]. In the United States, cancer remains the second leading cause of death after heart disease, with an estimated 1.9 million new cancer cases and 608,570 cancer-related deaths expected in 2021 alone [2]. The most diagnosed cancers in the US are breast, lung, prostate, and colorectal cancer [3]. On a global scale, lung cancer stands as the most commonly diagnosed cancer and remains the primary cause of cancer-related fatalities. It contributed to approximately 20% of all cancer deaths in the year 2020. Breast cancer is the second most common cancer globally, followed by colorectal and prostate cancer [1]. Furthermore, COVID-19 pandemic had significant impacts on cancer patient diagnosis, treatment, and outcomes. Delayed cancer screenings and disruptions to treatment have led to many cancer cases being diagnosed at later stages, resulting in poorer outcomes. A recent study estimated that delays in cancer screenings due to the pandemic could result in over 10,000 excess deaths from breast and colorectal cancer alone in the US over the next decade [4].

1.1 Implant Based Cancer therapy

Cancer immunotherapy works by enhancing the body's natural defences against cancer. This approach aims to activate the immune system, enabling it to identify and target cancer cells that might have previously evaded detection. It does so by either boosting the body's natural immune response or by introducing synthetic proteins that can recognize cancer cells specifically [5]. Immunotherapy has revolutionized cancer treatment by leveraging body's innate immune system to target cancerous cells with precision, resulting in a paradigm shift in the approach to cancer treatment. Compared to traditional chemotherapy or other therapeutic approaches that actively destroy both cancerous and healthy cells, immunotherapy has fewer off-target side effects and offers several advantages [6]. Immunotherapy has demonstrated encouraging outcomes in diverse cancer types, such as melanoma, lung cancer, and bladder cancer. Nonetheless, it is crucial

to acknowledge that not all patients exhibit a positive response to immunotherapy, and its efficacy may vary across different cancer types. Nevertheless, the use of immunotherapy has significantly improved the overall survival rate for some patients, and Continuous research endeavours persist in investigating novel approaches to leverage the potential of the immune system in the realm of cancer treatment. The innate immune system assumes a pivotal role in preserving overall bodily well-being through the detection of distinctive cell-surface pattern receptors that identify "pathogen-associated molecular patterns (PAMPs)" present in microorganisms and "damage-associated molecular patterns (DAMPs)" released from impaired cells. This coordinated immune response is crucial in mounting an effective immune response to combat infections and heal damaged tissues [7]. Immunotherapy is a ground-breaking treatment modality that has transformed the way cancer is treated, offering several advantages over traditional chemotherapy. By enhancing the body's natural defences against cancer, immunotherapy holds immense potential for improving cancer outcomes and the quality of life for patients.

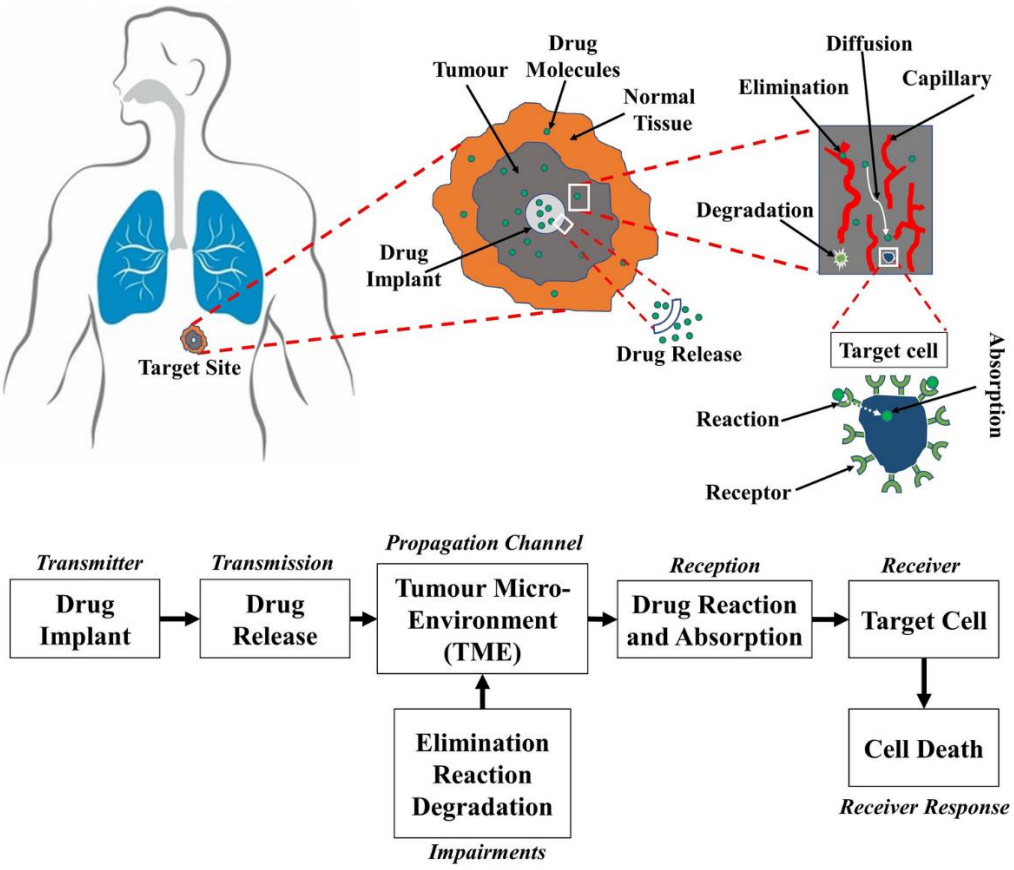


Figure 1.1: Implant based drug delivery for tumor (Source: A. Mohan, "Modelling of combination therapy using implantable anticancer drug delivery with thermal ablation in solid tumor," Sci. Rep., vol. 10, no. 1, pp. 1-16, 2020.)

1.1.1 Types of Implants based therapy

Implant-based immunotherapy for cancer refers to the use of implantable devices to deliver therapeutic agents that stimulate the immune system to identify and launch an attack against cancer cells. Here are three types of implant-based immunotherapy for cancer:

1. **Immune-stimulating implants:** These implants release immunostimulatory agents, which act as triggers to activate the immune system, stimulating it to target and eliminate cancer cells. For example, one type of immune-stimulating implant is a polymeric device that releases “interleukin-2 (IL-2)”, a cytokine, known for its ability to promote the proliferation of T cells and natural killer (NK) cells, is utilized. This type of implant has been shown to enhance the anti-tumor immune response and improve survival in animal models of melanoma and breast cancer [8,9].

2. **Tumor microenvironment modifying implants:** These implants modify the tumor microenvironment to make it more favourable for immune cell infiltration and attack. For example, one type of tumor microenvironment modifying implant is a hydrogel that releases “transforming growth factor-beta (TGF-β)” inhibitors and hyaluronidase, which breaks down the extracellular matrix in the tumor microenvironment. This type of implant has been shown to increase immune cell infiltration and enhance the anti-tumor immune response in mouse models of breast cancer and melanoma [10,11].

3. **Tumor-targeting implants:** These implants deliver immune-modulating agents directly to the tumor site, allowing for localized immune stimulation. For example, one type of tumor-targeting implant is a polymeric evice that releases toll-like receptor (TLR) agonists, these T cells and natural killer (NK) cells, when stimulated, play a crucial role in activating and mobilizing the immune system to specifically target and eliminate cancer cells. This type of implant has been shown to improve the anti-tumor immune response and increase survival in mouse models of breast cancer and melanoma [12,13].

1.1.2 Biomaterials for cancer therapy

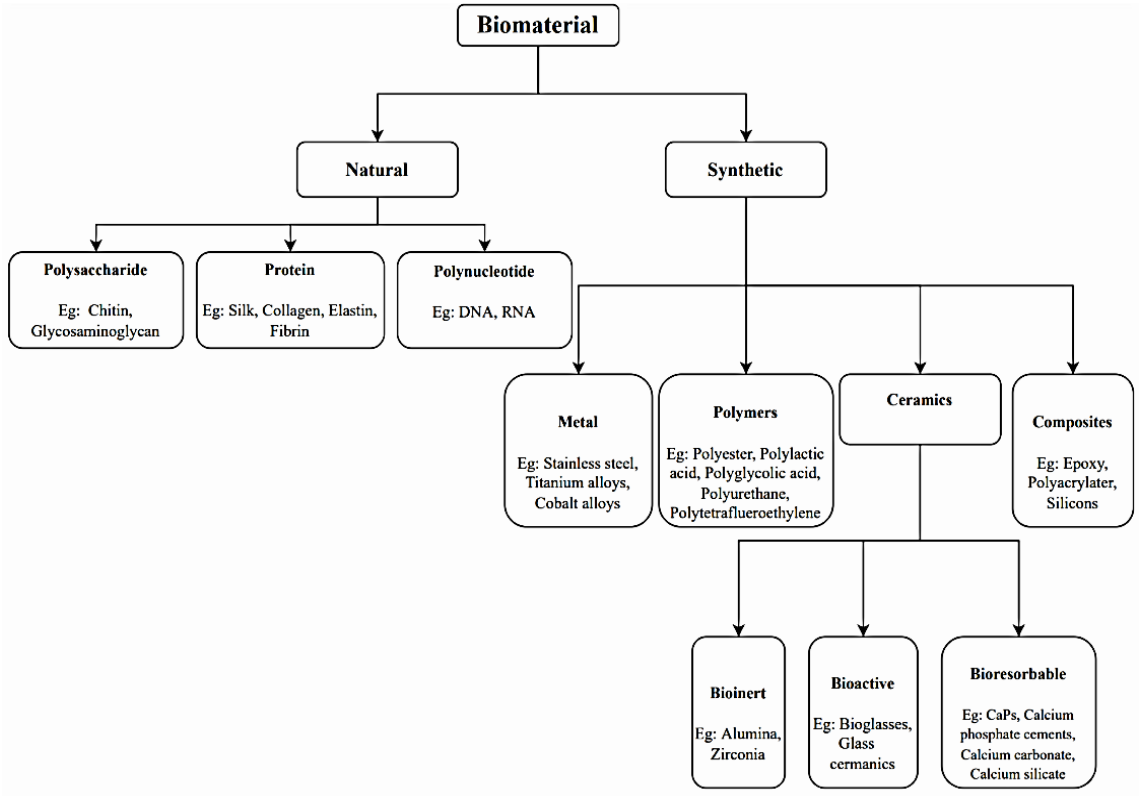


Figure 1.2: Types of biomaterials

Biomaterials have emerged as crucial tools in cancer immunotherapies, as they offer several advantages such as drug delivery, immune response triggering, and structural support for immune cells. The choice of biomaterials used in cancer immunotherapies depends on several factors, including the type of cancer being treated, the intended method of drug administration, and the compatibility of the material with the human body [14]. Recent advances in the development of biomaterials for cancer immunotherapies have led to the creation of innovative materials and techniques, which can enhance the efficacy of immunotherapies. For example, biomaterials such as liposomes, nanoparticles, and hydrogels have been extensively used as drug carriers in cancer immunotherapies, providing targeted drug delivery and controlled release of drugs to the tumor site. Furthermore, biomaterials can be engineered to stimulate the immune system and enhance its response against cancer cells [15]. For instance, materials that mimic the extracellular matrix of tumors can help activate immune cells and promote their infiltration into the tumor microenvironment. Additionally, biomaterials can also provide structural support for immune cells and aid in the creation of immunological synapses,

which facilitate interactions between immune cells and cancer cells. Overall, the use of biomaterials in cancer immunotherapies holds great promise for improving the effectiveness of cancer treatment. As researchers continue to create novel materials and techniques, the field of biomaterials for cancer immunotherapies is rapidly growing and advancing [16, 17].

