Identifying Tinospora cordifolia Bioactives with the highest anti-inflammatory therapeutic potential to downregulate the NF-kB pathway

A DISSERTATION
SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE
OF

MASTER OF TECHNOLOGY

IN

Industrial Biotechnology

SUBMITTED BY
PREKSHA JAIN
(2K21/IBT/06)

UNDER THE SUPERSVISION Of

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CANDIDATE'S DECLARATION

I, **Preksha Jain**, Roll No. **2K21/IBT/06**, M.Tech. (Industrial Biotechnology), hereby declare that the project Dissertation titled "**Identifying Tinospora cordifolia Bioactive with the greatest inflammatory therapeutic potential**" 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 not imitated from any source without proper citation and is authentic. This work has not beforehand formed the root for the award of any Degree, Diploma, Fellowship, Associateship or any other similar title or acknowledgment.

Place: **Delhi**

Date: 9/06/2023

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CERTIFICATE

I, hereby certify that the Project Dissertation titled "**Identifying Tinospora cordifolia Bioactives with the greatest inflammatory therapeutic potential**" which is submitted by Preksha Jain, Roll No. 2K21/IBT/06, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master ofTechnology is a testimony of the project work carried out by the student under our supervision. To the best of our awareness this work has not been submitted in part or full for any Degree or Diploma to this University or to a different place.

09/06/2023

(Prof. Pravir Kumar)
Head of the Department
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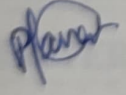
Assistant Professor
Department of

ACKNOWLEDGEMENT

I would like to express our sincere gratitude and appreciation to all those who have contributed to the successful completion of this project report. First and foremost, we extend our heartfelt thanks to our esteemed institution, ***Delhi Technological University*** for providing us with the opportunity to undertake this project. I am grateful for the knowledge, guidance, and resources that have been made available to us throughout this journey.

I would like to express my deep appreciation to my project supervisors, ***Dr. Asmita Das***, for their invaluable support, guidance, and mentorship. I would also like to extend our sincere thanks to the faculty members of the ***Biotechnology Department***, whose teachings and expertise have laid the foundation for our knowledge and skills.

My heartfelt appreciation goes to my classmates, friends and family who have been a constant source of support and motivation throughout this project. Their encouragement, collaboration, and discussions have been immensely valuable in overcoming challenges and finding innovative solutions.



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ABSTRACT

Our study focuses on the ability of bioactives contained in Tinospora cordifolia (Giloy) to decrease inflammation caused by an auto-immune response in the human body. Alkaloids, Diterpenes, Sterols, Glycosides, Aliphatic Compounds, and Hydroxycinnamic Acids are among the medicinal bioactives found in Giloy. The ability of these medicinal bioactives to control NF-kB is being investigated. In the immune system, NF-kB is a dimeric transcription regulator found in the cytoplasm of all cell types. It is engaged in many metabolic activities and plays an important part in many biological processes, but its malfunction leads to auto-inflammatory disorders. As a result, our study looked into the possibility of bioactives in giloy aiding in NF-kB downregulation. The approach of finding possible drug candidates was based on molecular docking. The one with good ADME characteristics and binding affinity, as well as the existence of polar interaction, was discovered.

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CHAPTER 1: INTRODUCTION

Body of a human is made up of many metabolic processes that keep the body running smoothly. Some of these metabolic processes are governed by the immune system. The immune system is a complex network of tissues, organs, and cells that cooperate to protect the body from pathogens such as harmful bacteria, viruses, fungi, and parasites. Its primary function is to distinguish between self and non-self-compounds and to eliminate non-self-invaders while preserving the body's own cells and tissues. (1). The immune system is separated into two categories, first one is innate and second one is adaptive immune systems. Physical barriers such as the skin and mucosal walls, as well as immune cells such as neutrophils, macrophages (phagocytic cells), and natural killer cells, comprise the innate system. These cells have the ability to recognise and eliminate a wide variety of infections. In contrast, the adaptive immune system is an extremely niche-specific defense mechanism that adapts over time. It is distinguished by immunological memory and enables an exclusive response to specific infections. The adaptive immune system is made up of immune cells known as lymphocytes, which include B and T cells. B cells create antibodies capable of recognising and neutralizing certain antigens, whereas T cells participate in cell-mediated immunity, assisting in the elimination of infected or aberrant cells(2). The process of immunological recognition is important to the adaptive immune system. Immune cells contain receptors that can recognise certain compounds on pathogen surfaces called as antigens. When an antigen is recognised, it sets off a chain of events that includes the development of particular antibodies for sending signals to immune cells for removing the invaders (3). A complex network of signalling molecules, including cytokines and chemokines, regulates the immune system, coordinating immune responses and maintaining immunological homeostasis. It can also tell the difference between hazardous infections and safe things like the human's own cells and helpful microorganisms in the gut. However, the immune system is not perfect, and it might malfunction at times.

In process of maintaining the immunological homeostasis, inflammation play an important role, it is an important immune system defensive mechanism, and it works by eliminating harmful stimuli and starting the recovery process. Inflammation can be acute or chronic, acute inflammation is distinguished by a quick reaction, which often

occurs minutes or hours following tissue damage or infection(4). The affected region may develop redness, swelling, warmth to the touch, and discomfort. These symptoms are caused by increased blood flow to the region, blood vessel dilatation, and the release of chemical mediators such histamines, prostaglandins, and cytokines. This reaction aids in the delivery of immune cells and nutrients to the site of injury, the isolation and destruction of invading pathogens, and the initiation of the healing process. Chronic inflammation, on the other hand, is a long-term inflammatory response that can last weeks, months, or even years. Chronic inflammation, unlike acute inflammation, does not dissipate readily and can cause tissue damage. It is frequently linked to autoimmune illnesses, long-term exposure to irritants or chemicals, obesity, and diseases such as rheumatoid arthritis, inflammatory bowel disease, and cardiovascular disease (5). Chronic inflammation has numerous underlying causes that entail immune system dysfunction. The immune response becomes hyperactive in this condition, and immune cells erroneously assault normal organs and tissues, resulting in continuous inflammation. Chronic inflammation can cause tissue loss, scarring, and reduced organ function over time.

NF-κB, or nuclear factor kappa light chain enhancer of activated B cells, is a 35-year-old pathway that plays critical regulatory roles in both innate and adaptive immune responses. There are many stimuli that can activate the NF-κB pathway, which then activates the various biological targets (6).

Pathogen-associated molecular patterns (PAMPs) recognised by pattern recognition receptors (PRRs) on diverse immune cells, such as macrophages (also known as the phagocytic cells), dendritic cells, and neutrophils, initiate the immune system's innate response in NF-kB activation. Pathogen attack triggers an innate response that translocates NF-kB to the nucleus and stimulates the production of genes encoding cytokines that are pro-inflammatory, chemokines, peptides that are antimicrobial, and other immune mediators. In reaction, pathogens are eliminated, immune cells are recruited at the infection site, phagocytosis is enhanced, and an inflammatory response is induced (7). Furthermore, NF-kB is involved in crosstalk between the immune system's components (innate and adaptive). Inflammatory signals generated from NF-kB activation in innate immune cells can impact adaptive component activation and function. Excited T cells, on the other hand, can create cytokines that influence NF-kB activity in innate immunity cells, further altering the immune response. NF-kB activity

has been linked to chronic inflammatory illnesses, auto-immune conditions, and malignancies. Defects in NF-kB activation, on the other hand, might result in immunological inadequacies and increased susceptibility to infections (8).

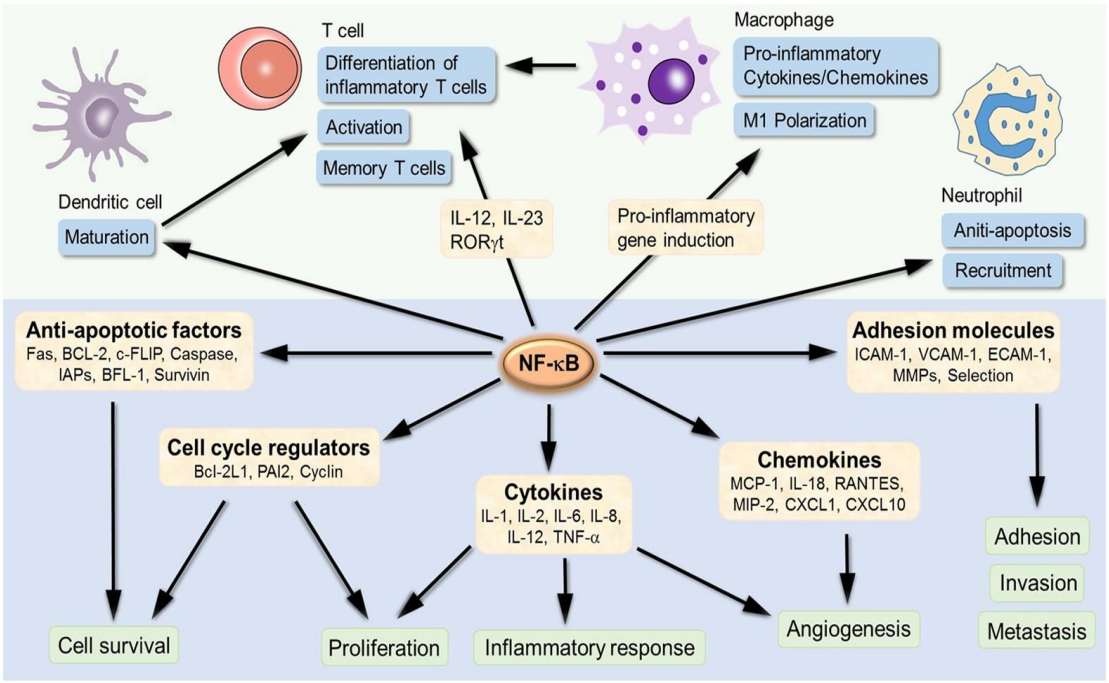


Fig 1.1: The NF-κB regulator genes involved in the onset and progression of inflammation(9) .

