"*Computational Analysis of Therapeutic Potential Of Herb Target Interactions Against Arthritis*"

A DISSERTATION

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

OF

MASTER OF TECHNOLOGY

IN

INDUSTRIAL BIOTECHNOLOGY

Submitted By:

HARSHITA SINGH

2K16/IBT/04

Under the supervision of

Dr. Navneeta Bharadvaja

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi-110042

JUNE 2018

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi- 110042

CANDIDATE'S DECLARATION

I, **Harshita Singh**, Roll No. **2K16/IBT/04**, student of M. Tech Industrial Biotechnology, hereby declare that my project dissertation titled ***"Computational Analysis of Therapeutic Potential Of Herb Target Interactions Against Arthritis"*** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology is an original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any degree, diploma associate ship, fellowship or other similar title or recognition.

Place: Delhi **Harshita Singh**

Date:

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi- 110042

CERTIFICATE

I hereby certify that the project dissertation entitled ***"Computational Analysis of Therapeutic Potential Of Herb Target Interactions Against Arthritis"*** which is submitted by **Harshita Singh (2K16/IBT/04) Department of Biotechnology,** Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi),in the partial fulfilment of the requirement for the reward of the degree of Master of Technology, is an authentic record of the candidate's own work carried out by the student under my supervision. To the best of my knowledge this work has has not been submitted in part or full for any degree or diploma to this university or elsewhere.

Dr. Navneeta Bharadvaja **Prof. Jai Gopal Sharma**

SUPERVISOR **HEAD, DEPARTMENT OF BIOTECHNOLOGY**

ABSTRACT

Arthritis is a joint disease which mainly affects the knee and disables the person to walk due to pain in the joints. This disease is a type of chronic disease in which inflammation of knee causes pain. There are many medicinal plants with therapeutically active compound in them, which has property of anti-inflammatory response. Few important compounds are Lupeol, Arctiin, Tanetin etc., which is anti-inflammatory in response.

Docking of these compounds with a target protein TNF-α, which have an important function in inflammation suggest the result by giving the hydrogen bonding with binding affinity of 15.6 to 6.4 with number of hydrogen bond 6 to 0 in number. Docking has been done by the software 'Autodock Vina'. The more the number of hydrogen bond the better the affinity of target protein and the ligands. The complexes with good hydrogen bonding are the complex, which simulated gives good results. Docking helps to know the interactions of the ligands and the targets so that simulation of the complex is done to know its response to be used for drug designing after its stability in simulation. The simulation is done on Supercomputer at IIT Delhi. Simulation is done by the software GROMACS.

ACKNOWLEDGEMENT

It is beyond my word power to acknowledge for the guidance to those who have cooperated and inspired me during the completion of this project. Still I would like to take this opportunity to express my sincere thanks to all those people who have, in various ways, helped in successful completion of this project. I must express my heartiest gratitude to respected Prof. Jai Gopal Sharma, Head of Department, Department of Biotechnology, Delhi Technological University. I express my gratitude toward Shashank Shekhar Senior Project Scientist ,Supercomputing Facility for Bioinformatics &, Computational Biology, Indian Institute of Technology, Delhi who allowed me to work freely in their department and without which I couldn't have completed my work. I feel immense pleasure in expressing my cordial gratitude towards my supervisor and guide Dr. Navneeta Bharadvaja, Assistant Professor, Department of Biotechnology, Delhi Technological University whose continuous help and encouraging support, fully participation in my work and invaluable suggestions helped me in completing my work and gaining knowledge in the field of research. I feel very proud and fortunate to be counted as one of her student in my career.

Further I would like to thank Nupur Jauhari (PhD. Scholar) Nidhi Katyal (PhD. Scholar), who had helped me in Major report and research work, and have guided me in various field when required.

I would like to thank my friends, my parents especially who helped me during this project work by their encouragement and support.

Harshita Singh

CONTENTS

LIST OF TABLES

LISTS OF GRAPHS

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND OBJECTIVES

1.1 Introduction

Arthritis is a disease of joints. The word arthritis is a Greek word which means joint inflammation. Inflammation affects the tendon and the ligaments. It is a collection of disease namely Osteoarthritis, Rheumatoid arthritis etc. Osteoarthritis is a joint inflammation due to the loss of cartilage in the joint and the surrounding areas of joints. The cartilage top layer is damaged due to which the bones glide over each other and rub each other. This rubbing of bone to each other causes redness, swelling, pain and inflammation. Unlike other type of arthritis, the osteoarthritis affects the knee and the joints not the internal organs. The cartilage cushions the bones in joints wears away, so this is also called wear and tear arthritis. In USA disability due to arthritis is common diseases. The difficulty in walking is due to osteoarthritis which affects the knee and hip and causes difficulty for person to walk (Mikkelsen et al, 1967). Joint, hip and knee are affected. This disease is mostly found in USA. This disease is a old disease as it has been seen in the historical relics of the skeletal part of the Egyptian mummies (Braunstein et al, 1988). This disease is not found only in one of the geographical areas (Roberts et al, 1966).

The next is rheumatoid arthritis, which is a kind of autoimmune disease in which the body own immune system attack the body, in which pain, swelling and inflammation occurs in the joint of knee, hands, wrists, elbow and shoulders occurs. It is a chronic disease of joints (Pedersen et al, 2011). Disability and pain is one of the important features of this rheumatoid arthritis (Verstappen et al, 2013). The shape of the hands and feet misshapen as the tendon moves out, the muscles also weakens. This is a chronic disease, which has affected nearly all the races of the world and is ranking the first or second position in almost all the races. This disease affects about 8% of adults all over the world (Gabriel, 2001) and 0.8% of England (Symmons et al, 2002).