1.2 Phytotherapy for cancer using *Tinospora cordifolia (T. cordifolia)*

Recent studies have focused on the potential anticancer activity of natural compounds of plant origin [18]. In this regard, plants used in traditional medicine systems are considered a valuable source of active compounds and important supplements for chemotherapy/treatment [19]. *T. cordifolia* is a widely used plant in Ayurvedic medicine and has been found to possess several biological activities, including anticancer properties [20]. *T. cordifolia* is a plant with genetic diversity, and is also known as 'Giloy' or 'Guduchi'. It belongs to the Menispermaceae family and can be found growing at high altitudes, with greenish-yellow flowers [21,22]. It has been identified by the Indian Pharmacopoeia as having medicinal properties and is a vital component of several medicinal formulations used to manage a range of diseases and discomforts, such as pyrexia, dyspepsia, syphilis, gonorrhoea, diseases of the urinary tract, gout, viral hepatitis, anaemia, general weakness, urinary tract infections, dermatological diseases, loss of appetite, and asthma [23-26]. There have been several studies conducted on the plant, with researchers discovering that it possesses bioactive components in various *in vitro* models, these substances have demonstrated the ability to hinder “cellular proliferation”. Furthermore, they exhibit “antineoplastic, antitumor, anti-angiogenesis, and anti-metastatic properties” in diverse in vivo models. Isoquinoline alkaloids, particularly berberine, are the secondary metabolites produced by *T. cordifolia* that are believed It is believed that these compounds are primarily accountable for the majority of the beneficial effects associated with the plant. Berberine has been used in the treatment of different types of diarrhoea [27,28] and has shown efficacy in the management of gastroenteritis, diabetes, hyperlipidaemia, cardiovascular diseases, inflammatory conditions, hyperglycaemia, and obesity [29–32]. Furthermore, berberine has demonstrated anti-cancer activity on various cell lines [33]. Various studies have reported that different extracts of *T. cordifolia* contain bioactive components that can hinder cellular proliferation in various in vitro models and exhibit antineoplastic [34], antitumor [35–

37], anti-angiogenic [38,39], and anti-metastatic activity in various in vivo models [37,40].

Table 1.1: Some *T. cordifolia* Extracts used for different type of cancers

Compound/ Extracts	**Type of cancer/ Cancer cells**	**Action**	**Ref**
T. cordifolia Extract	Lung cancer	Enhancing dynamics of the surface membrane leading to improved apoptosis	[41]
	Oral cancer	Induces cell death through apoptosis	[42]
	Breast cancer	Anti-proliferative effects	[43]
	Pulmonary carcinogenesis	Chromatin condensation and apoptotic body formation	[44]
Berberine	Colon cancer	Antiproliferative activity	[45]
Chloroform and hexane extracts	Brain cancer	Slow down the pace of cell growth and trigger the process of cellular differentiation.	[46]
Aqueous alcoholic extract	Colon cancer	Induces mitochondrial apoptosis and autophagy.	[47]
	Neuroblastoma	Arrest cells in the G0/G1 phase	[48]
	Tumor	Boost tumor-associated macrophages's antitumor activity and promote their differentiation to dendritic cells	[49]
Polysaccharide	Melanoma cells	Prevent the formation of metastases in the lungs of syngeneic animals through inhibition	[50]
Palmatine	Skin carcinogenesis	The restoration of superoxide dismutase and catalase enzymes, along with a reduction in DNA damage, was observed in lymphocytes.	[51]
Dichloromethane	HeLa cells	Capable of inducing DNA damage in the form of micronuclei with high efficiency	[52]
Tinocordiside	KB and SiHa cells	Increased cytotoxicity	[53]

1.2.1 The mechanism of action of different phytocomponents of *T. cordifolia* against cancer cells

T. cordifolia is known to contain several active phytocomponents, such as alkaloids, glycosides, steroids, essential oils, and a combination of fatty acids, calcium,

phosphorous, protein, and polysaccharides [54]. A systematic review of various studies identified a range of phytocomponents present in *T. cordifolia* . These include “berberine, new clerodane furanodiptherineglycosidae, elligic acid, kaempferol, N-formylannonain, magnoflorine, jatrorrhizine palmatine, 11-hydroxymustakone, cordifolioside A, tinocordiside, yangambin, anthraquinones, terpenoids, saponins, phenol, pyrrole-based small molecules, quercetin, rutin, arabinogalectian, palmatine, clerodane-derived diterpenoids, and hexane fractions”. One of the identified phytocomponents, berberine, has been shown to possess anticancer properties. It inhibits the proliferation of cancer cells and induces apoptosis, partly by impeding cell cycle progression and elevating reactive oxygen species (ROS) levels. Similarly, kaempferol, a flavonoid, has been shown to induce cell cycle arrest and apoptosis in various cancer cell lines by modulating multiple signalling pathways [55-58].

The mechanism of action of Tc varies depending on the specific phytocomponents utilized. One such phytocomponent is the new clerodane furanodiptherineglycosidae, which exerts its anticancer activity by triggering reactive oxygen species and autophagy, leading to the induction of mitochondrial-mediated apoptosis [56]. Another group of phytocomponents, namely phenolic compounds, have been found to have Geno protective and antioxidant effects on cancer cells [58]. Studies have also shown that Tc ethanolic extracts induce apoptosis by increasing the number of cells in the sub G0 phase without altering the cell cycle [59]. Arabinogalactans, found in aqueous Tc extracts, exhibit “immunological and cytotoxic activities.” Phenols have been shown to possess “antimutagenic and anti-malignant effects.” Flavonoids, on the other hand, play a chemo-preventive role in cancer. Pyrrole-based molecules have been shown to induce apoptosis and cytotoxic effects [60]. Palmatine, another phytocomponent, has demonstrated enhanced antioxidant activity by increasing the levels of antioxidant enzymes and showed inhibition of lipid peroxidation, indicating a role in the detoxification pathway [61]. Berberine, a well-known phytocomponent, has been shown to inhibit tumor cell growth by reducing the secretion of growth factors [55]. Finally, hexane fractions have been found to induce apoptosis through the activation of caspase 3 and DNase [62].

1.3 Molecular Docking

Molecular docking is a computational method employed in structural biology and drug discovery. It predicts the binding orientation and strength (affinity) between a small

molecule (known as a ligand) and a target macromolecule, typically a protein, at the atomic level. It aims to simulate the formation of a stable complex between the ligand and the target molecule by predicting their optimal binding mode [63].

The process of molecular docking typically involves the following steps:

1. **Preparation of the ligand and target:** The ligand (small molecule) and target (usually a protein) are prepared for docking analysis. The molecules are processed by removing any extraneous atoms, adding necessary hydrogen atoms, and assigning appropriate charges. The target molecule, typically a protein, has its structure determined or predicted prior to the docking process.

2. **Generation of binding site:** The binding site on a target molecule is typically defined, often based on the active site or known residues involved in binding. This region represents the area where the ligand is expected to bind.

3. **Search algorithm:** During the docking process, a search algorithm is employed to explore the conformational space and position the ligand in various binding modes within the binding site. Typically, the ligand is treated as a rigid molecule, while the target molecule can either be flexible or rigid depending on the specific method utilized.

4. **Scoring and ranking:** During the docking process, each generated ligand pose undergoes evaluation and scoring using a scoring function. This function estimates the binding affinity between the ligand and the target by considering several factors including shape complementarity, hydrogen bonding, electrostatic interactions, and hydrophobic interactions.

5. **Analysis and selection:** The generated ligand poses are usually clustered based on their similarity, and the top-scoring poses are selected for further analysis. The binding modes of the ligand in the selected poses can provide insights into the interactions and binding affinity with the target molecule [64, 65].

1.3.1 Significance of Molecular Docking in Pharmaceutical Research

Molecular docking plays a crucial role in the field of drug discovery. It serves as a pivotal tool in the process of identifying potential therapeutic compounds. It helps researchers

understand the binding mechanisms of small molecules with target proteins, predict their binding affinity, and guide the design of new compounds with improved binding properties [66]. Molecular docking holds significant importance in computational drug development as it enables the prediction and examination of the binding interactions between small chemical compounds (ligands) and target biomolecules (typically proteins). This computational technique is widely used in the early stages of the drug discovery process to identify and optimize potential drug candidates[67].

Outlined below are several fundamental rationales for the indispensability of molecular docking in computer-aided drug design:

1. **Ligand binding prediction:** Molecular docking allows researchers to predict the binding affinity and orientation of a ligand within the binding site of a target protein. This data is crucial for comprehending the molecular connections implicated in the establishment of a stable complex between the ligand and the target. By predicting the binding modes, researchers can gain insights into how a ligand interacts with a target protein, including hydrogen bonding, electrostatic interactions, and hydrophobic interactions [68].

2. **Virtual Screening :** Virtual Screening is a methodology employed to screen extensive repositories of substances in order to pinpoint prospective pharmaceutical candidates. Molecular docking plays a pivotal role in virtual Screening , as it allows for the efficient and expeditious assessment of the binding strength and selectivity of numerous ligands against a target protein. By virtually screening many compounds, researchers can prioritize the most promising candidates for further experimental validation, thus saving time and resources.

3. **Lead optimization:** Once a potential drug candidate is identified, molecular docking can be used to guide the optimization process. Docking can help researchers understand the key interactions between the ligand and the target protein , allowing for the design and refinement of new compounds with improved binding affinity, selectivity, and pharmacokinetic properties. By iteratively docking and modifying ligands, researchers can fine-tune their chemical structures to enhance their potential as drug candidates.

4. **Polypharmacology and off-target effects:** Molecular docking can also be utilized to assess the potential off-target effects of a drug candidate. By docking the candidate ligand against a panel of protein targets, researchers can evaluate its binding affinity to various proteins and assess its potential interactions beyond the intended target. This analysis helps identify potential safety concerns and optimize the selectivity profile of the drug.