Cytokines are small messenger molecules produced by a number of immune cells during inflammatory processes, including macrophages, dendritic cells, and T cells. They help the immune system govern and coordinate the immune response. External sources, such as allopathic medications or herbal remedies, can influence these cytokines, which are responsible for modulating immune responses. Natural herbs or the herbal medicines are reoccurring now because they are physiologically active and function as substrates for one or more of the several metabolic systems that can transport the compounds to their intracellular location of action. One use for these natural medicines is to stimulate the immune system into an inflammatory reaction to an infection or illness and one such herb is Tinospora cordifolia, a Menispermeaceae herbaceous climber also known as Giloy, Amrita, Guduchi, Gulancha, Ambervel and Gulvel in various parts of India, is an Ayurvedic rasayana medicine that is indicated for

a variety of ailments and health enhancement. It is available across India, as well as in China, Burma, Bangladesh, and Sri Lanka (10).

Giloy, a traditional herb, has the potential to modulate the nuclear factor-κB (NF-κB) pathway, a signaling system that encompasses both noncanonical and canonical routes, both of which are important in many inflammatory reactions. The canonical route, when activated, is in charge of the transcriptional synthesis of chemokines, pro-inflammatory cytokines, and other inflammatory mediators in a range of innate and adaptive immune responses. Synthesis of chemokines and cytokines are modulated with the help of bioactives or phytochemicals and Giloy has a lot of bioactives such as alkaloids (Choline, Berberine, Palmatine, Tinosporin, Tembetarine, Magnoflorine, and Isocolumbin), glycosides (Tinocordiside, Cordioside), diterpenoid, steroids (beta-sitosterol), aliphatic molecule (Octacosanol), and other chemicals (11, 12).

Fig 1.2: Tinospora cordifolia

The objective of this thesis is to identify a possible bioactive from Tinospora cordifolia (giloy) that has the capacity to downregulate the nuclear factor kappa-B signalling pathway, which can be useful in auto-inflammatory illnesses.

CHAPTER 2: LITERATURE REVIEW

2.1 NF-kB Signalling System:

NF-kB is a dimeric transcription regulator that is located in the cytoplasm of all cell types. It is involved in many metabolic functions and plays a critical role in many biological processes, but its dysfunction leads to auto-inflammatory diseases (13). The ligand bound receptor's signal is conveyed to changes in the regulation of genes, which leads to increased production of effector molecules such as cytokines and adhesion molecules(14). In mammals, the NF-kB signalling system is composed of a total of five parts that form heterodimers or homodimers: RELA (p65), RELB, c-RER, NF-kB1 (p105/p50), and NF-kB2 (p100/p52) (15). It should be noted that NF-kB communication is complicated and associated with several other pathways, and there can be crosstalk and cooperation between the canonical and non-canonical pathways (Alternative and Atypical). Figure 2.1 depicts the mechanism of this NF-kB signalling pathway (16).

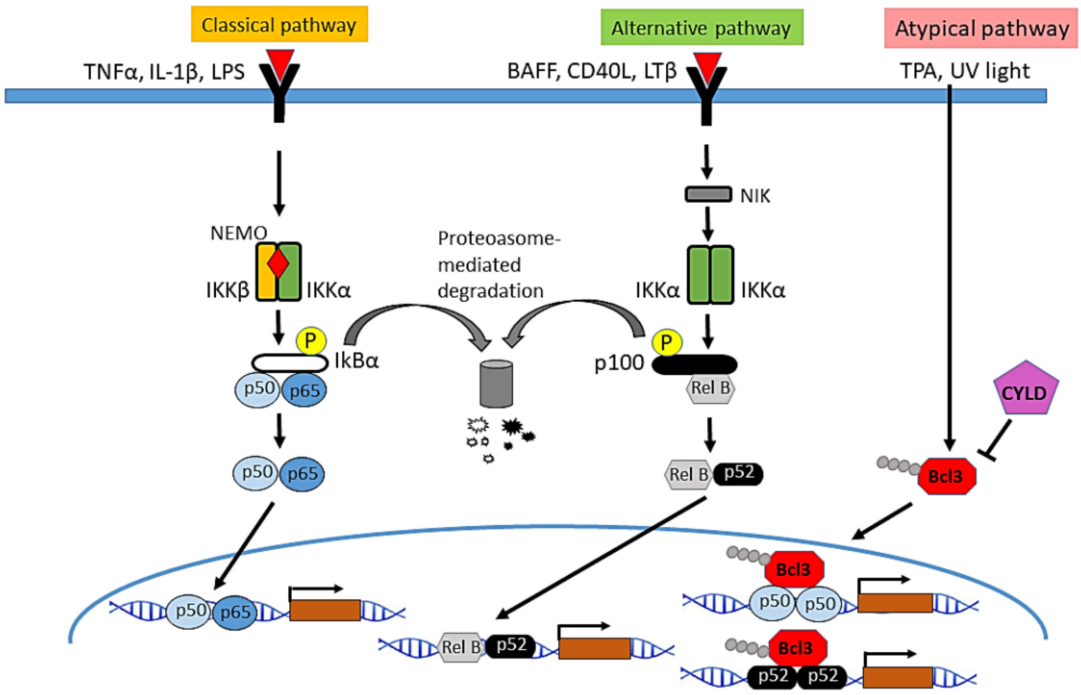


Fig 2.1: The primary signalling mechanisms involved in NF-kB pathway activation(16)

The classical downregulatory signaling mechanism incorporates the stimulation of immune cells by numerous external stimuli such as pathogen-associated molecular patterns (PAMPs) or pro-inflammatory cytokines (7). This route is largely dependent on the activation of the IKK complex, which results in the release and nuclear translocation of NF-kB. Cytokines downregulate NF-kB, several cytokines have been demonstrated to decrease NF-kB activity, resulting in reduced pro-inflammatory signalling. Cytokines bind to immune cell surface receptors, starting signalling cascades downstream. These receptors commonly activate intracellular pathways including Janus kinases (JAKs) and signal transducer and activator of transcription (STAT) proteins. Cytokines can cause the production of inhibitory proteins that directly interact with NF-kB subunits, restricting DNA binding and transcriptional activity (17, 18).

2.2 Bioactives in Tinospora cordifolia that can down regulate NF-kB pathway

Tinospora cordifolia, often known as Guduchi or Giloy, is a well-known Ayurvedic medicinal plant with anti-inflammatory properties. It has been found to contain bioactive compounds that impact inflammation in the body(19). Various bioactive components of Tinospora cordifolia have been studied for anti-inflammatory effects (20). The following are some bioactives in giloy that can modify multiple cytokine concentrations in the body and also result in down-regulation of the NF-kB pathway.

Table 2.2.1 Tinospora cordifolia bioactives that can activate cytokines resulting in downstream NF-κB signalling pathway

Class of Bioactive	Bioactive	Source	Cytokines resulting in downstream NF-κB signalling pathway	Ref
Alkaloids	Palmatine	Stem	Decreased efficacy of TNF- α	(21, 22)
			inhibited the release of TNF-α, IL-1β, IL-6 to down regulate pathway	(23)
	Berberine	Stem	decreases in IL-8, IFN-γ, and TNF-α protein concentrations in serum result in down regulation of pathway	(24, 25)