USA is the country where almost every family has arthritis disease like osteoarthritis and rheumatoid arthritis mostly. Fatigue is also caused due to this systemic disease. A report of 2010 by the National Rheumatoid Arthritis Society says that UK economy

productivity cost lost overall due to the rheumatoid arthritis namely about eight billion per year whereas the expenditure by NHS is about to seven hundred million per year. This concludes that this is causing huge economic loss to the country.

Juvenile arthritis occurs as a result of daily fever and low blood counts. This disease also has the impact on heart, lungs, eyes and the nervous system secondarily. Since this disease affects the children of age under or sixteen, so the name derives juvenile arthritis from the children. It is a kind of rheumatoid arthritis. This disease extends from few months in children to whole life in children. This disease represents itself in joint pain and swellings in the joints mainly knee, wrist and ankles. *Ankylosing spondylitis* causes pain and stiffness primarily, which affects the spine. Gout is another type of arthritis, which is caused due to increase in uric acid in the joints. It is painful in knee and joints. Fibromyalgia is an arthritis which causes pain in muscoskeletal part of the body. Fatigue also occurs in this arthritis. Pseudogout is a inflammatory kind of arthritis in which the crystal of calcium pyrophosphate accumulate in the joints of the knee.

Medicinal plants have a property to be used as medicine. These plants are source of infinite property present in them for the different disease. Different components of plants are used for different disease like stem, root, bark etc. from earlier ancient era these medicinal plants were used by the earlier people. Different kinds of drugs are made from the medicinal plants. The forest is a rich medicinal plant heritage. India is considered the richest in the world in terms of the medical tradition practiced over here especially by the people of the rural areas as they are mostly dependent on the medicinal plants for the treatment of disease and prevents from taking drugs and medicine marketed in the markets. (Wakdikar, 2004) They considered medicinal plant as the pure source of medicinal taken directly from the nature. This concept is also applicable to *Ayurveda* as all of their medicine are made from medicinal plants, herbs taken directly from the plant and are effective fastly and natural in nature.

An estimated about 1.5 million practiceners are keen involved in using medicinal plants for treatment of disease. There are about 2000 tones of herbs, which are being used yearly for drug (Ramkrishnappa, 2002). Medicinal plants estimated about 2500000 that are rich in bioactive compound. However, only few of them have been investigated for their diverse presence of vast sources of bioactive compounds (Wakdikar, 2004). About 80% of the world's populations are still using medicinal plants as medicine because

most of them are developing countries are mostly dependent on traditional plants (Hashim et al, 2010). Another reason of using traditional plants for medicine is its cheap cost and easy availability. Some common plants are present everywhere, which is easily accessible to people especially in rural areas.

Docking is a used to find the interaction between two molecules i.e. ligand and protein and the orientation in which the two molecules bind to form a complex molecule determined by their binding energy. Minimum the binding energy maximum the bonding between the two molecules i.e. complex and hence the best docking orientation. The ligand molecule binds with the protein at the active site of the protein through hydrogen bond. Docking helps in finding the best orientation of the binding complex and hence helps in pharmaceutical industry for drug designing. The binding complex is studied by software's such as Pymol, Rasmol etc. The Pymol mention the structure of protein ligand binding complex. It also has the residues to mention. Docking is of two sections i.e Search algorithm: this algorithm is used to find the optimal conformation of the binding complex, which is the orientation and the pose of the binding complex. This also calculates the binding energy of the binding complex interaction. There are different types of algorithm in docking as follows:

Monte Carlo methods

Molecular Dynamics

Genetic Algorithm

Distance Geometry methods

Scoring functions: This is a mathematical method that finds the potential of the non covalent interaction called as the binding affinity after the two molecules I.e. ligand and protein have docked. Empirical docking function of of any docking is:

Fitness =vdW + Hbond + Elec

Binding energy = $\Delta G_{vdw} + \Delta G_{hbond} + \Delta G_{elect} + \Delta G_{tor} + \Delta G_{conform} + \Delta G_{sol}$

There are two types of docking: Lock and or rigid docking: In this type of docking the internal geometry of the protein and the ligand is fixed. Induced fixed or flexible docking: In this type of docking the interaction between ligand and the protein is flexible and binding energy is calculated for the different conformation formed after the

complex bind to have minimum binding energy. Binding energy and the binding orientation are two main calculation for the docking. The docking energy determines the approximation of the minimum binding energy. Binding energy is used by letter Ed.

R+L + RL Eq 1

Where R is the free receptor and L is the free ligand. Ed is an approximation of the binding energy ΔG binding

$$Kd = \exp(\frac{Ed}{RT}) \quad \text{Eq 2}$$

Where R= 1.987* 10^{-3} [kcal mol-1 K-1] and t the fixed absolute temperature

The percentage relative error of the dissociation constant Kd due to docking energy absolute error is δEd =Edi-Edtrue is given by the following the equation.

$$Kd\ \%error = \frac{Kdi-Kdt}{Kdt} x100 = (\exp(\frac{\delta Ed}{RT}) -1) \times 100 \quad \text{Eq 3}$$

Where

- Kdi is the incorrect dissociation constant calculated by incorrect docking energy Edi
- KdT is the true dissociation constant calculated by the true docking energy EdT.
- δEd = Edi-EdT is the total absolute error on the docking energy.

Simulation is the motion of the molecules which is studied by the computer. The atoms and the molecules interact for a period of time and form complex with binding energy. This simulation is studied in physics and biology. It is studied for proteins and biomolecules. There are different types of software for simulations such as AMBER (Brooks et al, 1983) CHARMM (Weiner et al, 1981) NAMD (Nelson et al, 1996) GROMACS (Berendsen et al, 1995). All these software are based on newton's laws of motion for finding the at or the particle at future point in the time.

F= ma Eq 4

Where F is the force, m is the mass and a is the acceleration.