5. **Fragment-based drug design:** Molecular docking is frequently utilized in fragment-based drug design, a strategy that entails screening minuscule molecular fragments against a target protein. By docking fragments individually or in combination, researchers can identify high-affinity binding interactions and build upon these fragments to construct larger compounds with improved binding properties [69,70].

Overall, molecular docking plays a crucial role in computer-aided drug design by providing valuable insights into ligand-target interactions, enabling virtual screening, guiding lead optimization, assessing off-target effects, and facilitating fragment-based drug design. By integrating computational methods like docking with experimental techniques, by leveraging molecular docking and other advanced techniques, researchers have the potential to expedite the drug discovery process and enhance the likelihood of discovering innovative and effective therapeutic compounds.

1.3.2 Chemical drugs v/s phytocompounds using molecular docking

Comparing chemical drugs and phytocompounds using molecular docking can provide insights into their binding interactions with target proteins and help understand their potential as therapeutic agents. Here are some points of comparison [71]:

1. **Chemical Diversity:** Chemical drugs are typically small organic molecules designed and synthesized in the laboratory, while phytocompounds are derived from plants and encompass a wide range of chemical structures. Chemical drugs offer a vast diversity of structures due to the ability to modify them extensively, whereas phytocompounds exhibit natural structural diversity.

2. **Binding Affinity:** Molecular docking can assess the binding affinity between ligands and target proteins [72]. Comparing chemical drugs and phytocompounds

in terms of binding affinity can provide insights into their strength of interaction. Chemical drugs are often optimized for high binding affinity, while phytocompounds may exhibit a range of affinities depending on their structural features.

3. **Binding Modes:** Molecular docking has the capability to unveil the binding modes of ligands within the active site of the target protein. Comparing the binding modes of chemical drugs and phytocompounds can elucidate differences in their interaction patterns and potential for specific interactions such as hydrogen bonding, hydrophobic interactions, or metal coordination.

4. **Selectivity and Specificity:** Molecular docking can evaluate ligand selectivity and specificity towards target proteins. Chemical drugs are typically designed to interact with specific targets, whereas phytocompounds may exhibit a broader range of interactions due to their complex mixture of compounds. Molecular docking can shed light on the selectivity and potential off-target effects of both chemical drugs and phytocompounds [73,74].

5. **Optimization Potential:** Molecular docking can aid in the optimization of ligands by identifying key interactions and guiding structural modifications. Comparing chemical drugs and phytocompounds in terms of their docking profiles can provide insights into that can be used to optimize and design more potent and selective compounds.

6. **ADMET Characteristics: "**Absorption, distribution, metabolism, excretion, and toxicity (ADMET)" characteristics are vital factors in the process of drug development. Molecular docking serves as a valuable tool to anticipate certain ADMET properties, including binding strength and prospective toxic effects. Comparing the ADMET profiles of chemical drugs and phytocompounds can help assess their drug-likeness and potential safety concerns.

It is important to note that molecular docking is just one aspect of the overall evaluation process, and other experimental and computational methods are necessary to comprehensively compare chemical drugs and phytocompounds. Additionally, the specific target protein and ligands being investigated will greatly influence the outcomes and conclusions drawn from molecular docking studies [75].

CHAPTER 2

LITERATURE REVIEW

Cancer continues to pose a substantial global health challenge , characterized by rising incidence rates and a scarcity of available treatment options. Despite advancements in conventional therapies such as surgery, chemotherapy, and radiation, the search for more effective and less toxic treatments continues. In recent years , there has been growing interest in exploring natural compounds for their potential anticancer properties due to their diverse chemical structures and potential therapeutic benefits. Plant-based compounds have been particularly intriguing due to their long-standing traditional use in various medicinal practices and the rich diversity of bioactive molecules they contain.

One such plant of interest is *T. cordifolia* , commonly known as Giloy or Guduchi. It is a versatile plant widely used in traditional Indian Ayurvedic Medicine for its broad spectrum of therapeutic properties. *T. cordifolia* extracts have been reported to possess various biological activities, including immunomodulatory, antioxidant, anti-inflammatory, and anticancer effects [76]. Several studies have demonstrated the potential of *T. cordifolia* extracts in inhibiting cancer cell growth and inducing apoptosis in different cancer types, including breast, colon, liver, lung, and prostate cancer [77,78,79].

Another widely used chemotherapeutic drug in cancer treatment is doxorubicin, a potent anthracycline antibiotic. Doxorubicin has been effective against various cancers, but its use is limited due to severe side Effects , including cardiotoxicity, myelosuppression, and drug resistance. Therefore, there is a need to explore complementary treatment options that can enhance the efficacy of doxorubicin while minimizing its adverse effects [80].

Computational docking analysis is a valuable tool for predicting and evaluating the potential interactions between small molecules and target proteins. It allows for the identification of potential binding sites and the estimation of binding affinities, thereby aiding in the rational design and optimization of drug candidates [81]. In this study, our objective is to explore the effectiveness of plant extracts derived from *T. cordifolia* and their potential synergistic effects with doxorubicin in cancer treatment using computational docking analysis.

By performing molecular docking simulations, we can elucidate the binding modes of the bioactive compounds present in *T. cordifolia* with key proteins involved in cancer progression and drug resistance. Furthermore, we can assess the potential synergistic effects of these plant extracts with doxorubicin, shedding light on their combinatorial therapeutic potential. Such computational analyses can guide further experimental studies and provide valuable insights for the development of novel and improved strategies for cancer treatment that are more effective in combating the disease.

2.1 Anticancer effects of *T. cordifolia* and its compounds

Several phytochemicals have been identified and isolated from *T. cordifolia* , including tinocordiside, palmatine, dichloromethane, and berberine. Extensive investigations have been carried out to explore the potential anticancer effects of these plant extracts. Here is an overview of the anticancer properties associated with each compound:

1. **Tinocordiside:** Tinocordiside is a glycoside compound found in *T. cordifolia* . Although limited studies have specifically focused on tinocordiside's anticancer effects, research suggests that *T. cordifolia* extracts, which may contain tinocordiside, exhibit potential anticancer activities. They have been reported to induce apoptosis (programmed cell death) in various cancer cell lines, inhibit cell proliferation, and exhibit anti-metastatic properties [82].

2. **Palmatine:** Palmatine, an alkaloid found in *T. cordifolia* and various other plant species, has shown promising anticancer properties in numerous studies. It has been observed to “induce apoptosis” (programmed cell death), “inhibit cell proliferation,” and exhibit “anti-metastatic effects” in different cancer cell lines. It has also shown promise in enhancing the efficacy of chemotherapy drugs [83,84].

3. **Dichloromethane:** Dichloromethane is a solvent used for extracting phytochemicals from plant sources. It is not a specific compound found in *T. cordifolia* . However, it is worth mentioning that dichloromethane extracts of *T. cordifolia* have been investigated for their anticancer potential. These extracts have exhibited cytotoxic effects on cancer cells and have shown anti-proliferative and pro-apoptotic activities [85,86].

4. **Berberine:** Berberine is an isoquinoline alkaloid found in several plant species , including *T. cordifolia* . It has been extensively studied for its anticancer effects. Berberine has demonstrated promising anticancer properties, including inhibition of cell proliferation, induction of apoptosis, and suppression of tumor growth in various cancer types, such as breast, lung, liver, and colon cancer [87,88,89].

It is crucial to highlight that although these compounds have demonstrated potential anticancer effects in laboratory studies and preclinical models, additional research is required to validate their efficacy and safety in humans. This entails conducting *in vivo* studies and clinical trials to assess their therapeutic benefits and potential side effects accurately. Moreover, the mechanisms underlying their anticancer activities are still being investigated.

2.2 Role of Protein in cancer

Proteins play an indispensable role in the initiation and advancement of cancer. Several proteins have been identified and extensively studied for their involvement in various aspects of cancer biology. Proteins play diverse roles in cancer development and progression. They are involved in crucial cellular processes that are dysregulated in cancer cells, contributing to tumor initiation, growth, metastasis, and resistance to treatment. Here are some general roles of proteins in cancer:

1. **Cell Growth and Proliferation:** Proteins implicated in the regulation of the cell Cycle have a critical function in the development of cancer. Cyclins, “cyclin-dependent kinases (CDKs),” and their suppressors govern the advancement of the cell cycle. Dysregulation of these proteins can lead to uncontrolled cell growth and proliferation, a hallmark of cancer [90].

2. **Signal Transduction:** Proteins engaged in signal transduction pathways facilitate the transmission of signals from the outer membrane of the cell to the nucleus, regulating various cellular processes. Abnormal activation or inactivation of signaling proteins, such as receptor tyrosine kinases (RTKs), Ras, and PI3K/AKT pathway components, can promote cell survival, growth, and angiogenesis in cancer.

3. **DNA Repair and Genomic Stability**: Proteins participating in DNA repair pathways uphold the integrity of the genome and impede the accumulation of genetic mutations. Mutations or dysregulation of DNA repair proteins , such as "BRCA1/2" and mismatch repair proteins, can lead to genomic instability and increased susceptibility to cancer development.

4. **Apoptosis and Cell Death:** Proteins involved in apoptosis "(programmed cell death)" are essential for maintaining Tissue Homeostasis and eliminating damaged or unwanted cells. Dysregulation of apoptosis-related proteins, such as Bcl-2 family members, caspases, and p53, can lead to defective cell death signaling and promote cell survival in cancer [91-93].

5. **Angiogenesis:** Proteins engaged in angiogenesis, the process of generating new blood vessels, play a pivotal role in tumor growth and metastasis. "Vascular endothelial growth factor (VEGF)" and its receptors are particularly significant in promoting angiogenesis, facilitating the access of tumors to essential nutrients and oxygen.

6. **Cell Adhesion and Migration:** Proteins involved in cell adhesion and migration, such as integrins, cadherins, and "matrix metalloproteinases (MMPs)", have a important role in cancer metastasis. These proteins facilitate tumor cell invasion, migration, and interaction with the extracellular matrix and other cells.

7. **Immune Response:** Proteins involved in immune response, including "immune checkpoint proteins" such as "PD-1 and CTLA-4," regulate the interplay between cancer cells and the immune system. Dysregulation of these proteins can enable tumors to evade immune surveillance and hinder anti-tumor immune responses.

These roles highlight the intricate involvement of proteins in various aspects of cancer biology. Dysregulation or mutations in these proteins can disrupt normal cellular processes and contribute to tumor development and progression. Understanding the roles of specific proteins in cancer can guide the development of targeted therapies aimed at restoring or inhibiting their function, leading to more effective cancer treatments [94,95].

Here, we will discuss the roles of some important proteins in cancer, including Haemoglobin, Bcl-2, p53 protein, and Ras protein:

1. **Haemoglobin:** Haemoglobin is primarily known for its function in oxygen transport in red blood cells. However, emerging evidence suggests that haemoglobin and its subunits may have additional roles in cancer. Abnormal expression of haemoglobin subunits has been detected in specific types of cancer, and their expression levels have been associate ed with tumor growth, metastasis, and poor prognosis. The exact mechanisms by which haemoglobin contributes to cancer progression are still being investigated [96].