			Decreased protein levels of the pro-inflammatory cytokines (IL-1, IL-6, and TNF- α)	(26)
	Magnoflorine	Stem	Reduction in anti-inflammatory cytokine IL-6 and IL-8 which partly inhibits NF-κB signalling axis	(27, 28)
	Tetrahydropal matine	Root	IL-1β, IL-6, IL-18, and TNF-α the pro-inflammatory cytokines were lowered to cause anti-inflammatory effect	(29, 30)
	Choline	Stem	Supplemental methionine reduces IL-1 and IL-18 hepatic expression	(11, 31)
	Jatrorrhizine	Roots and stem	Reduction in IL-1β and TNF-α cause protective effect on blood vessels	(32-34)
Diterpenes	Columbin	Stem	Inhibition of IFN-γ to cause anti-inflammation	(35, 36)
	Clerodane	Stem	inhibited IL-1β, IL-6 and TNF-α	(37, 38)
	Ecdysterone	Stem	Inhibits the production of TNF-α	(11, 39)
Sterols	Stigmasterol	Stem	Suppress the expression of TNF-α, IL-6 and IL-1β which inhibit p-IKB-α activation	(40, 41)
	β-sitosterol	Stem	cytokines IL-6 and IL-12 were lowered	(42, 43)
Glycosides	Syringin	Stem	Reduction in IL-4, IL-5, IL-13 and IFN-y brought reduction in IgE concentration	(11, 44)
	Palmatosides	Stem	Reduction of IL-1β, TNF-α, IL-6, and IL-17 are responsible for anti-inflammatory effect	(45, 46)
	Furanoid diterpine glucoside	Stem	Produced inhibitory effect on TNF- α	(46, 47)
Aliphatic Compound	Octacosanol	Whole Plant	Increased expression of TNF-α, IL-1β and IL-6 can be seen	(48, 49)
Hydroxycinnamic acids	Sinapic Acid	Stem	enhanced IFN-γ level and decreased IL-4, IL-5 and IL-13 levels and resulted in reduced IgE levels	(50, 51)

The cytokines listed in the table above have a distinct inflammatory effect on the body. Tumor necrosis factor (TNF) family and interleukins (IL) family are the two cytokine families most responsible for NF-κB pathway downregulation in humans, resulting in anti-inflammatory actions. Inflammation regulator TNF belongs to the proinflammatory cytokine superfamily and is largely secreted by macrophages (52). It regulates biological processes such as differentiation, cell proliferation, death, coagulation, and lipid metabolism. TNF has been associated to autoimmune diseases, polycystic kidney disease, insulin resistance, rheumatoid arthritis, ankylosing spondylitis, Yellow fever, and cancer, among other things. TNF also has neuroprotective properties (53). Another important class of cytokines involved in inflammation are interleukins, among them IL-1/IL-1β a member of interleukin-1 cytokine family, is created as a proprotein by activated macrophages and is proteolytically transformed to an active form by caspase1 (54). IL-18, another cytokine of interleukin-1 family proinflammatory cytokine, can stimulate interferon gamma (IFN-γ) production and, both IFN-γ and IL-18 can induce T-cell responses. IFN-γ is the cytokine which is soluble in nature belongs to type-II class of interferons is produced by both adaptive and innate immune system in human body and response to any microbial infection, but if IFN-γ is secreted in large amount it can cause autoimmune disorders (55). The human body is also affected by increased production of the cytokine IL-5, which works as a growth and differentiation factor for both B cells and eosinophil's, may be linked to eosinophil-dependent inflammatory disorders.IL-5, IL-3 and IL-13 are known for the gene cluster they form on chromosome 5q, this gene cluster is cytokine cluster and all of them are responsible for acute inflammation and wound healing(56). Interleukin-6 (IL-6) also act as a pro-inflammatory cytokine that is essential for immunological function and inflammation. It is engaged in a variety of physiological activities, including immune cell function modulation, acute-phase responses, and the repair of tissue (57).

2.3: NF-κB Dimers:

NF-kB dimers are made up of many subunits, the well-studied and regularly encountered of which are the p50 and p65 subunits (58). The p50 subunit (NFKB1) is generated from the NFKB1 gene, as a precursor protein termed p105, which is then

proteolytically processed to become the mature p50 component. The p50 subunit lacks transactivation domain seen in other NF-kB subunits, it is transcriptionally weak on its own. It then forms heterodimers with other subunits, notably p65, to boost NF-kB transcriptional activity. The p65 (RelA) subunit is a transcriptional activator with a transactivation domain(59).

The NF-kB p65 and p50 subunits work together to regulate gene expression and orchestrate the inflammatory response. The p65 subunit is principally in charge of transactivation and gene induction, whilst the p50 subunit aids in DNA binding and improves the stability and specificity of NF-kB dimers. The p65/p50 heterodimer functions as a major transcription factor complex in the NF-kB signalling pathway, mediating the expression of several inflammatory and immune-related proteins (60).

Bioactives can disrupt p50/p65 DNA binding by interfering with the p50/p65 heterodimer's capacity to bind DNA. The disruption occurs by direct binding to the DNA binding domains of either the p50 or p65 subunits, or by changing the structure of the heterodimer itself, resulting in NF-kB-mediated gene expression downregulation (61).

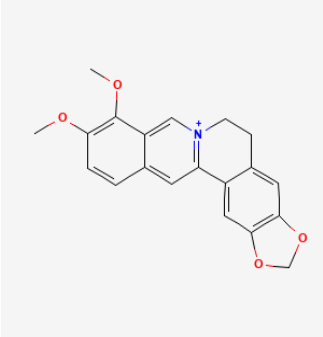
CHAPTER 3: METHODOLGY AND TOOLS USED

1. Retrieving ligand structure through PubChem. PubChem is a free database managed by the National Centre for Biotechnology Information (NCBI), which is part of the National Library of Medicine (NLM) in the United States. It contains detailed information on tiny compounds' biological activity, chemical structures, and characteristics (62).
2. ADME Predictions: To anticipate the pharmacokinetic and drug-like qualities of small molecules and to give insights into the Absorption, Distribution, Metabolism, and Excretion (ADME) features of substances, which are significant in drug discovery considerations swissADME tool was used (63).
3. Converting chemical file formats: Open Babel is a chemical file format converter. It can read and write a wide range of file types, including molecular file formats (such as PDB, SDF, MOL2, and SMILES) and computational chemistry file formats(64).
4. Docking Software: AutoDock Vina was used for the protein and ligand preparation. Both the ligands and proteins were pre-prepared and converted to pdbqt files for molecular docking. It is a popular molecular docking programme that predicts small molecule binding mechanisms and affinities with protein targets. It explores the conformational space and identifies favourable ligand-receptor interactions using an efficient search method and scoring function. (65).
5. Cavity Analysis and Selection: Cavities were detected and pockets were analysed using CASTp (66).
6. Visualizing and Analysing docked structure: PyMOL was used analyse the three-dimensional structures of biological macromolecules such as proteins, nucleic acids, and tiny molecules. It includes a comprehensive set of tools for molecular graphics, rendering, and analysis (67).

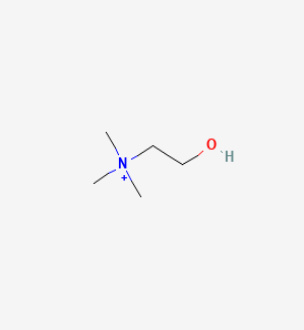
CHAPTER 4: RESULTS AND DISCUSSIONS

4.1: Ligand and Protein Structures used for Analysis

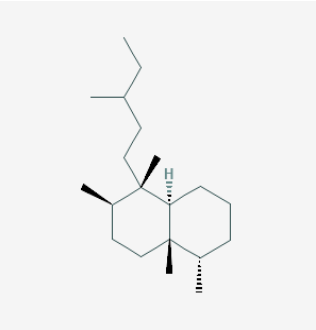
PubChem library was used to retrieve bioactive molecule structure that functions as ligand for p50 and p65 subunits of NF-kB, these structures are shown in Fig: 4.1.1. Whereas Protein Data Bank was used to retrieve p50 and p65 protein structure shown in Fig 4.1.2. And Fig 4.1.3 respectively.