There are many types of algorithm used in the molecular simulations. It is predicted that the forward and backward position of atoms or particles at a time is estimated by Taylor series expansions.

$$r(t + \Delta t) = r(t) + \Delta t \cdot v(t) + \frac{\Delta t^2 \cdot a(t)}{2} + \frac{\Delta t^3 \cdot b(t)}{6} \quad \text{Eq 5}$$

$$r(t - \Delta t) = r(t) - \Delta t \cdot v(t) + \frac{\Delta t^2 \cdot a(t)}{2} - \frac{\Delta t^3 \cdot b(t)}{6} \quad \text{Eq 6}$$

Where, r is the atomic position, t is the time, v is velocity, a is acceleration, and b is rate of change of acceleration. Adding the taylor expansions the atomic positions at $t + \Delta t$ is the verlet algorithm.

$$r(t + \Delta t) = 2r(t) - r(t - \Delta t) + \Delta t^2 a(t) \quad \text{Eq 7}$$

Verlet algorithm is explained by position and accelerations at time t i.e. from $t + \Delta t$ to new time $t - \Delta t$ (Verlet et al, 1967).

Leap frog algorithm: This algorithm is made of two steps like the verlet algorithm equation. (van Gunsteren et al, 1988) firstly the velocity is calculated in the first part of the equation which is later used for the second equation or the leapfrog equation.

$$v\left(t + \frac{\Delta t}{2}\right) = v\left(t - \frac{\Delta t}{2}\right) + [\Delta t.\, a(t)] \quad \text{Eq 8}$$

$$r(t + \Delta t) = r(t) + [\Delta t.\, v\left(t + \frac{\Delta t}{2}\right)] \quad \text{Eq 9}$$

There is absence of Δt^2 which means that this algorithm is more accurate as comparted to the verlet algorithm.

Force field : This is the interactions of the atoms in the molecular simulation.(Ponder et al, 2003) Force field include two types of interactions namely bonded and non bonded interactions which is explained as

$$V_{total} = V_{bonded} + V_{non\ bonded} \quad \text{Eq 10}$$

Where V is the potential energy. Bonded interactions: bonded interactions mainly have four different types of interactions collectively which include: Stretching along the bond (Estr), Bending between the bond (Eb), Planer distortion (Eimproper), Torsion (Et). Stretching along the bond states the energy required for stretching or compressing the

covalent bond. Stretching reconnects the length between the two particles and fluctuation from the equilibrium length. Fluctuation directly coordinates with the length between the particles, greater the fluctuation more the energy required to maintain the equilibrium length. The energy required to stretch or compress the length is stated by the hooks law of ideal spring as follows

$$Estr = \frac{1}{2}ks_{ij}(r_{ij}-r_{ij})^2 \qquad \text{Eq 11}$$

Ebend is the energy required in to bend the from its equilibrium angle θ is same as stretching but only differs in terms of length and angle of equilibrium so also stated by the hooks law of ideal spring with respect to angle as follows

$$Ebend = \frac{1}{2}k_{bijk}(q_{ijk}-qo)^2 \qquad \text{Eq 12}$$

Eimproper is the energy which deforms the planer group of atoms from its equilibrium angle ω which is equal to zero. The hooks law of ideal spring with respect to planer angle is as follows

$$Eimproper = \frac{1}{2}k_{oijkl}(\omega_{ijkl}- \omega o)^2 \qquad \text{Eq 13}$$

Etor is the energy for rotation around the bond. the equation is as follows

$$Etor = \Sigma\ 1.4\ pairs\ K\acute{o}\ (1 - \cos(n\acute{o}) \qquad \text{Eq 14}$$

Two common algorithms that are used by the GROMACS software are verlet and leap frog (Allen et al, 1987).

1.2 Objectives

- To explore potential molecular targets for arthritis.
- Finding novel natural molecule with the potential to inhibit the functional activity of the identified molecular target.
- To get inside into the molecular interactions of pattern of the most potential protein ligand complexes using the approach of molecular dynamic simulations.

CHAPTER 2

REVIEW OF LITERATURE

Rheumatoid arthritis is an autoimmune disease in which the body immune system attacks the own body. Chinese medicinal bioactive compound kirenol from the plant herbs *siegesbeckiae.* This medicinal compound is docked with protein TNF-α, IL 1and IL 6. The auto dock tool is used for docking the compound which is having default parameters for finding the binding energies of the complex. The binding energies from all the binding complex are from -6.75 to -2.68. Among the results IL is having the good binding energy as compared to the other two (Wu et al) lupeol is a triterpene from cabbage, grapes and many medicinal plants. This natural product is binded with human serum albumin and alpha 1 acid glycoprotein has the features of drugs. (Kallubai et al, 2015)

Medicinal plants have medicinal compound. Euphorbiaceae is a family of plant kingdom which is a large family consisting of 7500 species. These plants are flowering plants. *Ricinus communis* is a plant among this euphorbeaceae, which is also known as castor plant is a medicinal plant with great medicinal value. This plant has anti-inflammatory effects. This anti inflammatory effect is due to different kinds of flavonoids, glycosides in this plant. (Jena et al, 2012) Rheumatoid arthritis is an autoimmune disease that has effect on the joint mainly knee. The inflammation that is caused due to rheumatoid arthritis is the cytokine in joints. This cytokine has inflammatory effects. The different kinds of cytokine are TNF-α, IL 1, IL6. TNF-α and IL 1 are the two important compound that is responsible for inflammation in the joints. TNF-α is a kind of pro inflammatory compound that stimulate other signaling pathways for inflammatory response. Curcumin is a medicinal plant which has medicinal compound that is responsible for anti-inflammatory effects. This curcumin stop the inflammation caused by the TNF-α and hence is a anti inflammatory compound. Binding of the curcumin with the TNF-α to have a binding complex gives a good binding energy (Chabib et al, 2017).