2. **Bcl-2:** "B-cell lymphoma 2 (Bcl-2)" is a protein that plays a significant role in regulating apoptosis, which is the process of programmed cell death. It belongs to the Bcl-2 family of proteins, which consists of both pro-apoptotic and anti-apoptotic members. Bcl-2 functions as an anti-apoptotic protein by impeding apoptosis and promoting cell survival. Dysregulation of Bcl-2, such as overexpression or genetic alterations, is commonly observed in various cancers and is associated with increased resistance to apoptosis, allowing cancer cells to survive and proliferate [97].

3. **p53 protein:** p53 is a crucial tumor suppressor protein that has a vital function in safeguarding genomic stability and preventing the development of cancer. It operates as a "transcription factor", regulating the "expression of genes" involved in controlling the "cell cycle", facilitating "DNA repair", inducing "apoptosis", and promoting cellular senescence. Notably, mutated TP53 gene encodes for p53, are prevalent in a substantial proportion of human cancers. . Loss or inactivation of p53 function leads to the disruption of cellular processes that maintain genomic integrity, thereby promoting tumor formation and progression [98].

4. **Ras protein:** Ras is a family of small GTPases that serves as a molecular switch involved in "signal transduction pathways regulating cell growth", proliferation, and differentiation. Mutated Ras genes, specially KRAS, are among the mostly observed genetic alterations in cancer. These mutations result in the continuous activation of Ras signalling, which promotes uncontrolled cell growth, survival, and tumor progression. Dysregulated Ras signalling is associated with various cancer types and is often linked to poor prognosis and resistance to certain cancer treatments [99].

It is crucial to acknowledge that the roles and functions of these proteins in cancer are intricate and contingent on the specific context. Extensive research is ongoing to unravel the intricate mechanisms underlying their contributions to cancer development and progression. Understanding the involvement of these proteins in cancer biology may provide insights for the development of novel targeted therapies and diagnostic approaches.

2.3 Tools used for molecular docking

Molecular docking is an essential computational technique employed to forecast and analyse the binding interactions between a small molecule (ligand) and a target-protein or receptor. It plays a vital role in drug discovery and design by providing valuable insights into the binding affinity and positioning of ligands within the active site of a protein.

Numerous software tools and algorithms have been developed to facilitate molecular docking simulations. These tools employ diverse algorithms and scoring functions to predict the most favourable binding-poses and estimate the binding-affinities among the ligand and protein. Some commonly used molecular docking tools are:

1. **Auto-Dock:** Auto-Dock is a widely adopted docking software that utilizes a "Lamarckian genetic algorithm" to explore the conformational space of the ligand and conduct flexible docking simulations. It is known for its ability to handle large libraries of ligands and flexible receptor models [68].

2. **AutoDock Vina**: Auto-Dock Vina is an improved version of Auto-Dock that utilizes an iterated local search global optimizer combined with an efficient scoring function. It offers faster and more accurate docking predictions compared to its predecessor [100].

3. **DOCK:** DOCK (ligand) is a molecular docking program that uses a combination of systematic search algorithms with scoring functions to analyse ligand-protein binding. It is known for its efficiency in handling flexible ligands and receptor models [101].

4. **GOLD**: "Genetic Optimization for Ligand Docking" is a widely used docking software that employs a genetic algorithm for flexible ligand docking. It

incorporates a range of scoring functions and search algorithms to explore ligand conformational space effectively [102].

5. **Glide:** Glide is a molecular docking program developed by Schrödinger. It utilizes a combination of docking and scoring algorithms to analyse ligand-binding modes and affinities. It offers high-speed docking and has been widely used in virtual screening and lead optimization [103].

These tools, along with others, provide a user-friendly interface, diverse docking Algorithms , and scoring functions to facilitate efficient molecular docking studies. Researchers can choose the most suitable tool based on their specific requirements and preferences.

2.4 Tools used for docking in this project

2.4.1 AutoDock Vina 2.0

AutoDock Vina 2.0 is an advanced molecular docking software that has gained popularity among researchers due to its efficiency and accuracy in predicting ligand-protein binding interactions. It is an improved version of the AutoDock software and was developed by Oleg Trott and Arthur J. Olson in 2010. AutoDock Vina incorporates an iterated local search global optimizer combined with an efficient scoring function. This combination allows for faster and more accurate docking simulations compared to its predecessor. The software is designed to handle both rigid and flexible ligands and receptors, providing flexibility in modeling different molecular systems.

Key features of AutoDock Vina include:

1. **Global Optimization**: AutoDock Vina employs an iterated local search algorithm, which combines global and local optimization strategies. It explores the conformational space of the ligand by performing systematic searches and refining the solutions iteratively to find the best docking poses.

2. **Scoring Function**: The scoring function used in AutoDock Vina combines different terms, including steric clashes, hydrogen bonding, and electrostatic interactions, to evaluate the binding affinity between the ligand and the

receptor. The scoring function aims to predict the most favorable binding pose and estimate the binding energy.

3. **Flexible Receptor and Ligand Handling**: AutoDock Vina allows for flexible docking of both the receptor and the ligand. It can handle various types of ligand flexibility, including rotatable bonds and flexible side chains, allowing for a more accurate representation of the conformational space.

4. **User-Friendly Interface**: AutoDock Vina provides a user-friendly graphical interface that simplifies the preparation of input files, parameter settings, and visualization of docking results. This makes it accessible to researchers with varying levels of expertise in computational docking.

5. **Open-Source and Availability**: AutoDock Vina is an open-source software, distributed under the "GNU General Public License". It is freely accessible for academic and non-commercial use, allowing researchers worldwide to utilize and contribute to its development.

Auto-Dock Vina has been widely applied in various research areas, including drug discovery, virtual screening, and lead optimization. It has shown success in identifying and characterizing ligand binding modes, predicting binding affinities, and aiding in the rational design of new compounds. Auto-Dock Vina's combination of efficient algorithms, accurate scoring functions, and user-friendly interface makes it a valuable tool for molecular docking studies, facilitating the exploration of ligand-protein interactions and the discovery of potential drug candidates [104].

2.4.2 PyMol

PyMOL is a powerful and widely used molecular visualization software that allows researchers to analyze and visualize 3D structures of biological macromolecules such as "proteins, nucleic acids, and small molecules". It provides a range of tools and features for manipulating and displaying molecular structures, making it an essential tool in structural biology and drug discovery research [105]

Key features and capabilities of PyMOL include:

1. **Molecular Visualization:** PyMOL enables the interactive visualization of molecular structures in various representations, including wireframe, sticks, cartoons, surfaces, and more. It offers extensive control over the color scheme, transparency, and style of molecular representations, allowing for detailed and customized visualizations.

2. **Structure Analysis**: PyMOL provides tools for measuring distances, angles, and torsion angles within a molecule. It allows for the calculation of surface areas, hydrogen bonding interactions, and electrostatic potentials. Additionally, PyMOL offers functionalities for aligning multiple structures, superimposing molecules, and exploring structural similarities and differences.

3. **Image Rendering and Animation**: PyMOL offers high-quality image rendering capabilities, allowing to produce publication-ready images and presentations. It supports ray tracing and ambient occlusion techniques to enhance the visual quality of molecular scenes. PyMOL also supports the creation of molecular animations, which can be useful for conveying dynamic processes and molecular mechanisms.

4. **Scripting and Customization:** PyMOL is highly programmable and provides a Python-based scripting interface. This allows users to automate tasks, create custom workflows, and extend the software's functionalities. Users can also develop plugins and macros to enhance PyMOL's capabilities or integrate it with other computational tools.

5. **Community Support and Resources**: PyMOL has an active and supportive user community, which has contributed to the development of various plugins, scripts, and tutorials. Additionally, the PyMOL Wiki website provides a wealth of resources, including documentation, tutorials, and examples, to help users effectively utilize the software [106,107].

PyMOL has been widely adopted in various research fields, including structural biology, drug discovery, molecular modelling, and education. It has been extensively cited in scientific literature and has played a significant role in visualizing and analysing complex molecular structures.

2.4.3 Swiss ADME

Swiss ADME is a web-based platform that provides a range of tools and calculations for predicting various drug-like properties and pharmacokinetic parameters of small molecules. It is designed to aid in drug discovery and development by assisting researchers in evaluating the drug-likeness and optimizing the pharmacokinetic properties of potential drug candidates [108].

The key features and functionalities of Swiss ADME include:

1. Drug-Likeness Prediction: Swiss ADME utilizes the Lipinski's rule of five, which is a widely used guideline in medicinal Chemistry , to evaluate the drug-likeness of compounds. It assesses factors such as “molecular weight, lipophilicity, number of hydrogen bond donors and acceptors, and logP (partition coefficient)” to determine the likelihood of a compound to possess good oral bioavailability.

2. Pharmacokinetic Property Prediction: Swiss ADME calculates a range of pharmacokinetic parameters, including aqueous solubility, “blood-brain barrier (BBB)” permeability, “P-glycoprotein (P-gp)” substrate and inhibitor potential, human intestinal absorption, cytochrome P450 (CYP) enzyme inhibition, and plasma protein binding. These predictions assist in understanding how a compound is ADMET in the body.

3. Toxicity Prediction: Swiss ADME offers tools to estimate potential toxicological properties of compounds, such as mutagenicity, carcinogenicity, hepatotoxicity, and “hERG (human Ether-à-go-go-Related Gene)” inhibition. These predictions help researchers assess the safety profile and potential risks associated with drug candidates.

4. Interactive Visualization: Swiss ADME provides interactive visualizations of molecular structures, physicochemical properties, and predicted pharmacokinetic parameters. This allows users to explore and analyze the results in a user-friendly and intuitive manner.

5. Batch Mode and Integration: Swiss ADME allows users to submit multiple compounds in batch mode for simultaneous analysis. Additionally, it supports

integration with other computational tools and workflows through its web services and application programming interface (API) [109,110].

Swiss ADME is freely accessible through a web interface, making it readily available to researchers without the need for local software installation. It is based on a collection of predictive models developed using diverse data sources and machine learning algorithms. However, these predictions are computational and should be interpreted cautiously, and experimental validation is still necessary. Swiss ADME has proven to be a valuable tool for preliminary evaluation and optimization of drug-like properties, aiding researchers in early-stage drug discovery efforts [111].

2.5 Database used for data collection

2.5.1 The Protein Data Bank (PDB)

PDB is a database that provides a collection of three-dimensional (3D) structures of proteins, nucleic acids, and complex assemblies. It is the primary resource for researchers and scientists working in the field of structural biology. The PDB database contains experimentally determined structures obtained through techniques such as X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, and cryo-electron microscopy (cryo-EM). These structures provide valuable insights into the function and interactions of biomolecules.