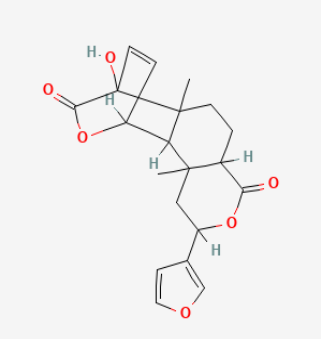
(a) Berberine



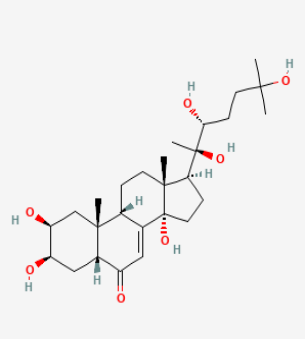
(b) Choline



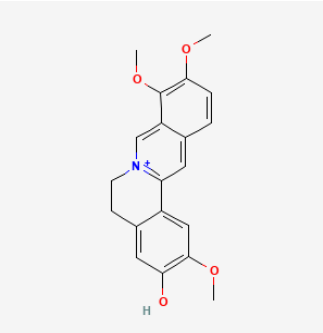
(c) Clerodane



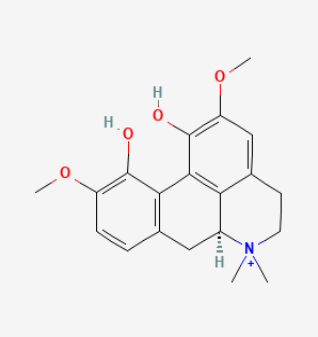
(d) Columbin



(e) Ecdysterone

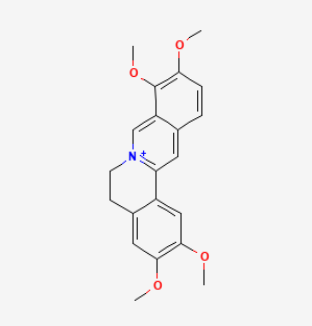


(f) Jatrorrhizine

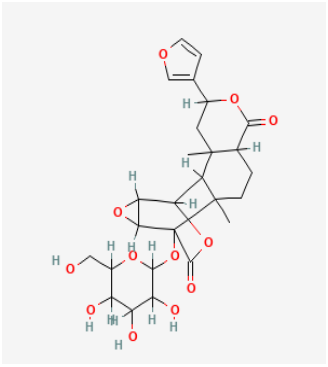


(g) Magnoflorine

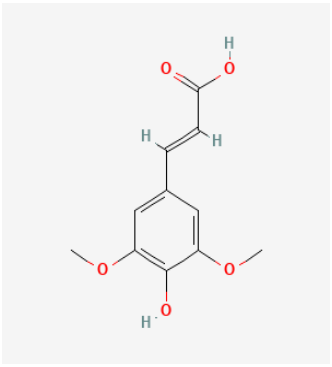
(h) Octasanol



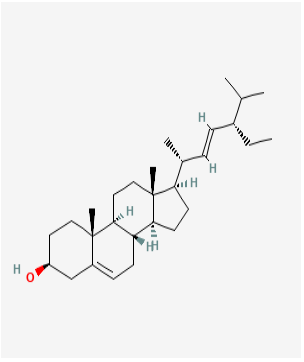
(i) Palmatine



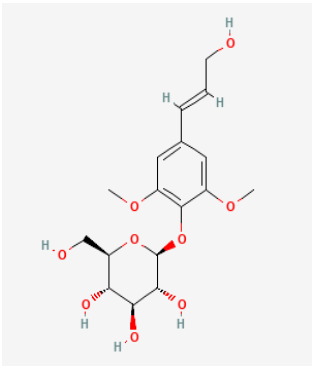
(j) Palmatosides



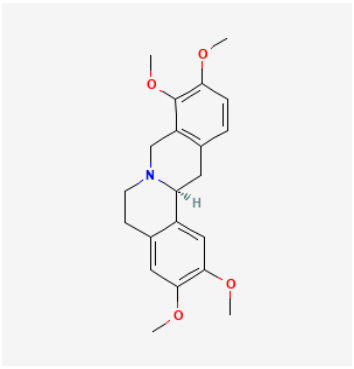
(k) Sinapic acid



(l) Stigmasterol



(m) Syringin



(n) Tetrahydropalmatine

Fig 4.1.1 Structure of Bioactives in Tinospora cordifolia with potential to downregulate NF-kB canonical pathway

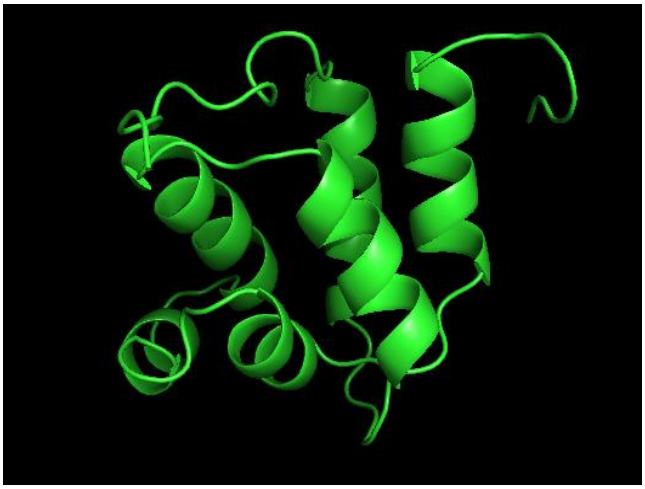


Fig 4.1.2: Structure of human's nuclear factor NF-kappa-B subunit p105/p50

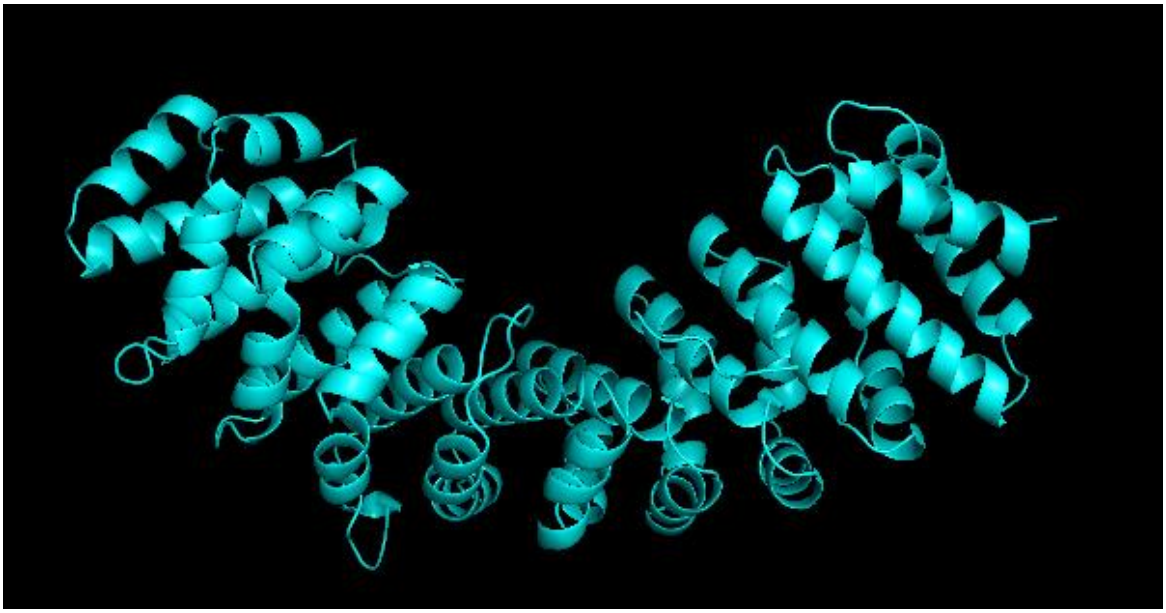


Fig 4.1.3: Structure of human's nuclear factor NF-kappa-B subunit p65

4.2: ADME analysis of Bioactives

ADME analysis is a critical component of drug discovery and development. It stands for Absorption, Distribution, Metabolism, and Excretion, all of which are important elements in determining a drug's fate within the body. ADME analysis assists pharmaceutical researchers and scientists in understanding how a medication is absorbed, distributed, metabolised, and removed in the body, which determines its efficacy and safety profile.

Table 4.2.1 ADME analysis of Bioactives (by swissADME)

Bioactives	**Lipo-philicity**	**Water Solubility**	**GI - absorption**	**Log Kp (skin permeation)**	**Druglikeness**		**Bio-availability Score**
					Lipinski	**Violation**	
Palmatine	3.75	Moderate	High	-5.79 cm/s	yes	0	0.55
Berberine	3.62	Moderate	High	-5.78 cm/s	yes	0	0.55
Magnoflorine	2.74	Moderate	High	-6.44 cm/s	yes	0	0.55
Tetrahydropalmatine	3.24	Moderate	High	-6.17 cm/s	Yes	0	0.55
Choline	-0.40	Highly	Low	-7.22 cm/s	Yes	0	0.55
Jatrorrhizine	3.42	Moderate	High	-5.94 cm/s	yes	0	0.55
Columbin	2.16	Soluble	High	-6.95 cm/s	Yes	0	0.55
Clerodane	8.59	Poorly	Low	-1.90 cm/s	Yes	1	0.55
Ecdysterone	0.45	Soluble	High	-8.91 cm/s	Yes	1	0.55
Stigmasterol	8.56	Poorly	Low	-2.74 cm/s	Yes	1	0.55
β-sitosterol	7.74	Poorly	Low	-4.32 cm/s	Yes	1	0.55
Syringin	-1.31	moderate	Low	-9.50 cm/s	Yes	0	0.55
Palmatosides	-0.27	Soluble	Low	-9.76 cm/s	No	2	0.17
Octacosanol	13.61	Insoluble	Low	0.86 cm/s	Yes	1	0.55
Sinapic Acid	1.46	Soluble	High	-6.63 cm/s	Yes	0	0.56

The table 4.2.1 has compiled analysis of swissADME tool, it contains lipophilicity, water solubility, GI-absorption, Log Kp (skin permeation), drug-likeliness and bioavailability. Lipophilicity is a molecule's propensity to dissolve in lipid-based environments such as cell membranes. It is an essential factor that influences medication absorption. The optimal range of lipophilicity might vary, however for many medications, a logP value between -1 and 5 is commonly regarded suitable (68). Water solubility another essential feature of a medicine since it influences dissolution, absorption, and bioavailability. A drug's optimal range of water solubility is determined by a number of criteria, including the planned route of administration and the therapeutic indication (69). Swiss ADME analysis show The Log Kp number which

reflects a substance's capacity to permeate the skin. A greater Log Kp value indicates better skin penetration, suggesting that the substance may pass through the skin barrier more easily. It should be noted that the actual Log Kp values might vary greatly depending on the molecule being tested, the experimental settings, and the measuring methodologies. However, as a general rule, the Log Kp values for various compounds may be classified as follows:

- Low permeability (Log Kp < -4): Low skin permeability substances have a harder time passing the skin barrier and are less likely to be absorbed into the systemic circulation.
- Moderate permeability (-4 < Log Kp < -1): Substances with moderate skin permeability can permeate the skin barrier and may absorb in a modest amount.
- High permeability (Log Kp > -1): High skin permeability substances can easily pass through the skin barrier and have a higher potential for absorption into the systemic circulation (70).