TNF-α is an inflammatory compound that is responsible for pain in joints. The binding of TNF-α with triterpene, saponin is done by the auto dock tools. The binding results

gave a good binding affinity with TNF-α. The binding energy were between -7.1 to -9.1. These results gave the conclusion that good affinity means this compound is a good against the inflammatory compound TNF-α. The simulation of the binding complex is done to for 100 ns in computer simulation which gave a good result as the binding complex is stable in the simulation results. These results gave a conclusion that this compound bind with inflammatory compound in body condition in good binding complex. (Oanh et al, 2016)

The rheumatoid arthritis is mainly the degradation of a cartilage which affects the joint of the knee. The binding of EGCG with the bovine β lactoglubulin by the protein ligand docking with auto dock tools. The binding between the two compounds was hydrophobic which was stable due to hydrogen binding (Xuli et al, 2012). The docking of compound β lacto globulin with the ligand Arctiin give the result that Arctiin is stable in the binding site of the β lacto globulin. This results in turn give the result that hydrogen bond is the main bond that is responsible for the binding stability of the complex. This result is further simulated in computer simulation to know the complex binding in the body like condition.

The analysis of simulation give the results that the root mean square deviation of the reached the value of the equilibrium and also fluctuated around the means value of 4000ps. The time evolution of the radius of gyration also gives the result that complex of β lacto globulin and Arctiin around 2500 Pico seconds. The fluctuation of atoms in the computer simulation give the results that the complex of β lacto globulin and Arctiin is stable in and the binding site where the complex is binded is rigid (Khosravi et al, 2014).The dynamic properties include the molecular geometries and energies of the proteins, rate of changes in the configuration, free energies and the motions of the particle (Mcgeagh et al, 2011, Dodson et al, 2008, Grossfield, 2011, Stansfeld, 2011). Hardware and software in the recent have increased the complexity of the structure and the time scale for the simulations (Lindorff-Larsen, 2011, Gumbart et al, 2011, Dror, 2011, Shaw et al, 2010, Sgourakis et al, 2010). Simulation predicts the structure of the proteins and molecules and their interactions with each other for their binding energy. It finds the dynamic properties of the proteins along with the equlilibrium status of the protein (Mcgeagh et al, 2011, Dodson et al, 2008, Grossfield, 2011, Stansfeld, 2011).

People are like a disable person who is unable to walk due to joint pain and therefore is a disability worldwide. People of middle and elder age are the most affected people with arthritis. This disease is also called degenerative joint disease. This disease has affected nearly 27 milliion adults in USA of elder age. This disease is caused due to misuse of anabolic steroid, being overweight which is responsible for joint problems especially in knee. This disease is also affecting the economy of a country as people are not able to walk so retires from their job or leave the job and take rest which in turn affects the gross domestic product (GDP) of the country. In USA the total loss of money due to this arthritis costs 90 billion dollar every year (Leigh et al, 2001) indirectly the loss of income accounts 7 dollar billion.(Ricci et al, 2005). Hip is the another most important joint which is taken into account for this arthritis (Felson et al, 2004.) In 2005, the percentage of muscular problems was 24 % of total all health problems listed. (Muskel et al, 2007) All kinds of races were affected due to this arthritis. (Bremner et al, 1968).

Arthritis sometimes also affects other parts of the body besides these such as lung, nerves and eyes. It is found more in female than in men. This disease has a tendency to occur in any age but is most common at the age above 30 which deteoriate the life of people due to more pain in knee and weakened muscles. This disease has taken in account 1% of the population (kirenol). An estimated of more than 80% of people in Asia are dependent on medicinal plants for their daily life medical needs (Fransworth et al, 1985). About of 1.1 billion population of India comprising of 70% of the India's are using medicinal plants as a medicine.(Vaidya et al, 2007).

Most of the plants contain secondary metabolites as active constituents. One of the oldest book dated about 1000 BC has also described the use of this traditional medicinal plants of about 20000 herbs for medicinal. (Meenal et al, 2010). Medicinal plants are invaluable resource which are getting destructed due to floods, afforestation etc. as a result the flora biodiversity is getting lost due to (Rahman, 1999). Medicinal plants are of great importance. Docking is affected by a number of problems such as instability and due to this problem there is a difference between the binding energy and orientation of the binding complex in docking scores and experimentally determined binding affinity.(Wang et al, 2003).

According to a report by IUCN 34000 plants are on verge of threat (Walter and Gillet, 1998, Verma et al, 2002). Conservationist are looking after only those plants which are

beneficial from the point of food, timber and etc but they are overlooking the aspects of medicinal plants which needs to be conserved (Natesh, 2001).About 80% of the world population are still using medicinal plants as medicine because most of them are developing countries are mostly dependent on traditional plants (Hashim et al, 2010). Another reason of using traditional plants for medicine is its cheap cost and easy availability. Some common plants are present everywhere which is easily accessible to people especially in rural areas.

The first simulation was done for liquid to simulate liquid by the help of hard sphere models. (Rahman, 1964) which was later developed for the modeling of the proteins earlier in the absence of the solvent (McCammon et al, 1977) and later in simple lipid bilayer membrane. (Van der Ploeg at el, 1982).

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Protein Preparation

The structures of proteins in crystallographic forms are obtained from RSCB PDB i.e. the protein database website which is www.rcsb.org. The pdb contains database of structural data of biological macromolecules. It was established by Brookhaven national (BNL). The protein selected is TNF-α. The pdb id for TNF-α is 2az5. The protein with their pdb id is downloaded in pdb format from pdb website. The pdb structures also have some missing information such as formal charges or bond orders. This needs to be added before docking. The structures of the protein are refined before the docking. The refinement includes addition of the hydrogen mainly polar hydrogen, additions of geistger charges.