Researchers can access the PDB database online through the official website at https://www.rcsb.org/. The website provides a user-friendly interface for searching, browsing, and downloading the 3D structures of interest. It also offers various tools and resources for analysing and visualizing the structural data. The PDB database is continuously updated as new structures are deposited by scientists worldwide. Each entry in the database includes detailed information about the structure, such as the amino acid or nucleotide sequence, experimental methods used, resolution or quality of the structure, and any associated scientific publications.

In addition to the PDB maintained by the RCSB Protein Data Bank (RCSB PDB), there are other regional PDB centers around the world, including PDBe (Protein Data Bank in Europe) and PDBj (Protein Data Bank Japan). These centres collaborate to ensure the consistent archiving and dissemination of structural data globally. Researchers and

scientists use the PDB database to study protein structures, perform structure-based drug design, understand protein-ligand interactions, and gain insights into various biological processes.

2.5.2 PubChem database

The PubChem database is a comprehensive and publicly accessible resource that provides information about the biological activities, chemical properties, and structures of small organic molecules. It is maintained by the National Centre for Biotechnology Information (NCBI), which is part of the U.S. National Library of Medicine. PubChem contains a vast collection of chemical compounds, including substances of medicinal interest, environmental pollutants, natural products, and more. It aggregates data from a variety of sources, including scientific literature, chemical catalogues, and databases, making it a valuable tool for researchers, scientists, and students in various fields such as chemistry, biology, pharmacology, and drug discovery.

The database consists of three primary interconnected databases:

1. **PubChem Compound:** This database provides detailed information on individual chemical compounds, including their names, synonyms, chemical structures, molecular formulas, physical and chemical properties, safety data, and links to other databases and resources.

2. **PubChem Substance:** This database contains information about the various forms or samples of a chemical compound, such as different salt forms, stereochemical variants, and mixtures. It serves as a hub for linking the different representations of a compound in various resources.

3. **PubChem Bio Assay:** This database stores data related to biological activities and screening results of chemical compounds. It includes information on assays, targets, screening protocols, and bioactivity data, allowing users to explore the relationship between chemical structures and biological effects.

CHAPTER 3

METHODOLOGY

3.1 Protein Preparation

The study selected four types of proteins related to cancer in Homo sapiens and their 3D The structures of the proteins were acquired from the "Protein Data Bank (PDB)" (https://www.rcsb.org/). They were Haemoglobin (HB, PDB ID: 6BB5) as Cancer patients undergoing chemotherapy often develop anaemia, which has been linked to unfavourable clinical results [112], Mutant B-cell lymphoma 2 (Bcl-2, PDB ID: 5FMI) as Bcl-2 protein family members play a significant role in apoptosis regulation, tumorigenesis, and cellular responses to anticancer therapy by exerting either pro- or anti-apoptotic effects [113], Mutant p53 protein (PDB ID: 6FF9) because Certain p53 mutations can promote cell proliferation and malignant transformation by blocking the pro-apoptotic function of p53 [114], and Ras protein (PDB ID: 7MOC) play a crucial role as key components of signalling pathways that regulate cell proliferation, differentiation, and survival [115]. These proteins were downloaded in PDB format, and were processed using PyMol by deleting water molecules and multiple chains. The proteins were subsequently converted to the PDBQT format. with the addition of polar hydrogens and a grid box was used to identify the docking sites of proteins using AutoDock tools. The dimensions are shown in Table 2.1. The protein-ligand conformations were visualized using PyMol.

Table 3.1: Grid box dimensions of all the selected protein

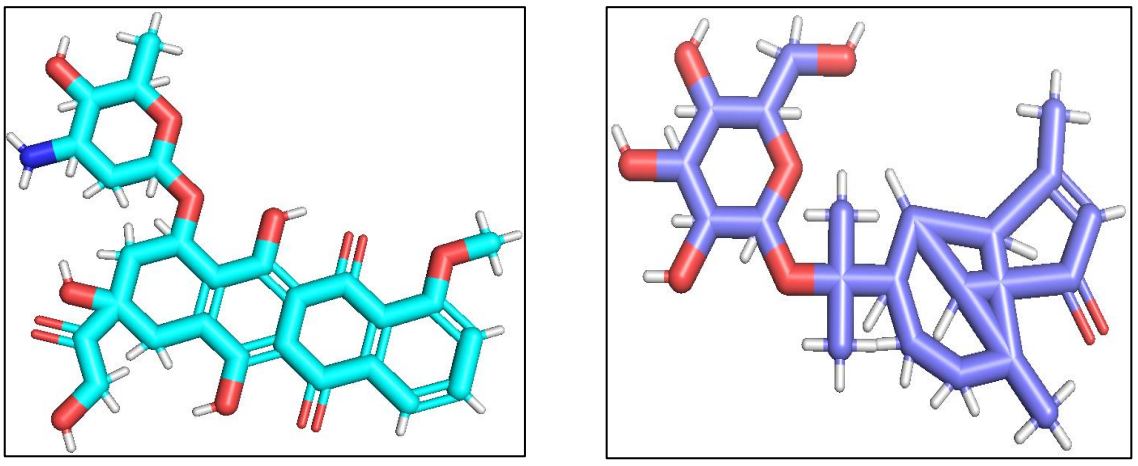
Protein	Centre x	Centre y	Centre z	Size x (Å)	Size y (Å)	Size z (Å)
HB	35.15	34.208	16.091	44	58	48
Bcl-2	3.682	-4.181	1.974	80	90	68
p-53	86.994	-6.847	95.891	126	126	126
Ras	183.407	153.807	168.314	106	126	88

3.2 Ligand Preparation

The selected ligands for the study include a drug compound called doxorubicin (PubChem CID: 31703), which is commonly used for cancer treatment. In addition, four plant compounds extracted from *T. cordifolia* were also chosen. These compounds are Tinocordiside (PubChemID: 177384), Palmatine (PubChemID: 19009), Dichloromethane (PubChemID: 6344), and Berberine (PubChemID: 2353). These plant extracts have demonstrated anticancer effects. To prepare the ligands for docking simulations, The 3D structures of these compounds were obtained by downloading them in SDF format from the PubChem database, accessible at https://pubchem.ncbi.nlm.nih.gov/. The structures were then analysed using PyMOL software, where they were converted to PDB format. Subsequently, these PDB files were opened as ligands in AutoDock, a molecular docking tool. Afterwards, the downloaded 3D structures of these compounds were converted to the PDBQT file format. adding torsion angles. The process involved converting the downloaded SDF files to PDB format, followed by further conversion to PDBQT format with added torsion angles using AutoDock tools. This allows for the ligands to be utilized in subsequent molecular docking simulations. The docking was performed using AutoDock vina [100].

3.3 ADMET Analysis

The ligands' 2D structures were downloaded from PubChem and subjected to ADME analysis using two software tools: SwissADME (http://www.swissadme.ch/) and ADMETLab 2.0 (https://admetmesh.scbdd.com/service/evaluation/index) [116]. The SDF file format containing the 2D structures was uploaded and converted to SMILES format for analysis. The structures are given in figure 3.1.



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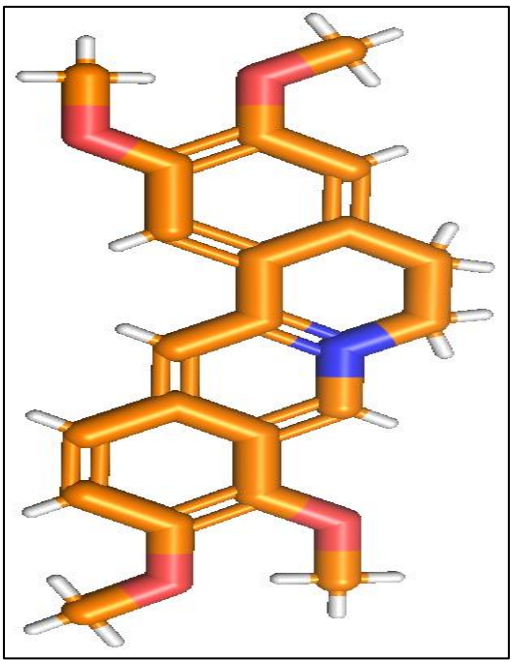

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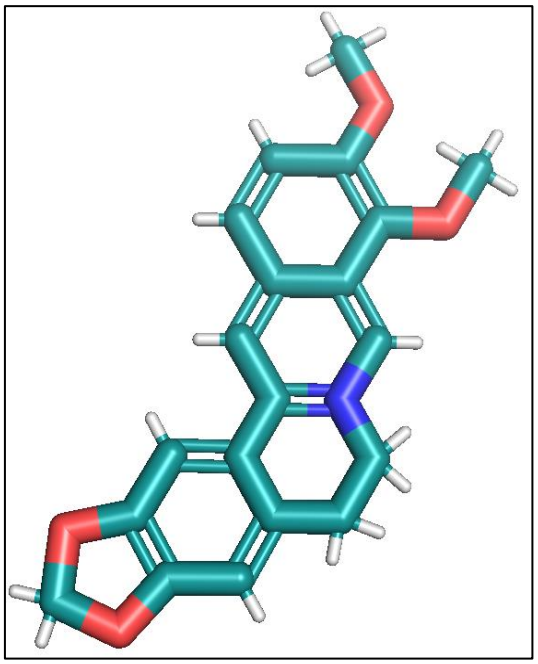

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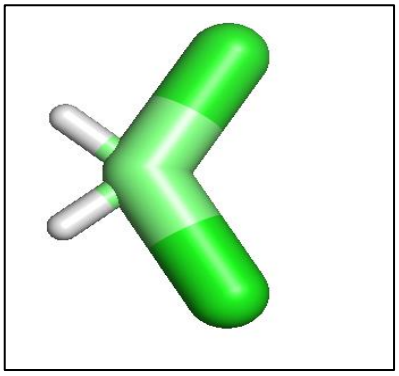

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Figure 3.1.: 2D structures of ligands. a.) Doxorubicin, b.) Tinocordiside, c.) Palmatine , d.) Berberine ,e.) Dichloromethane

CHAPTER 4

RESULT AND DISCUSSION

Molecular docking of 5 ligands with Haemoglobin

Doxorubicin and four phytochemicals found in *T. cordifolia* were studied in terms of their interaction with haemoglobin (HB) protein through a process called docking. Doxorubicin showed an affinity of -6.9 with a "root mean square deviation (RMSD)" value of 0, indicating a strong interaction. It formed four polar bonds with HB protein at distances of 2.3, 2.3, 2.6, and 3.1, interacting with specific amino acids such as Histidine, Glycine, and Glutamic Acid. The phytochemicals from *T. cordifolia* also displayed promising results in their interaction with HB protein. Palmatine and Berberine exhibited the highest affinities with scores of -8.1 and -7.6, respectively as shown in Table 3.1. On the other hand, dichloromethane showed the lowest affinity, forming only non-polar bonds. The binding amino acids for Doxorubicin and the plant compounds were different, indicating that they do not compete for the identical binding site. Notably, the affinities observed for the phytochemicals were higher than that of Doxorubicin. This suggests that a combination therapy utilizing both Doxorubicin and the phytochemicals from *T. cordifolia* could be beneficial. The different binding sites and higher affinities of the phytochemicals support their potential use in combination therapy.