The evaluation of a compound's possibility of possessing the requisite qualities for successful drug development is referred to as drug likeness. It entails assessing a compound's numerous molecular and physicochemical properties in order to establish its potential as a therapeutic candidate. The goal of drug likeness analysis is to find compounds that are more likely to display good ADME features(71). Another feature of drug that is bioavailability is a critical metric in ADME study. It is the fraction or percentage of a drug's given dosage that enters into the bloodstream in an unaltered or active state (72).

Following the swissADME study, bioactives with drug-likeness violations are excluded from molecular docking analysis. Clerodane, ecdysterone, stigmasterol, octacosanol, palmatosides, and β-sitosterol are the bioactives with infringement. Other than these syringin with excessive lipophilicity (more than -1) and poor GI-absorption have not been considered.

4.3: Molecular docking analysis

AutoDock Vina is a popular software for docking of the biological molecules that predicts the binding affinity between a small molecule ligand and a target protein. The binding affinity in AutoDock Vina is represented by the binding energy score, often

known as the "Vina score". The Vina score is a numerical number that represents the expected strength of ligand-protein binding. A lower Vina score implies a higher binding affinity, meaning that the ligand has higher chances to bind to the protein with greater stability.

Table 4.3.1: Binding affinity/Vina score of bioactives with p50 and p65 subunits

Bioactive	**Vina score with p50 subunit (kcal/mol)**	**Vina score with p65 subunit (kcal/mol)**
Sinapic Acid	-5	-5.4
Palmatine	-5.8	-6.3
Berberine	-6.6	-7.5
Magnoflorine	-5.8	-6.7
Tetrahydropalmatine	-6.4	-6.5
Jatrorrhizine	-6.6	-6.1
Columbin	-6.6	-7.1

The interaction of a protein and a ligand is critical for understanding the binding process, identifying important interactions, and predicting binding affinity. These investigations shed light on molecular recognition and can help with medication development and design. Several methods are routinely used to study protein-ligand interactions:

- **X-ray crystallography:** This approach includes crystallising the protein-ligand complex and using X-ray diffraction to determine the three-dimensional structure. It gives high-resolution information on the binding site and the particular interactions between the protein and ligand.
- **Spectroscopy via NMR:** Protein-ligand interactions in solution are studied using Nuclear Magnetic Resonance (NMR) spectroscopy. It can offer information on the protein's binding site, conformational changes, kinetics, and particular interactions with the ligand.
- **Surface Plasmon Resonance (SPR):** SPR monitors real-time binding events by measuring change of refractive index at the exposed boundary of a sensor chip. It enables the calculation of binding kinetics (association and dissociation rates) and affinity (K_d) between a protein and a ligand.
- **Isothermal Titration Calorimetry (ITC):** ITC quantifies binding affinity (K_d) and stoichiometry by measuring the heat generated or absorbed during a binding

event. It can also reveal information on the thermodynamics of the binding process.

- **Fluorescence Spectroscopy:** To analyse protein-ligand interactions, fluorescence-based approaches such as FRET (Fluorescence Resonance Energy Transfer) or FP (Florescence Polarisation) can be used. Changes in fluorescence characteristics are used to monitor binding events, characterise binding kinetics, and calculate dissociation constants.
- **Molecular docking:** Fixed commands or algorithms are used in computational docking approaches such as AutoDock Vina to predict the binding mechanisms and affinities of protein-ligand complexes. Docking calculations model protein-ligand interactions to yield realistic binding conformations.