3.1.1 Addition of Hydrogen

Hydrogen bond is one of the important bonds in docking as it helps in the stabilization of the binding between protein and the ligands. The capacity of the functional groups to form hydrogen bond depends on the hydrogen in proteins. There are choices of addition of polar hydrogen or polar and apolar hydrogen only. After addition of the hydrogen the PDB file is saved in pdbqt format before docking.

3.2 Ligand Preparation

The ligands are the part of docking where ligands bind with the protein for the docking. Hydrogen bonds are between ligands and the proteins. Ligands are taken from NCBI pubchem database. The ligands are downloaded from the website and are downloaded format other than pdb since pdb format is not available. The format is then converted into pdb with the help of open babble software which converts the format to pdb. This pdb is the format for the docking. This pdb ligand is added torsion tree root before docking and then is saved in the format of pdbqt. Both the pdbqt in the same folder format of protein and ligand are saved.

The ligands are structures are open by Pymol viewer. Pymol shows the structures and the residues of the ligands and different structures such as sphere structure, cartoon structures are also present from the Pymol viewer. Ligands selected are as follows:

Table 1: Ligands description

Ligands	Local name	Plants source	PubChem CID	Formula	Compound
Lupeol	Castor oil plants	*Ricinus cmmunis*	259846	$C_{30}H_{50}O$	Triterpenoid
Boswellic acids	Indian olibanum, Dhup, Salai, Salai, Guggul, Shallaki	*Boswellia serrata*	168928	$C_{30}H_{48}O_3$	Triterpene
EGCG(epigallocatechin-3-gallate)	Cháhuā	Tea plants *Camellia sinensis*	65064	$C_{22}H_{18}O_{11}$	Catechin
Arctiin	Greater burdock, Gobō, Edible burdock lappa, Thorny burr, Happy major	*Arctium lappa*	10 0528	$C_{27}H_{34}O_{11}$	Lignin
Gingerol	Ginger	*Zingiber officinale*	442793	$C_{17}H_{26}O_4$	Phenol
Tanetin	Feverfew, Featherfew, Featherfoil, Febrifuge plant, Midsummer daisy, Nosebleed, Wild chamomile, Wild quinine	*Tanacetum parthenium*	10043097	$C_{18}H_{16}O_7$	Flavonoid
Stigmastreol	American ginseng, pinyin, Cantonese Yale, Flower Flag	*Panax quinquefolius*	5280794	$C_{29}H_{48}O$	Phytosterol.
Bicalin	Huang quin	*Scutellaria baicalensis*			Flavone Glycoside.
Ethylcinnamate	Kencur, Aromatic ginger, Sand ginger, Cutcherry, or Resurrection lily	*Kaempferia galanga*	637758	$C_{11}H_{12}O_2$	Cinnamic Acid

The protein is TNF-α a homo tetramer protein which is responsible for inflammation. This protein is activated by macrophages. The primary work of this protein is to regulate the immune cells. Since this protein is a endogenous pyrogen so induce fever and inflammation. This protein binds to two receptors TNFR1 and TNFR2. Binding of TNF to TNFR2 causes a change in the structure of the receptor, which causes dissociation of inhibiting protein SODD. This dissociation helps the protein TRADD to recruits the protein TRAF2 which in turn recruit the IKK.IKBα binds to NFKB to inhibit its going is phosphorylated by IKK, so NFKB causes cell proliferation and inflammation figure 1.

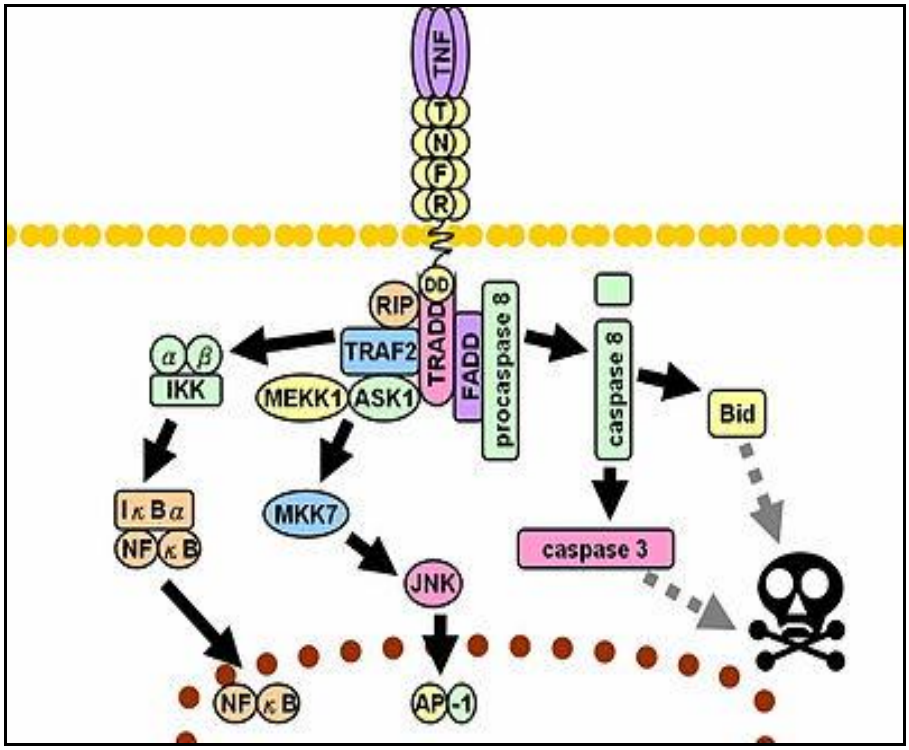


Figure 1: TNF-α pathways

3.3 Docking

The docking of ligand and protein are done with autodock Viena. Autodock vina is a type of open source program for doing docking.(http://vina.scripps.edu) this software was designed by Dr. Oleg trot. The Scripts Research Institute. It improves the accuracy of binding mode predictions as compare to Auto dock 4. Vina uses the pdbqt format which is same as that of autodock 4. Auto grid parameter such as GPF and DPF are not required in autoock Vina which is essential in auto dock 4.It is also faster than auto dock 4 (Trott et al, 2010). It available under apache license.