Table 4.1: Docking Results for Ligands with Haemoglobin Protein

Ligand	**Affinity (kcal/mol)**	**Number of H-bonds**	**Distance of H-bonds (Å)**	**Bonding Amino acid**
Doxorubicin	-6.9	4	2.3-3.1	HIS-50, GLY-51, GLU-23
Berberine	-7.6	1	2.4	HIS-87
Tinocordiside	-7.2	2	2.4	HIS 87
Dichloromethane	-2.5	-	-	-
Palmatine	-8.1	1	2.3	HIS-87

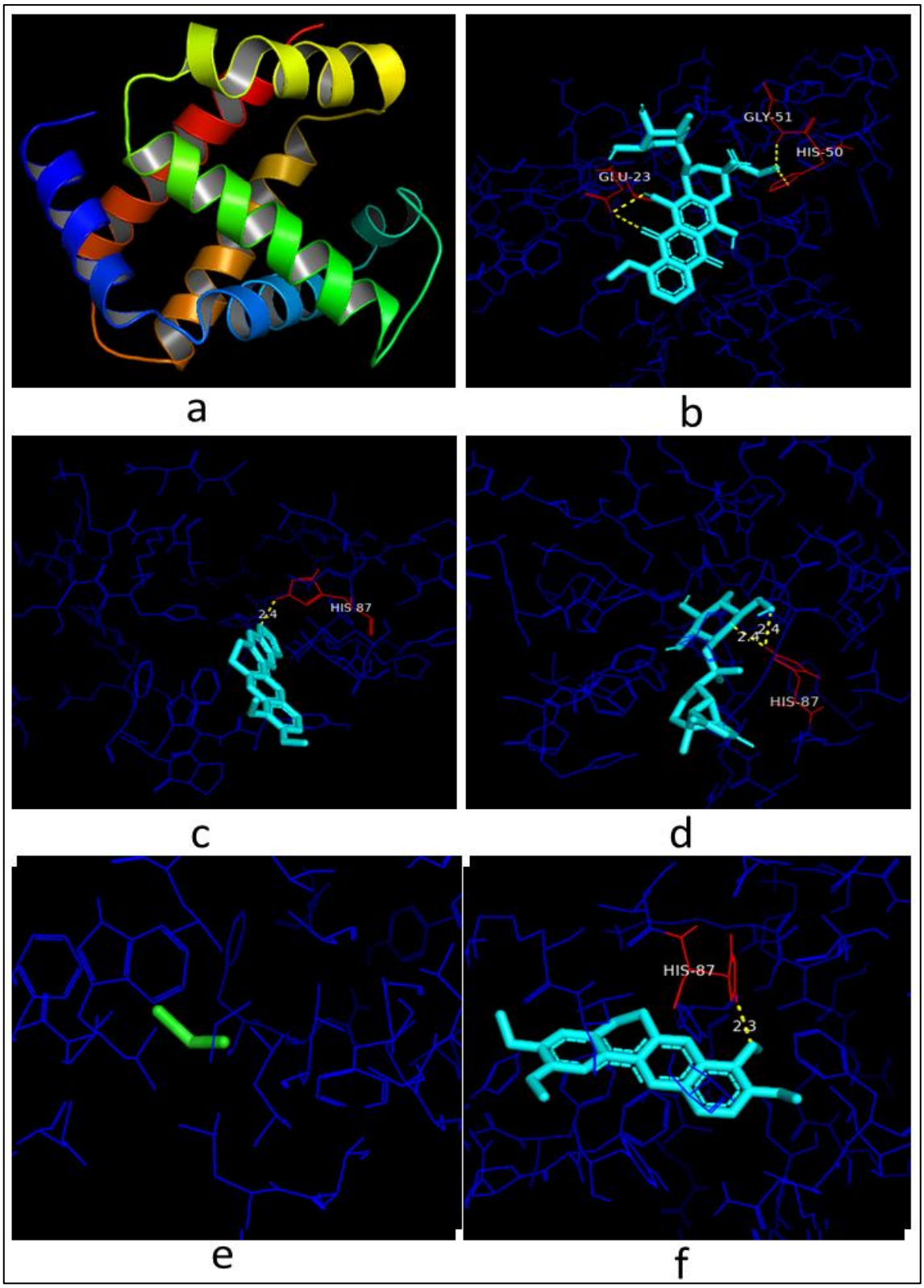

Figure 4.1: Ligands docking with HB. a) Doxorubicin, b) Berberine, c) Tinocordiside , d) Dichloromethane, e) Palmatine. *The Cyan/Green colour represent the ligands, Red represents the polar binding amino acid, Yellow colour represents H-bond and Blue colour represent non-polar binding amino acid*

Molecular docking of 5 ligands with Bcl-2

The interaction between Doxorubicin and four phytochemicals found in *T. cordifolia* with the Bcl-2 protein. The affinity of Doxorubicin towards Bcl-2 was found to be -6.9, indicating a strong interaction between the two. In the study, the RMSD value was 0 for all five ligands, indicating that the ligands had a stable and consistent interaction with the Bcl-2 protein. Specifically, Doxorubicin and Tinocordiside formed four hydrogen bonds with different amino acids of the Bcl-2 protein. This suggests that these two compounds have a strong binding affinity and interact effectively with Bcl-2 protein.

On the other hand, Dichloromethane showed no polar bond and exhibited the least affinity with Bcl-2 among the tested ligands. This implies that Dichloromethane may have a weaker interaction or no significant interaction with Bcl-2 compared to the other compounds studied. Overall, the study offers valuable insights into the interaction between these ligands and the Bcl-2 protein, suggesting their potential as therapeutic agents or tools for further research in relation to Bcl-2-related processes or conditions.

Table 4.2: Docking Results for Ligands with Bcl-2 Protein

Ligand	**Affinity (kcal/mol)**	**Number of H-bonds**	**Distance of H-bonds (Å)**	**Bonding Amino acid**
Doxorubicin	-6.9	4	1.8-3.5	ASP-57, LEU-140, THR-155
Berberine	-6.4	1	3.4	ARG-169
Tinocordiside	-6.4	4	2.2-3.2	SER-117, SER-121
Dichloromethane	-2.1	-	-	-
Palmatine	-6.1	1	3.3	SER-117

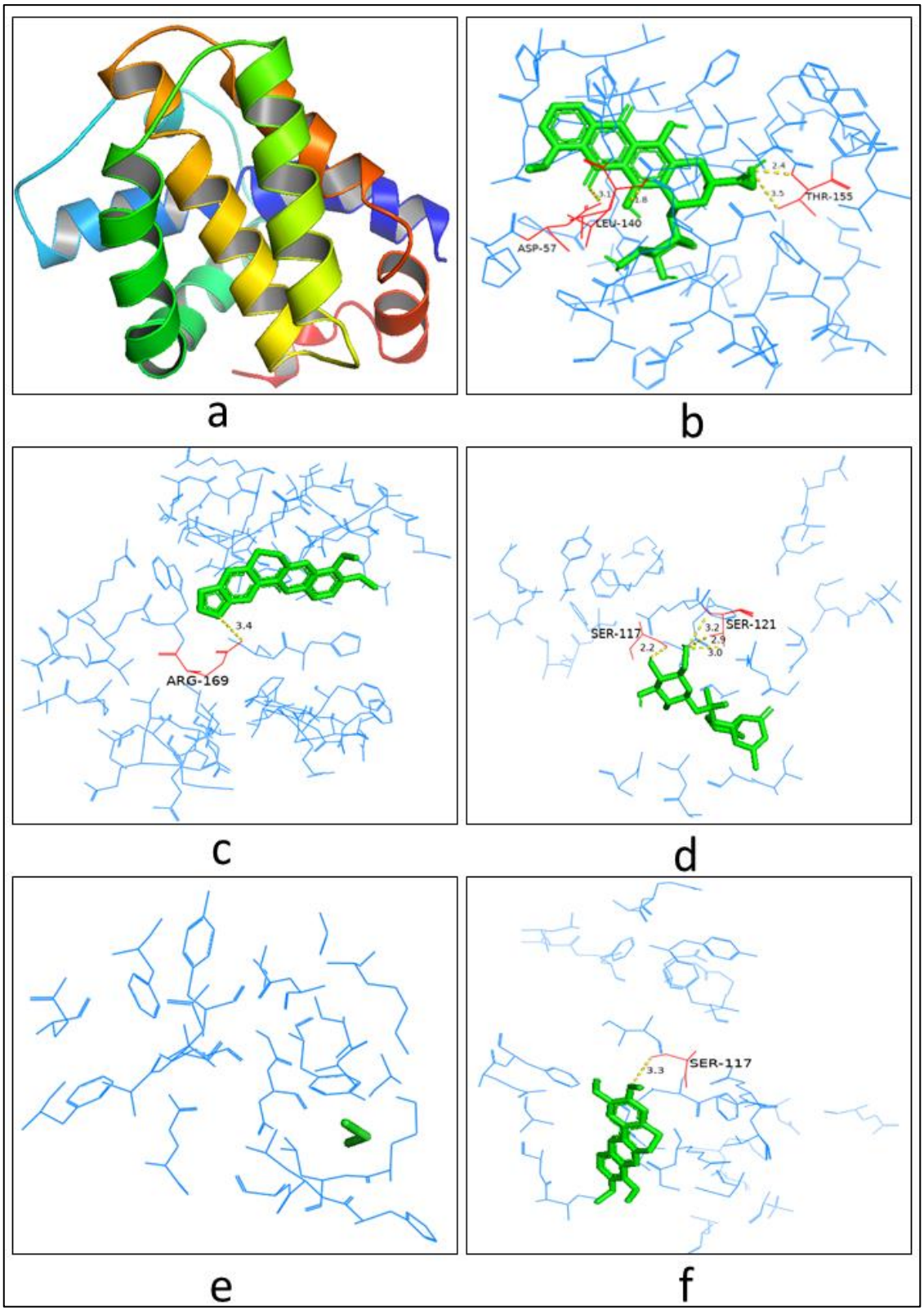

Figure 4.2: Ligands docking with Bcl-2. a) Bcl-2 protein, b) Doxorubicin, c) Berberine, d) Tinocordiside, e) Dichloromethane, f) Palmatine. *The Green colour represent the ligands, Red represents the polar binding amino acid, Yellow colour represents H-bond and Blue colour represent non-polar binding amino acid*

Molecular docking of 5 ligands with p-53

Based on these results, it appears that Doxorubicin has the highest binding Affinity (-9.8 kcal/mol) with the p-53 protein among the ligands tested. However, Tinocordiside also shows good binding affinity (-8.0 kcal/mol) and forms the most hydrogen bonds (4) compared to Doxorubicin. The ligands bind to different amino acids, with arginine (ARG-110), leucine (LEU-111, LEU-197), and serine (SER-106, SER-149) being common binding sites. All the details are given in table 3.3 and figure 3.3.

the RMSD value was 0 for all five ligands, this finding suggests that all five ligands, including Doxorubicin, berberine, tinocordiside, dichloromethane, and palmatine, have a strong and reliable binding mode with the p-53 protein. It further supports the notion that these ligands form stable complexes and interact consistently with the target Protein . It is important to note that a lower RMSD value indicates a closer match between the ligand's predicted binding pose and the experimentally determined structure. Therefore, an RMSD value of 0 indicates an exact match, which is a desirable outcome in docking studies, as it suggests a highly accurate and reliable prediction of ligand binding.