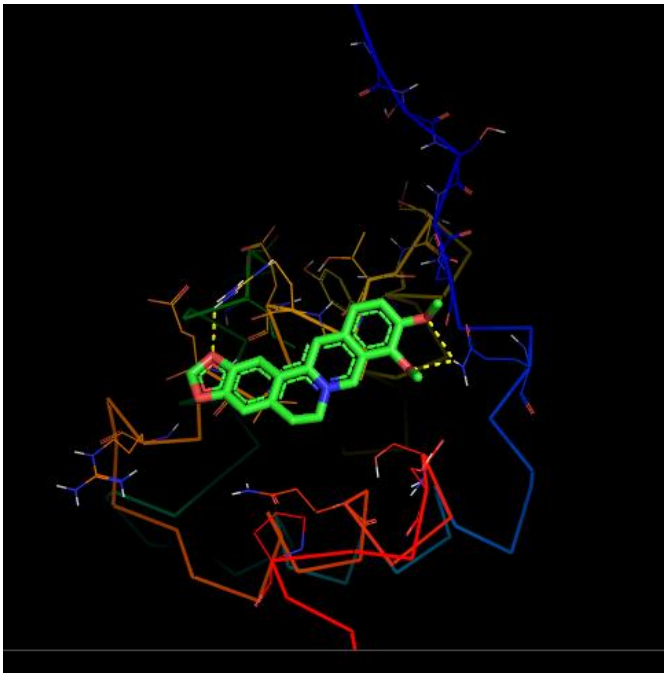


Fig 4.3.1: Berberine interaction with p50- 3 polar bonds are formed

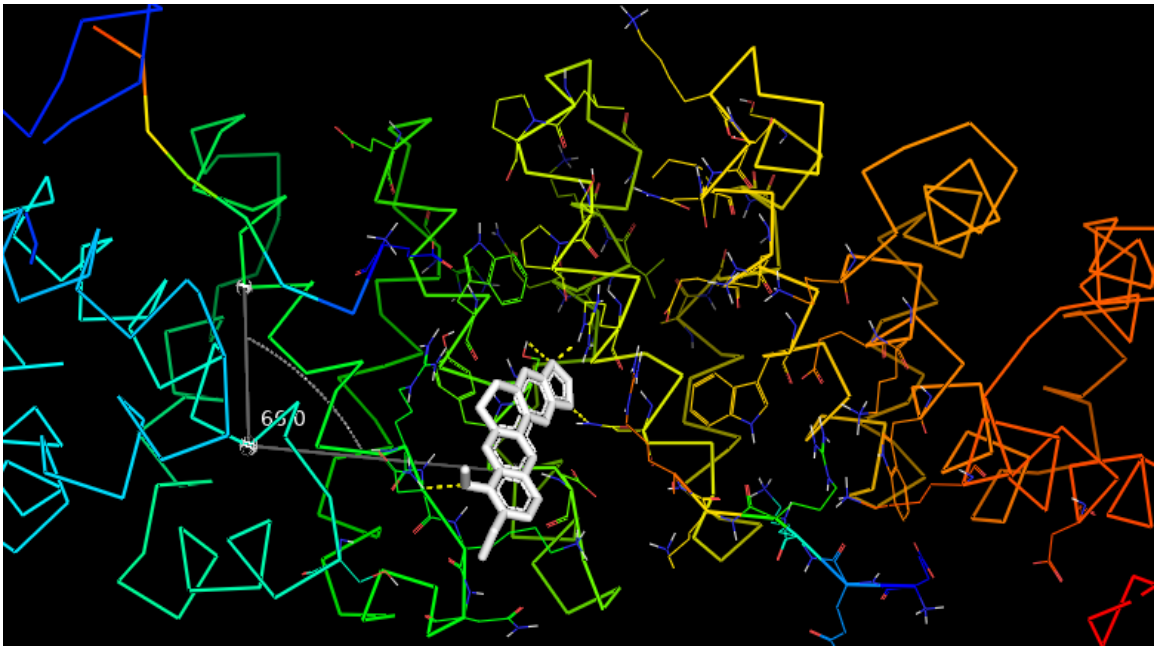


Fig 4.3.2: Berberine interaction with p65- 4 polar bonds are formed

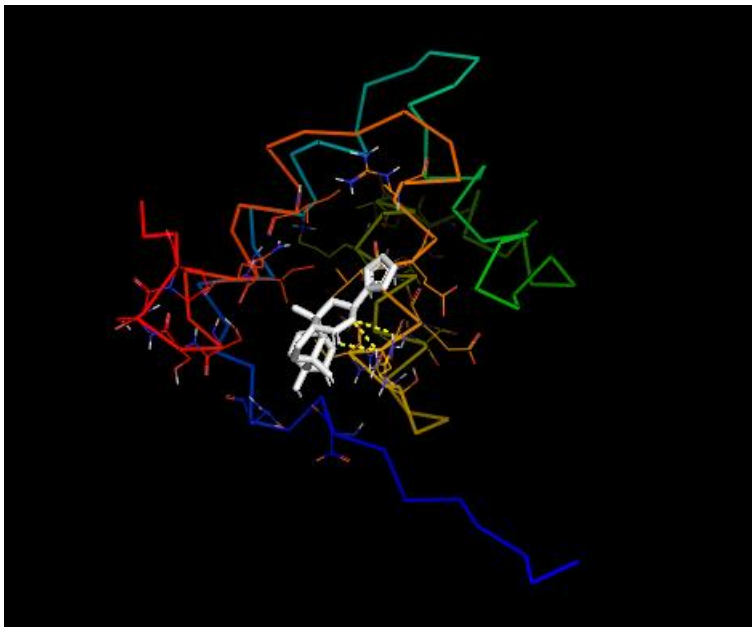


Fig 4.3.3: Columbin interaction with p50- 3 polar bonds are formed

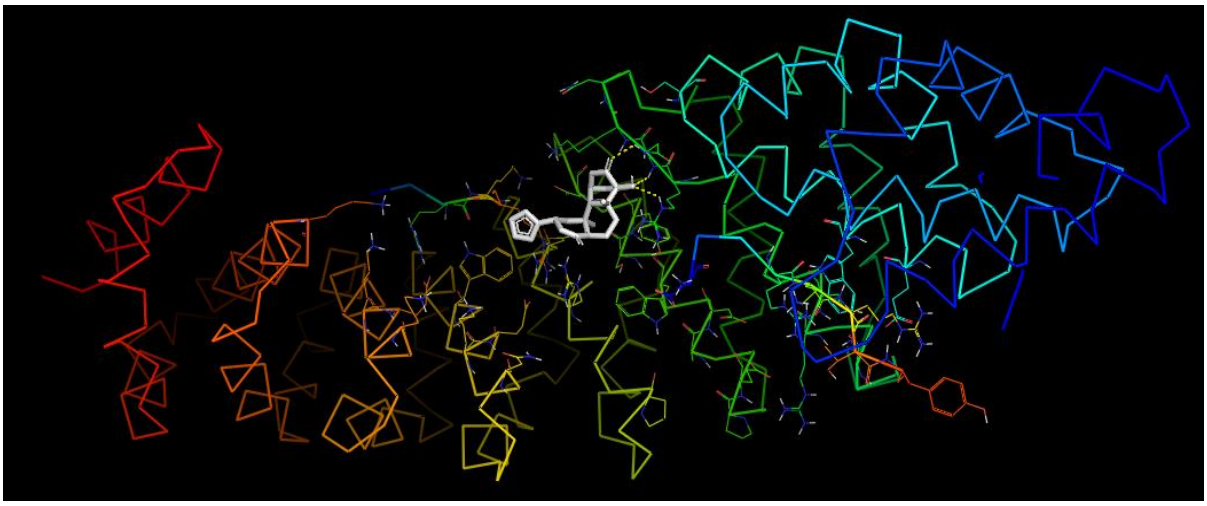


Fig 4.3.4: Columbin interaction with p65- 3 polar bonds are formed

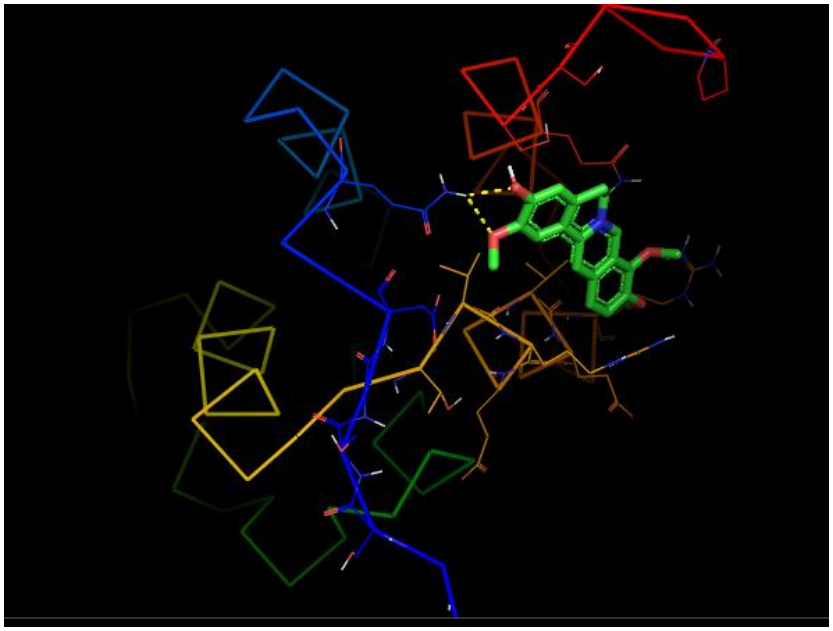


Fig 4.3.5: Jatrorrhizine interaction with p50: 2 polar bonds are formed

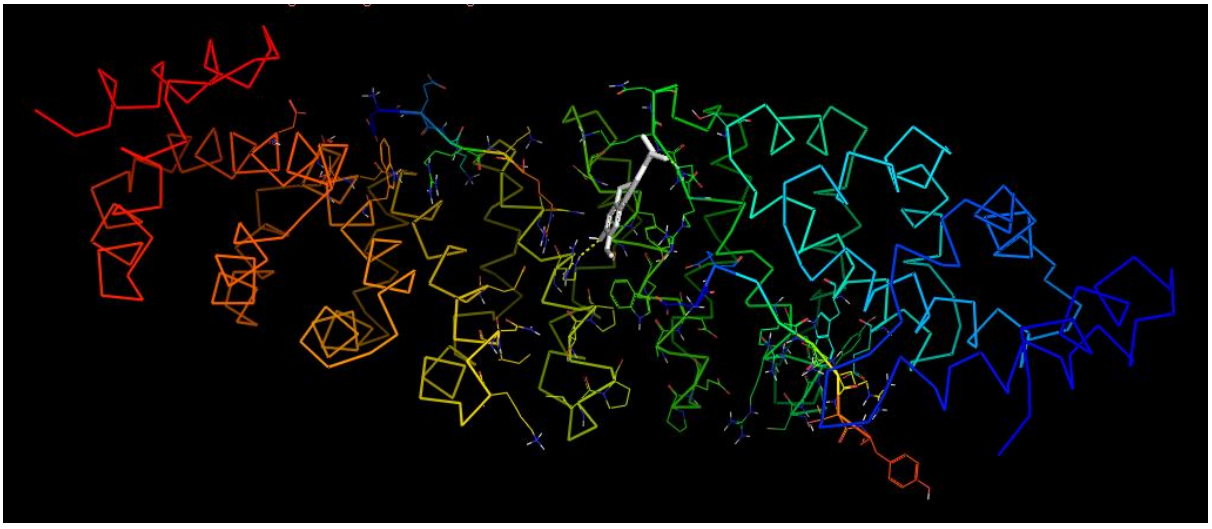


Fig 4.3.6: Jatrorrhizine interaction with p65 - 2 polar bonds are formed

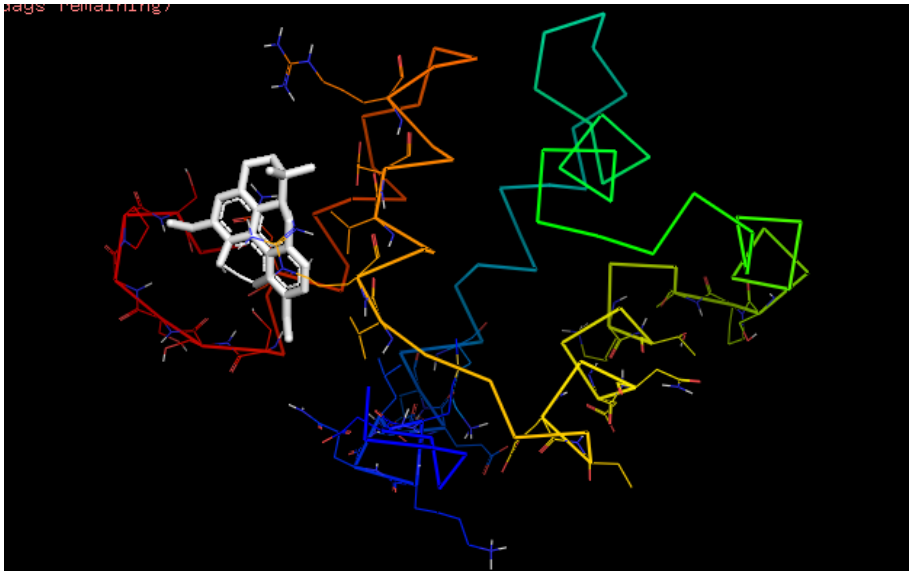


Fig 4.3.7: Magnoflorine has no interaction with p50

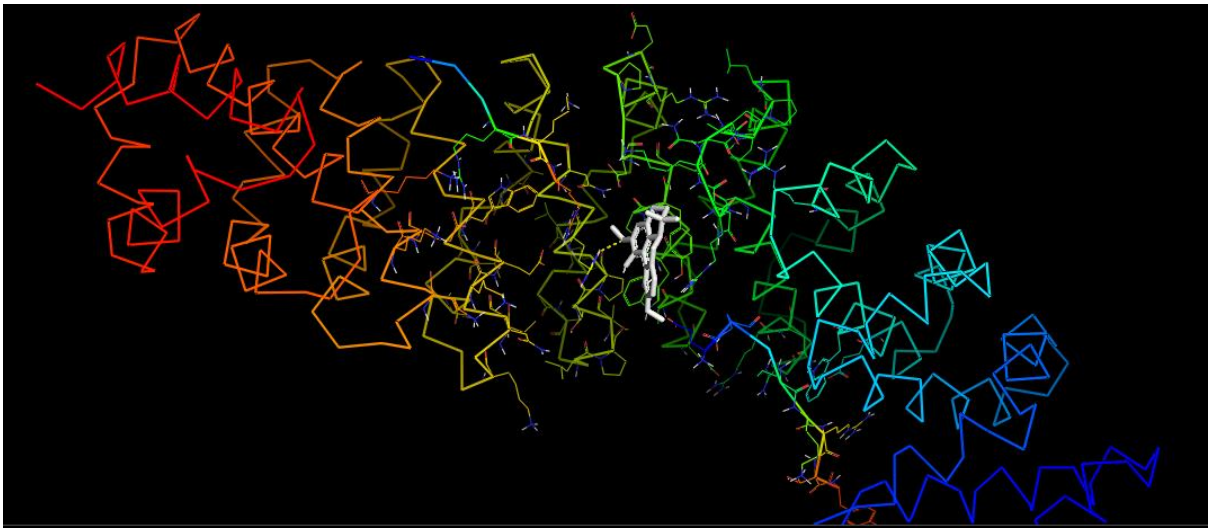


Fig 4.3.8: Magnoflorine interaction with p65-1 polar bond formed

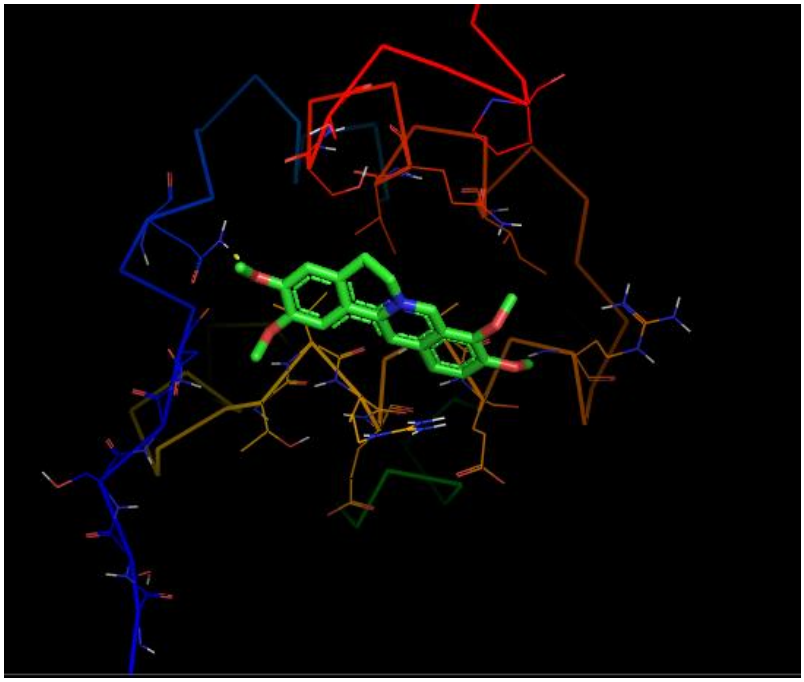


Fig 4.3.9: Palmatine interaction with p50- 1 polar bond formed

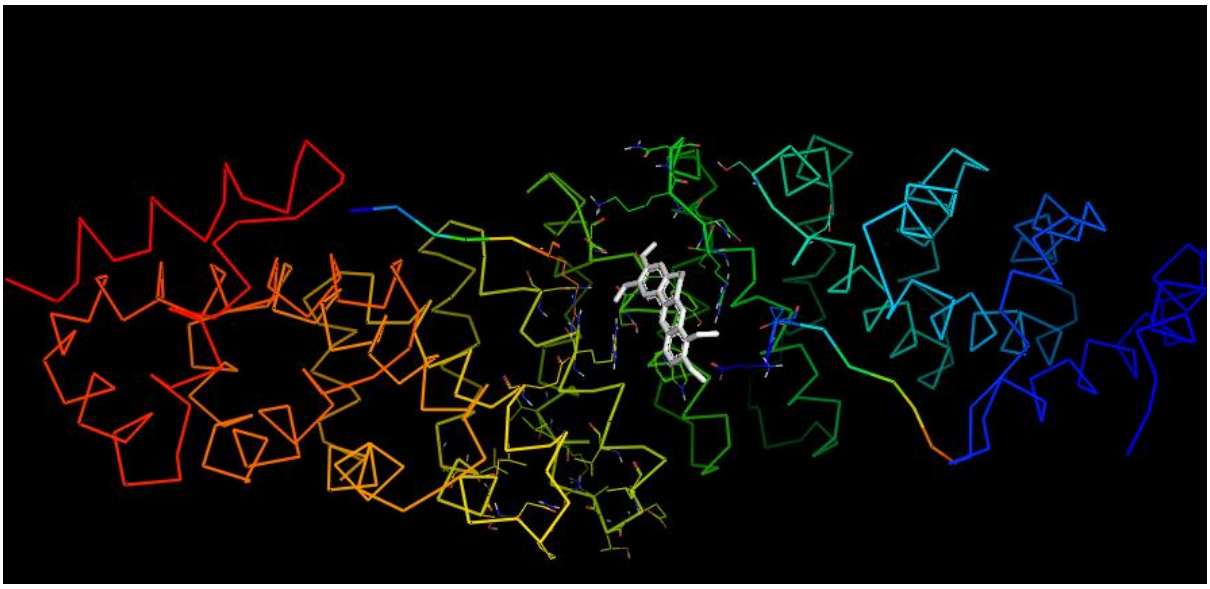


Fig 4.3.10: Palmatine has no interaction with p65

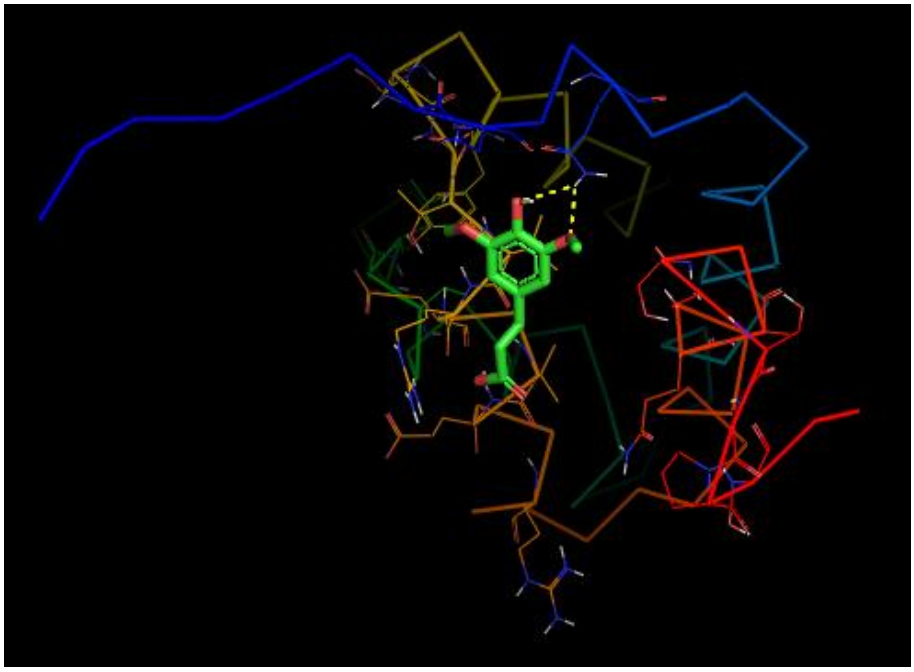


Fig 4.3.11: Sinapic acid interaction with p50- 2 polar bonds are formed

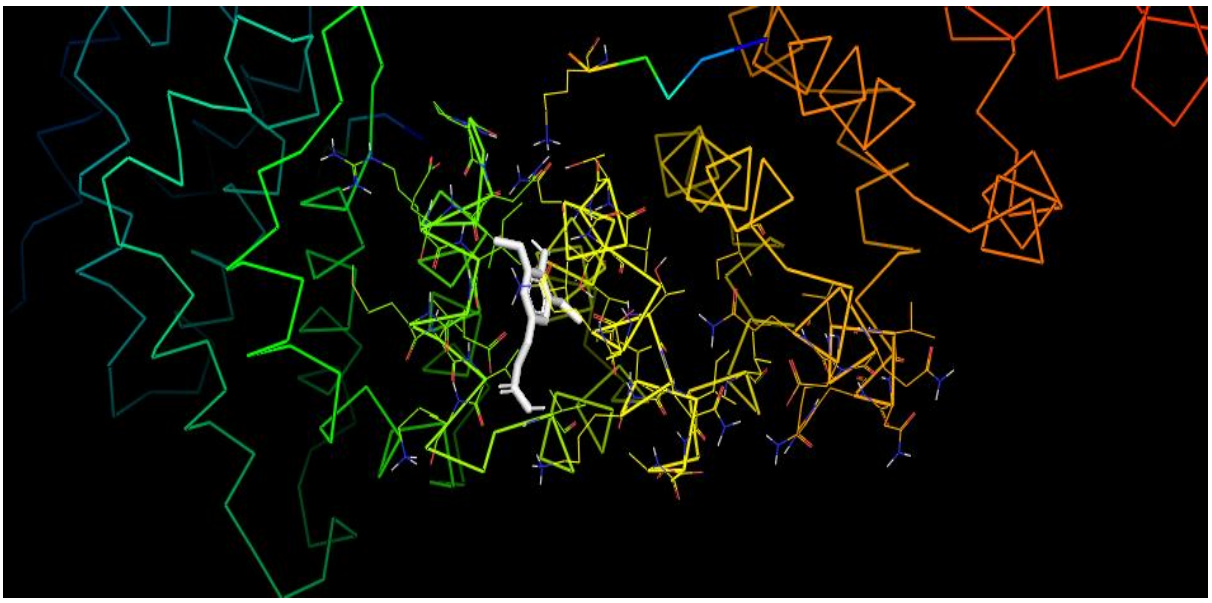


Fig 4.3.12: Sinapic acid has no interaction with p65

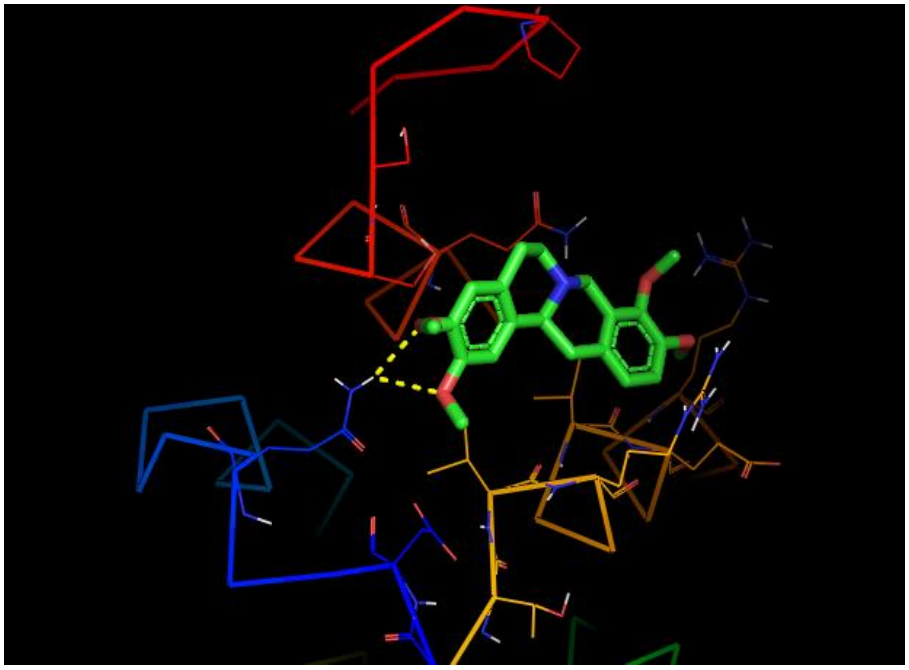


Fig 4.3.13: Tetrahydropalmatine interaction with p50- 2 polar bonds are formed

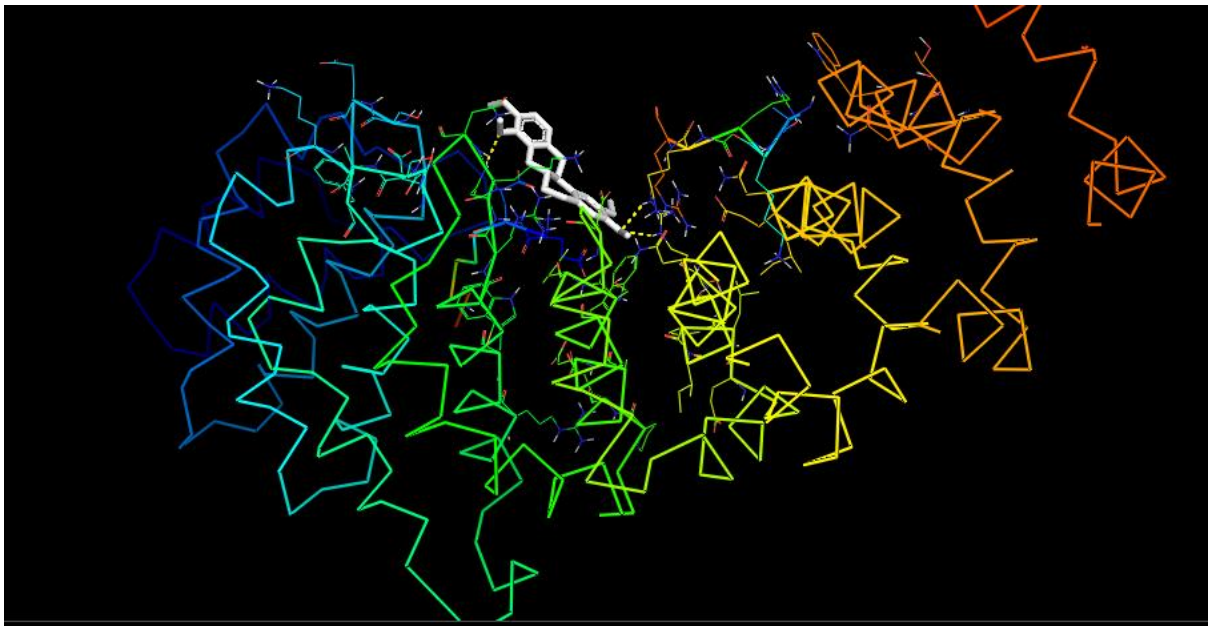


Fig 4.3.14: Tetrahydropalmatine interaction with p65- 3 polar bonds are formed