Docking

Protein and ligand are downloaded from the website and converted into their pdb format. Both the files are kept in the same folder. The autodock 1.5.6 version of the software is downloaded along with mgl tools and autodock vina tools. Autodock is opened and the protein is read, it is added with hydrogen of polar hydrogen and kollomon charges are added. It is saved in the format of pdbqt which is saved in the same folder. Ligand is opened and torsion tree root is added and is saved in pdbqt format. The ligand is also saved in the same folder. The string is selected from the string where active site of the protein id is mentioned and added. The display option adds the name and number of the protein. The name and number are added. The structure of the protein shows the yellow colour plus sign which shows the active site of the residue. This is the residue which is included in the grid box.

The grid box is selected which include the active site of the protein in the box with the help of mouse. The X, Y and Z coordinate changes with the adjustment of the grid box. The grid box is saved with save current option. The X, Y and Z coordinates of the grid box is added in a conf file which is a text file. This file is saved in the same folder. This text file also has the name of the ligand and the receptor in the pdb format.

The cmd window is opened. The path of the file is mentioned in the cmd window. The conf file is also added. The cmd window has the text for the docking The docking is done in 10 to 16 minutes and the affinity of the binding complex is saved in log file in the same format. A ligandout file is also saved in the same folder. This ligand out file is saved after the docking.

The pdb file of the protein is opened in Pymol viewer and the ligand out file is also opened in the same Pymol viewer. The molecule is saved by export molecule in pdb format. The ligplot of the pdb is obtained from the website pdbsum generate. The ligplot mention the hydrogen bond between the ligand and the protein.

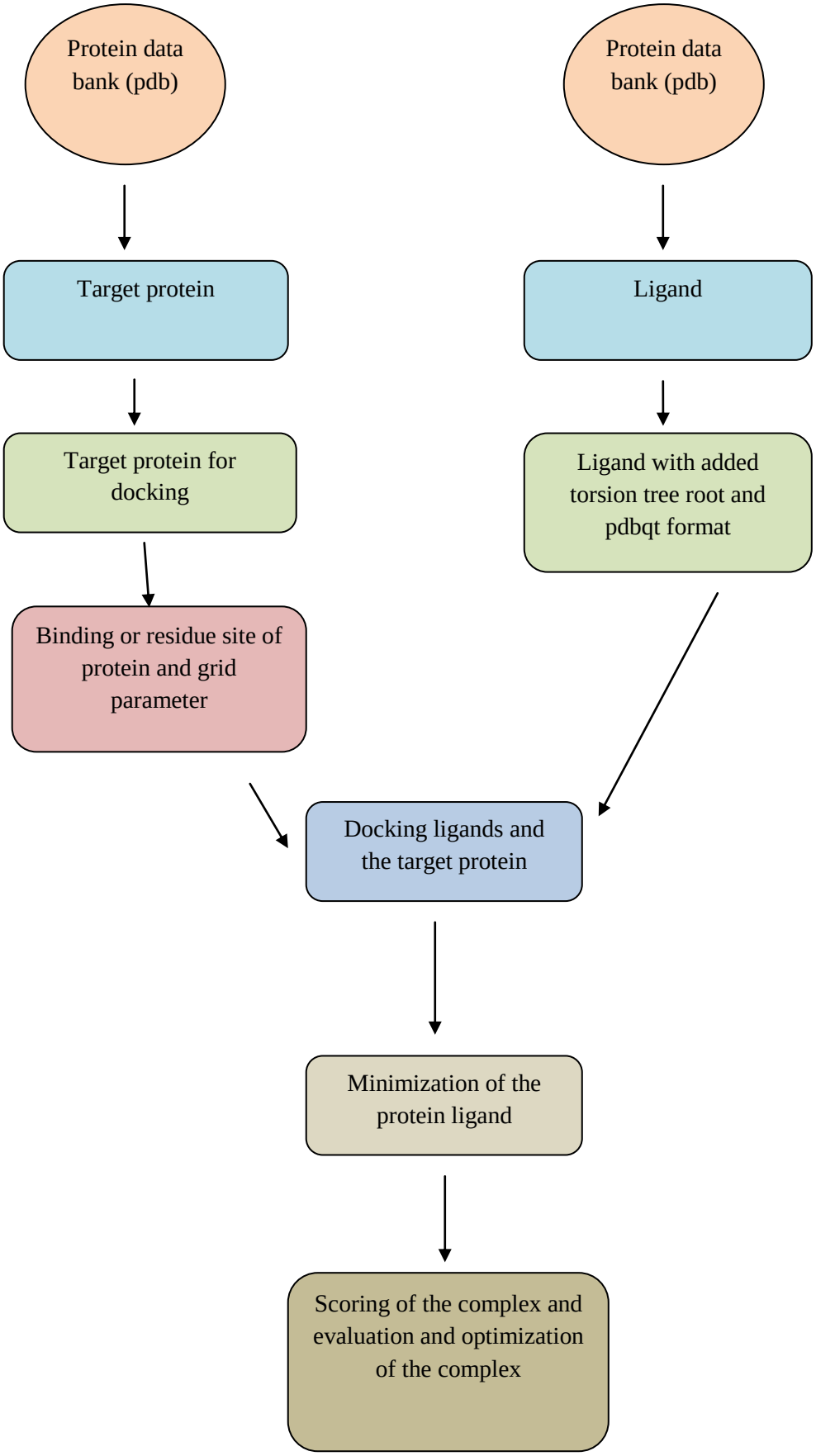


Figure 2: Flowchart of ligand protein docking using Autodock vina

3.4 Simulation

The simulation of protein lysozyme in water with PDB ID 1aki.