Table 4.3: Docking Results for Ligands with p-53 Protein

Ligand	**Affinity (kcal/mol)**	**Number of H-bonds**	**Distance of H-bonds (Å)**	**Bonding Amino acid**
Doxorubicin	-9.8	3	1.9-2.2	ARG-110, LEU-111, HIS-115
Berberine	-7.7	1	2.2	PHE-113
Tinocordiside	-8.0	4	2.0-2.4	SER-149, LEU-197, SER-106, ASN- 239
Dichloromethane	-2.5	-	-	-
Palmatine	-7.3	2	2.0, 2.5	TYR-126, ARG-110

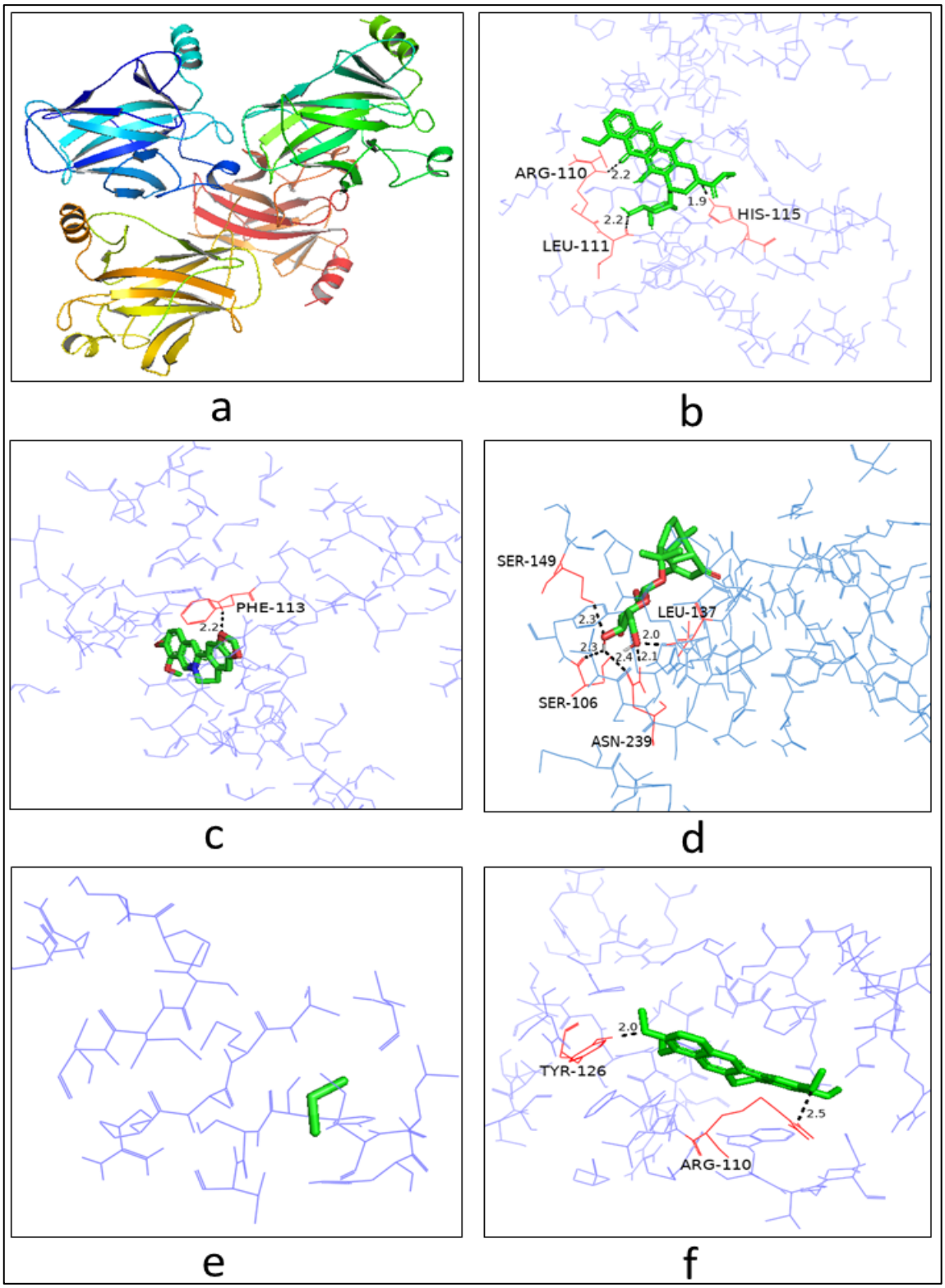

Figure 4.3: Ligands docking with p-53. a) p-53 protein, b) Doxorubicin, c) Berberine, d) Tinocordiside, e) Dichloromethane, f) Palmatine. *The Green colour represent the ligands, Red represents the polar binding amino acid, Black dotted line represents H-bond and Blue colour represent non-polar binding amino acid*

Molecular docking of 5 ligands with Ras

The docking results for the ligands with the Ras protein reveals, Doxorubicin has an affinity of -7.1 kcal/mol in relation to the Ras protein. It establishes three hydrogen bonds with amino acids Threonine (THR-737), Glutamic acid (GLU-977), and (HIS-982). Overall, these docking results indicate that Doxorubicin, berberine, Tinocordiside, and Palmatine have relatively favourable binding affinities with the Ras protein. They form hydrogen bonds with specific amino acids, suggesting potential interactions and stabilization of the ligand-protein complexes. However, the absence of information regarding hydrogen bonding for dichloromethane limits our understanding of its binding mode.

Table 4.4: Docking Results for Ligands with Ras Protein

Ligand	**Affinity (kcal/mol)**	**Number of H-bonds**	**Distance of H-bonds (Å)**	**Bonding Amino acid**
Doxorubicin	-7.1	3	1.9-3.2	THR-737, GLU-977, HIS-982
Berberine	-6.5	1	3.3	ASN-957
Tinocordiside	-6.2	4	2.2-3.4	ASN-974, HIS-975, ASP-973
Dichloromethane	-2.3	-	-	-
Palmatine	-6.2	2	3.2, 3.3	GLN-684

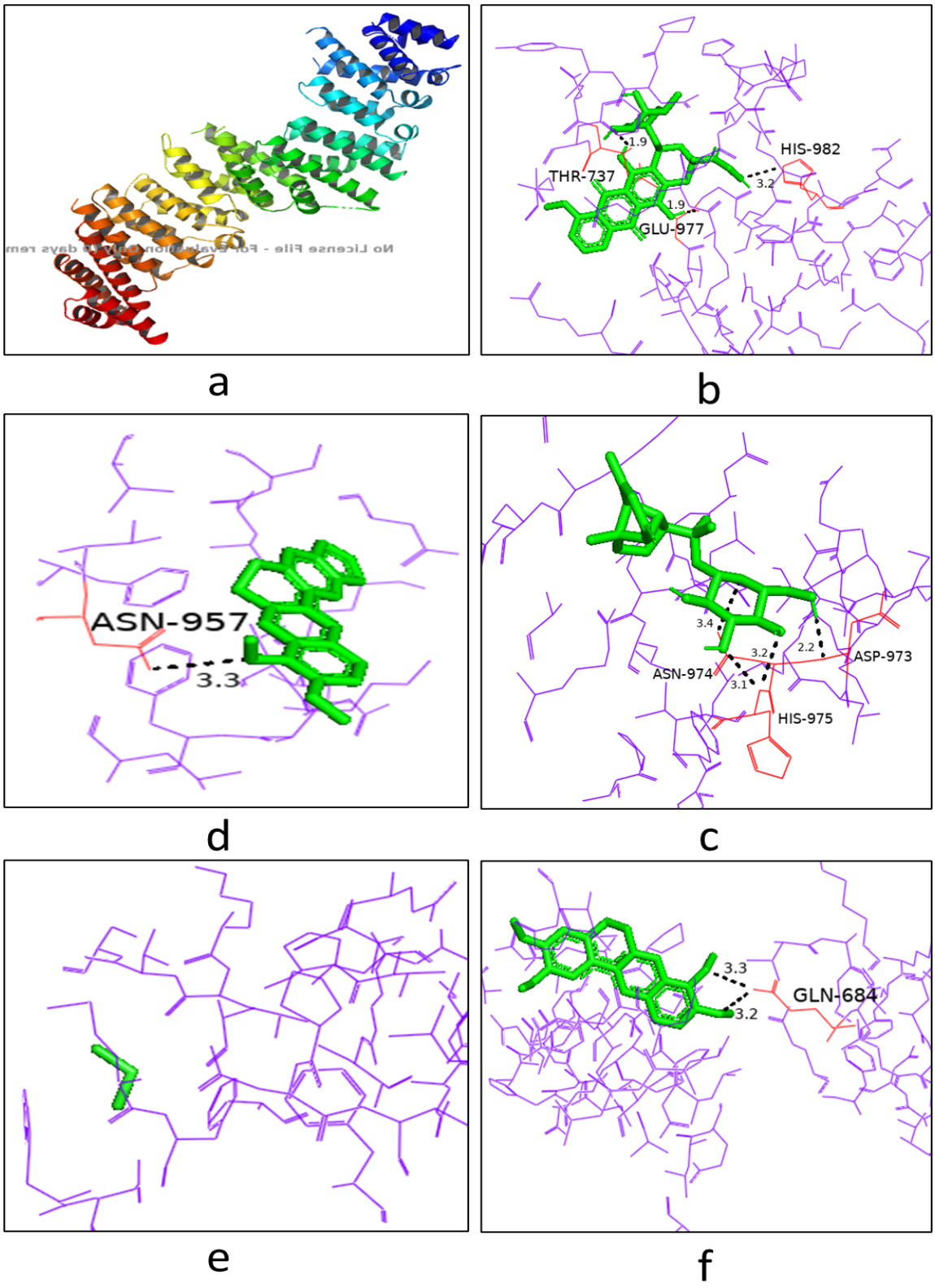

Figure 4.4: Ligands docking with Ras. a) Ras protein, b) Doxorubicin, c) Berberine, d) Tinocordiside, e) Dichloromethane, f) Palmatine. *The Green colour represent the ligands, Red represents the polar binding amino acid, Black dotted line represents H-bond and Blue colour represent non-polar binding amino acid*

ADMET Analysis

This analysis plays a vital role in drug discovery and development by assessing the potential pharmacokinetic and pharmacodynamic characteristics of a compound, as well as its safety and potential for adverse effects. ADMET analysis is typically conducted using a combination of experimental studies, computational modeling, and in silico prediction methods. By considering the ADMET properties of a compound, scientists can prioritize drug candidates with favorable characteristics, improve their efficacy, reduce toxicity risks, and increase the chances of success in clinical trials and eventual patient use. The ADMET analysis was conducted for the best candidates that are Doxorubicin, Berberine, Tinocordiside and Palmatine. The result was provided in table 4.5.