After ADME analysis, binding affinity score and interaction studies of all the bioactives with NF- kB downregulation potential the best results are found for the Berberine bioactive. It that 3.62 lipophilicity score, -5.78 cm/s skin permeation, 0.55 bioavailability score ,0 violation in drug-likeness and 3 polar bond formation with p50 subunit & 4 polar bond formation with p65 subunit. These analysis prove it a potential drug for NF-kB downregulation and a good alternative for auto-inflammatory disorders.

Table 4.3.2: Binding interaction studies of bioactives

Bioactive	**Interaction with p50 subunit**		**Interaction with p65 subunit**	
	Number of Polar Bonds	**Interacting Amino acid**	**Number of Polar Bonds**	**Interacting Amino acid**
Sinapic Acid	2	Q	0	No interaction
Palmatine	1	Q	0	No interaction
Berberine	3	R,Q	4	R,I,V,S
Magnoflorine	0	No interaction	1	G
Tetrahydropalmatine	2	Q	3	S,G
Jatrorrhizine	2	Q	2	S,G
Columbin	3	R	3	S

CHAPTER 5: CONCLUSION

Tinospora cordifolia (Giloy) is a fantastic Ayurvedic remedy for auto-immune disorders like cancer, rheumatoid arthritis, and Crohn's disease. The bioactives in giloy are the main component that works against inflammation. Out of all the biologically active compounds in Tinospora cordifolia the one's which are responsible for downregulation of major inflammatory pathway NF- κB are Palmatine, Berberine, Magnoflorine, Tetrahydropalmatine, Choline, Jatrorrhizine, Columbin, Clerodane, Ecdysterone, Stigmasterol, β-sitosterol, Syringin, Palmatosides, Furanoid diterpine glucoside ,Octacosanol and Sinapic Acid.