GROMACS is the software in which simulation is done from the server of SCFBIO IIT Delhi .Access from SCFBio supercomputer (Linux) 3rd floor, Synergy building, IIT Delhi, Hauz Khas, New Delhi.

The infrastructure is as follows:

The resources in IIT are as follows:. 328 core AMD cluster with solaris operating system over a Infiniband switch. 288 core AMD cluster on Linux operating system over a infiniband switch. There is 104 Processor AMD cluster over a Gigabit switch. There is also a 16 node P4 processor clusters. Also have a Four Sun Fire V20z Server.200 Tera Bytes of Parallel File System based Storage. There is also a 2 X 40 KVA UPS. 30 Mbps dedicated internet connectivity on a optical fiber link. The aggregate compute power of the facility is over 15 Tflops and the data storage capacity is over 200 Terabytes. Procurement of another 70 Tera Flops is in process and will be implemented by August, 2018 (IIT DELHI 29). The local or remote computer is connected by the server computer of supercomputer of IIT by the putty software which is given in figure 3.

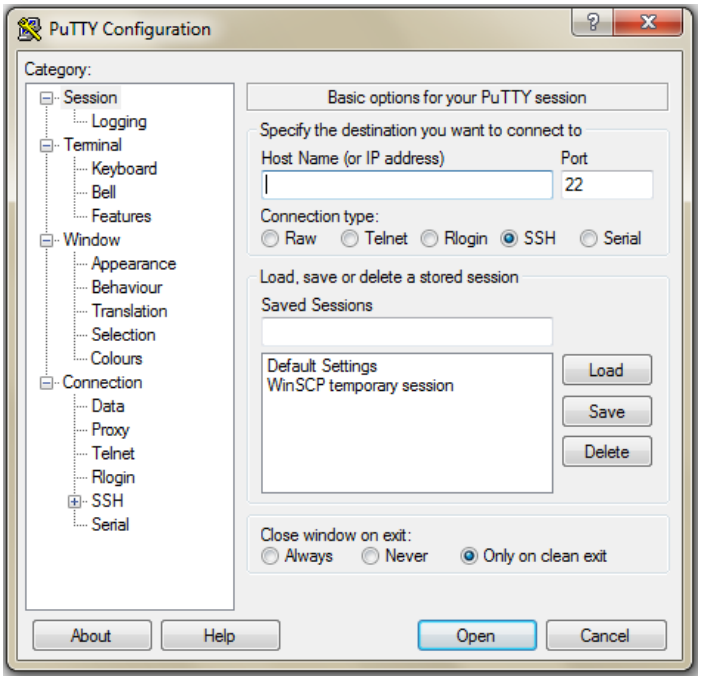


Figure 3: Putty Software

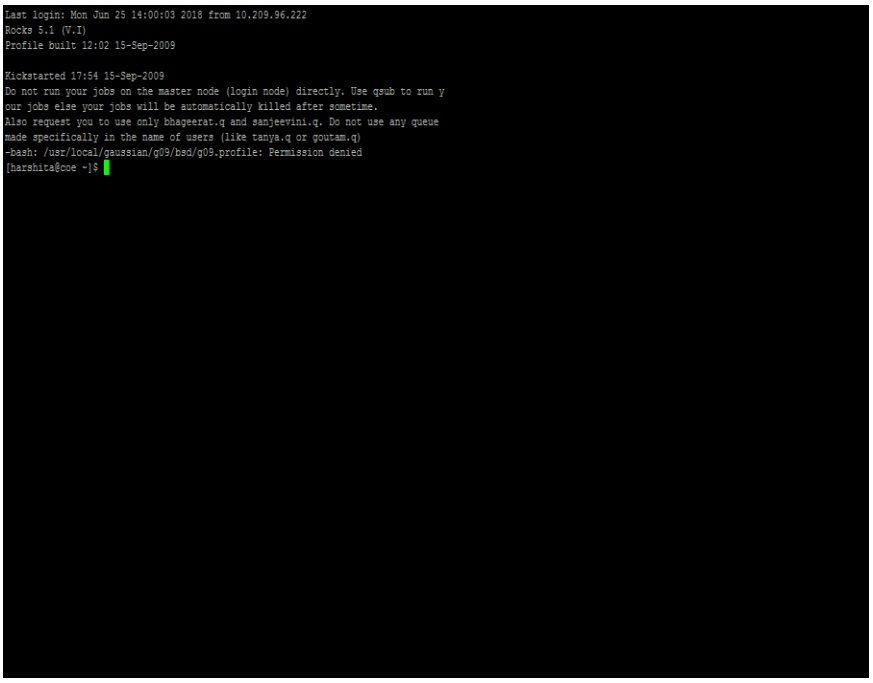


Figure 4: The server of supercomputer where commands are done

(After connecting the local to server the following comes on the windows as follows)

There are different kinds of commands which are done on supercomputer for simulations are as follows: For changing the directories: cd puts the users in the home directory of the users. The directory name as argument, the command gone to that directory is $>cd directory path. Copy files: There are two ways in the cp makes the copy of the files is $>cp file1 file2. According to this command a new copy of file 1 is done and is named as file 2is $>cp [list of files] directory .This command put all the files listed into the directory named. The mv command renames a file. Making a link: These command link two files are $>ln –s /usr/include incl. Make a new directory This makes a subdirectory in the directory. The command is $>mkdir directory name. Remove files: rm removes each files in a list from the directory. By default option-i or rm inquires whether each files to be removed or not. –rm options delete the directories along with files and directories present. The command is $>rm filename.

Remove directory: This removes an empty directory from the current directory. The command is $>rmdir directory name. This removes the subdirectory named directory name. Listing files and directories. ls list the files in the directory. The command is ls -a [directory]. List all the files. The command is ls – c [directory]. List files by date of creation. The command is ls -l [directory]. Lists files in long forms: links the owner,

size, date, name. The command is ls -p[directory]. List subdirectories: the command is ls –s[directory] this Lists the size of the files in the blocks.