Table 4.5: ADMET Analysis of favourable compounds

ADMET Analysis	**Doxorubicin**	**Berberine**	**Tinocordiside**	**Palmatine**	**Standard Range**
Physiochemical Properties					
Molecular weight (g/mol)	543.17	336.12	396.21	352.15	Optimal:100~600
n HA	12	5	7	5	Optimal:0~12
n HD	7	0	4	0	Optimal:0~7
Log S (log mol/L)	-3.419	-6.311	-2.9	-6.42	Optimal: -4~0.5
Medicinal Chemistry					
QED	0.235	0.674	0.543	0.675	Desirable:>0.67; undesirable:0.49~0.67; Complicated: < 0.34
SA score	4.486	2.787	5.978	2.62	SA score≥6, difficult to synthesize ; SA.score <6, easy to synthesize
NP score	1.811	1.302	2.731	1.139	-5 to 5

Lipinski Rule	No	Yes	Yes	Yes	MW ≤ 500; logP ≤ 5; Hacc ≤ 10; Hdon ≤ 5
Pfizer Rule	Yes	No	Yes	No	logP > 3; TPSA < 75
GSK Rule	No	No	Yes	Yes	MW ≤ 400; logP ≤ 4
Golden Triangle	No	Yes	Yes	Yes	200 ≤ MW ≤ 50; -2 ≤ logD ≤ 5
Bioavailability Score	0.55	0.55	0.55	0.55	0 to 1
Absorption					
Caco-2 Permeability	-6.086	-5.202	-4.913	-5.132	Optimal: > 5.15 Log unit
MDCK Permeability	6×10^{-6}	3.5×10^{-5}	6.6×10^{-5}	3.7×10^{-5}	Less permeability < $2–20 \times 10^{-6}$ cm/s < Highly permeable
HIA	Low	High	High	High	-
$F_{20\%}$	0.982	0.014	0.01	0.299	(Bioavailability ≥ 20%) 0-1 (bioavailability < 20%)
P-gp substrate	Yes	Yes	Yes	Yes	-
Distribution					
PPB	89.40%	93.10%	70.74%	74.56%	Optimal: < 90%
VD	1.147	1.217	1.544	1.199	Optimal: 0.04-20 L/kg
BBB penetration	No	Yes	No	Yes	-
Fu	Middle	Low	Middle	Middle	-
Metabolism					
CYP1A2 inhibitor	No	Yes	No	No	-
CYP2C19 inhibitor	No	No	No	No	-

CYP2C9 inhibitor	No	No	No	No	-
CYP2D6 inhibitor	No	Yes	No	Yes	-
CYP3A4 inhibitor	No	Yes	No	Yes	-
Excretion					
Clearance (mL/min/kg)	Moderate	High	Low	Moderate	High: >15; moderate: 5-15; low: <5
$T_{1/2}$	0.046	0.219	0.63	0.373	-
Toxicity					
H-HT	0.456	0.097	0.25	0.094	(H-HT negative) 0-1 (H-HT positive)
DILI	0.962	0.696	0.035	0.809	Low 0- 1 High
Skin Sensitization	0.711	0.869	0.196	0.882	Low 0- 1 High
Carcinogenicity	0.916	0.906	0.038	0.074	Low 0- 1 High
Eye Irritation	Low	Low	Low	Low	Low 0- 1 High
Respiratory Toxicity	0.898	0.84	0.85	0.503	Low 0- 1 High

Description of values of table 4.5:

- nHA : Number of hydrogen bond acceptors
- nHD : Number of hydrogen bond donors.
- LogS : Log of the aqueous solubility.
- QED : A measure of drug-likeness based on the concept of desirability
- SA score : The synthetic accessibility score is specifically developed to assess the ease of synthesizing drug-like molecules. It serves as a measure to estimate the feasibility and efficiency of synthesizing these molecules in a laboratory setting.
- NP score : Natural product-likeness score. The higher the score is, the higher the probability is that the molecule is a NP.
- Lipinski Rule : MW ≤ 500; logP ≤ 5; Hacc ≤ 10; Hdon ≤ 5. If two properties are out of range, a poor absorption or permeability is possible, one is acceptable.
- Pfizer Rule : logP > 3; TPSA < 75 Compounds with a high log P (>3) and low TPSA (<75) are likely to be toxic.
- GSK Rule : MW ≤ 400; logP ≤ 4. Compounds satisfying the GSK rule may have a more favourable ADMET profile

- Golden triangle : $200 \leq MW \leq 50$; $-2 \leq logD \leq 5$. Compounds satisfying the Golden Triangle rule may have a more favourable ADMET profile.
- Caco-2 Permeability: Assessment of potential bioavailability of drugs in the human body.
- MDCK Permeability: Membrane permeability screening.
- HIA : Human Intestinal Absorption
- $F_{20\%}$: 20% Bioavailability.
- PPB : Plasma Protein Binding. Drugs with high protein binding may have a narrow therapeutic index.
- VD : Volume Distribution.
- BBB penetration: Blood-Brain Barrier Penetration .
- Fu : The fraction unbound in plasms.
- $T_{1/2}$: Half-life. long half-life: >3h; short half-life: <3h.
- H-HT : Human Hepatotoxicity.
- DILI : Drug Induced Liver Injury

Based on the provided ADMET analysis table, the following deductions can be made for the compounds Doxorubicin, Berberine, Tinocordiside, and Palmatine. The physiochemical properties show, Doxorubicin has the highest “number of hydrogen acceptors” (n HA) and “hydrogen donors” (n HD) among the compounds. Log S values indicate that Berberine and Tinocordiside have relatively low solubility, while Doxorubicin and Palmatine have moderate solubility. According to medicinal chemistry, Berberine, Tinocordiside, and Palmatine have attractive quantitative estimation of drug-likeness (QED) values (>0.67), while Doxorubicin falls below the attractive range. SA scores suggest that Tinocordiside has the highest synthetic accessibility, while Doxorubicin and Berberine have relatively lower scores. NP scores indicate that Tinocordiside has the highest natural product-likeness among the compounds. Lipinski and Drug-like Rules state that Doxorubicin fails Lipinski's Rule of Five, while Berberine, Tinocordiside, and Palmatine satisfy these criteria. Doxorubicin and Palmatine pass Pfizer's Rule, but Berberine and Tinocordiside do not meet the criteria. Berberine, Tinocordiside, and Palmatine meet GSK's Rule, while Doxorubicin does not. All compounds satisfy the Golden Triangle rule. Bioavailability and Absorption results deduce that All compounds have the same bioavailability score. Caco-2 permeability values indicate that Berberine and Palmatine have relatively higher permeability than

Tinocordiside and Doxorubicin. MDCK permeability suggests that Berberine, Tinocordiside, and Palmatine have high permeability, while Doxorubicin has low permeability. Berberine, Tinocordiside, and Palmatine exhibit high HIA (human intestinal absorption), while Doxorubicin's HIA is categorized as low. Berberine and Palmatine have bioavailability above 20% (F 20%), while Tinocordiside and Doxorubicin fall below this threshold. All compounds are P-gp substrates. Distribution and Metabolism results show that Berberine and Palmatine have higher plasma protein binding (PPB) than the other compounds. All compounds have acceptable volume of distribution (VD) values. Berberine and Palmatine are predicted to penetrate the "blood-brain barrier (BBB)", while Doxorubicin and Tinocordiside are not. Fu (fraction unbound) is categorized as middle for all compounds. Doxorubicin, Berberine, and Palmatine are inhibitors of CYP2D6 and CYP3A4, while Tinocordiside is not. Excretion and Toxicity results suggest Berberine and Palmatine have higher clearance rates compared to Doxorubicin and Tinocordiside. Doxorubicin has the lowest half-life (T1/2), while Tinocordiside has the highest. Doxorubicin and Tinocordiside show higher hepatotoxicity (H-HT) values, while Berberine and Palmatine have lower values. Doxorubicin and Tinocordiside exhibit higher drug-induced liver injury (DILI) potential compared to Berberine and Palmatine. Berberine and Palmatine have low skin sensitization, carcinogenicity, eye irritation, and respiratory toxicity potential.

CHAPTER 5
CONCLUSION

In this computational docking analysis, we explored the efficacy of plant extracts from *T. cordifolia* , specifically Tinocordiside, Palmatine, Dichloromethane, along with the chemotherapeutic drug Doxorubicin, in cancer treatment. Through molecular docking simulations, we examined the binding interactions between these compounds and key cancer-related proteins, including Haemoglobin, Bcl-2, p53, and Ras. Berberine, Tinocordiside, and Palmatine show consistent affinity and hydrogen bonding interactions with the studied proteins, indicating their potential as ligands for these targets. Doxorubicin also exhibits strong affinity and hydrogen bonding, particularly with p53 and Haemoglobin. On the other hand, Dichloromethane demonstrates weak affinity and does not form significant hydrogen bonding interactions with any of the proteinsThese discoveries offer valuable understandings into the molecular associations between the ligands and the proteins, indicating their potential capabilities in altering protein function within the scope of cancer therapy. The ADMET analysis yields valuable information regarding the pharmacokinetic properties and toxicological profiles of the compounds. Berberine and Palmatine show more favorable properties in terms of drug-likeness, permeability, distribution, and lower toxicity potential. Tinocordiside exhibits promising synthetic accessibility, while Doxorubicin shows limitations in terms of drug-likeness, permeability, and higher toxicity potential. These findings can guide further investigations into the therapeutic potential and optimization of these compounds for anticancer applications.

However, these findings are derived from computational docking analysis, but it is essential to conduct further experimental studies to authenticate the effectiveness of these compounds in cancer treatment. In vitro and in vivo studies are imperative to evaluate their cytotoxicity, pharmacokinetic properties, and overall therapeutic potential. Additionally, additional investigations are warranted to uncover the exact mechanisms of action and subsequent impacts of these compounds on cancer cells. Overall, this computational docking analysis serves as a preliminary step in evaluating the potential efficacy of *T. cordifolia* plant extracts and Doxorubicin in cancer treatment. The findings highlight the importance of further research to validate these results and explore the therapeutic applications of these compounds in a clinical setting.

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