Clerodane, ecdysterone, stigmasterol, octacosanol, palmatosides, and β-sitosterol are the bioactives with ADME violation among all the bioactives discovered with down-regulation potential. As a result of the breaches in the ADME study, they are unsuitable as drug candidates.

Sinapic acid, Palmatine, Berberine, Magnoflorine, Tetrahydropalmatine, Jatrorrhizine, and Columbin were discovered to have Lipo-philicity ranging from -1 to 5, good water solubility, a Bio-availability Score of 0.55, a negative Log Kp (skin permeability) value, high GI absorption, and a 0 Violation.

After docking and interaction studies of potential bioactives that passed the SwissADME test, all of these potential bioactives have negative binding affinity values with the p50 and p65 subunits. However, during interaction experiments using PyMOL, the best interaction combined with binding score was achieved for Berberine. Berberine has the greatest binding affinity of any bioactive, measuring -7.5 kcal/mol for the p65 subunit and -6.6 kcal/mol for the p50 subunit. Berberine interaction revealed three polar interactions with the p50 subunit and four polar binds with the p65 subunit.

Tetrahydropalmatine, Jatrorrhizine and Columbin are the bioactives which too showed interaction with both p50 and p65. These bioactives interaction with p50/p65 heterodimer’s both subunit increases the chances of NF-kB downregulation and also in case only binding with one subunit occurs than also downregulation chances are high.

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