File uploading: File from local host to remote host. The command is $>put filename. File downloading: File from remote hosts to local hosts. The command is $>get filename. Closing the connection. The command is $>bye. Compiling programs: The compilers are C(cc) whose command is $>cc source.c. For C++ (CC), the command is $>cc source.cpp. FORTRAN 90(f90) whose command is $>f90 source.f. For FORTRAN 77 (f77) the command is $>70 source.f. For JAVA, the command is $>javac source.java. From all the compilers a.out file is executed. The java executes a byte code source class. Running the programs $>./a.out .From java $>java source.class. Submitting the job the command is $>qsub submit.sh.

Simulation Steps in Water

1 Generation of topology

2 Defining box and solvate

3 Adding ions

4 Energy Minimization

5 Equilibrium

6 Production simulation

7 Analysis

Generation of Topology

The protein topology has all the information about the molecule for the simulation. The topology has bonded and non bonded parameters where the bonded include atom types and charges whereas the non bonded include bond, angles and the dihedrals. Topology is executed by the command: gmx pdb2gmx –f protein name _clean.pdb –o protein processed. gro –water spce after this execution of command, force field is asked to select. There are two steps in defining of box and filling the box with solvent a defining of the box dimensions by editconf module. b Filling the box with water by solvate module. The command for editconf module is as follows: gmx editconf -f protein_processed.gro –o protein_newbox.gro –c –d 1.0 –bt cubic. For filling the box with solvent the command is as follows: gmx solvate –cp protein_newbox.gro –cs

spc216.gro –o protein_solv.gro –p topol.top .tpr file is assembled by following commands gmx grompp –f ions.mdp –c protein_solv.gro –p topol.top –o ions.tpr. gmx genion –s ions.tpr –o protein_solv_ions.gro –p topol.top –pname NA –nname CL –neutral. The binary input is assembled by command as follows : gmx grompp –f minim.mdp –c protein_solv_ions.gro –p topol.top –o em.tpr The mdrun command for energy minimization is as follows : gmx mdrun –v –deffnm em. The energy module command is as follows: gmx energy –f em.edr –o potential.xvg.

The command for equilibrium is gmx grompp –f nvt.mdp –c em.gro –r em.gro –p topol.top –o nvt.tpr. Next is gmx mdrun –deffnm nvt. The temperature module command graph is given. Final output of nvt is as follows: gmx grompp –f npt.mdp –c nvt.gro –r nvt.gro –t nvt.cpt -p topol.top –o nvt.tpr. Next is gmx mdrun –deffnm npt. The pressure module command is as follows: gmx energy –f npt.edr –o pressure.xvg. The command for density module is as follows: gmx energy –f npt.edr –o density.xvg. The command for running 1 ns simulation is gmx grompp –f md.mdp –c npt.gro –t npt.cpt –p topol.top –o md_θ_1.tpr. The command for execution of mdrun is as follows:

- gmx mdrun –deffnm md_θ_1
- gmx trjconv –s md_θ_1.tpr –f md_θ_1.xtc _o md_θ_1_noPBC.xtc –pbc mol –center
- gmx rms –s md_θ_1.tpr –f md_θ_1_noPBC.xtc –o rmsd.xvg –tu ns
- gmx rms –s em.tpr –f md_θ_noPBC.xtc –o rmsd_xtal.xvg –tu ns

  gmx gyrate –s md_θ_1.tpr –f md_θ_1_noPBC.xtc –o gyrate.xvg

CHAPTER 4

RESULTS AND DISCUSSION

Results :

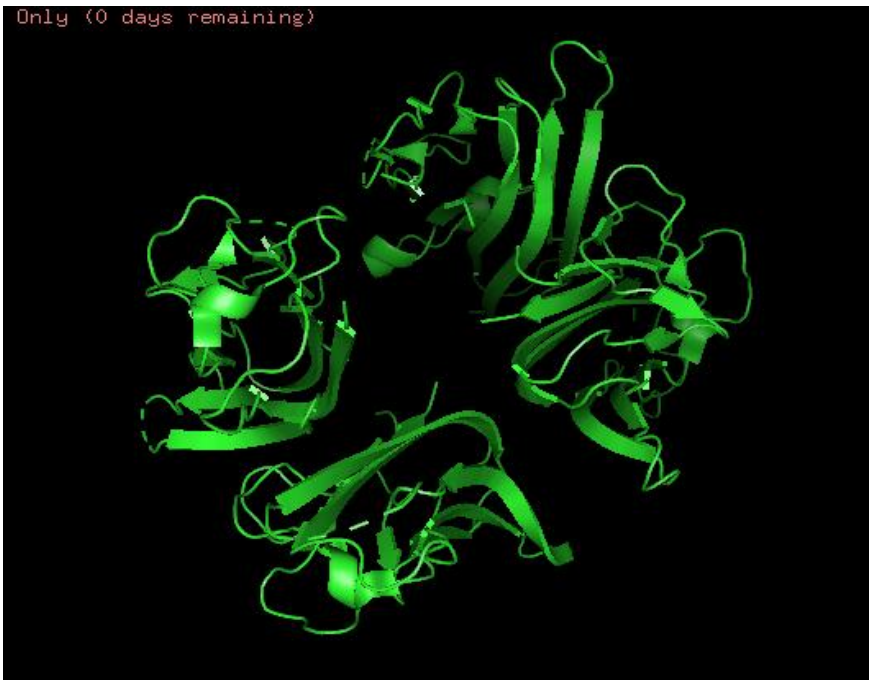


Figure 5: 3D Structure of TNF-α

TNF-α lupeol complex

Binding Energy: 15.6 (kcal/mol)

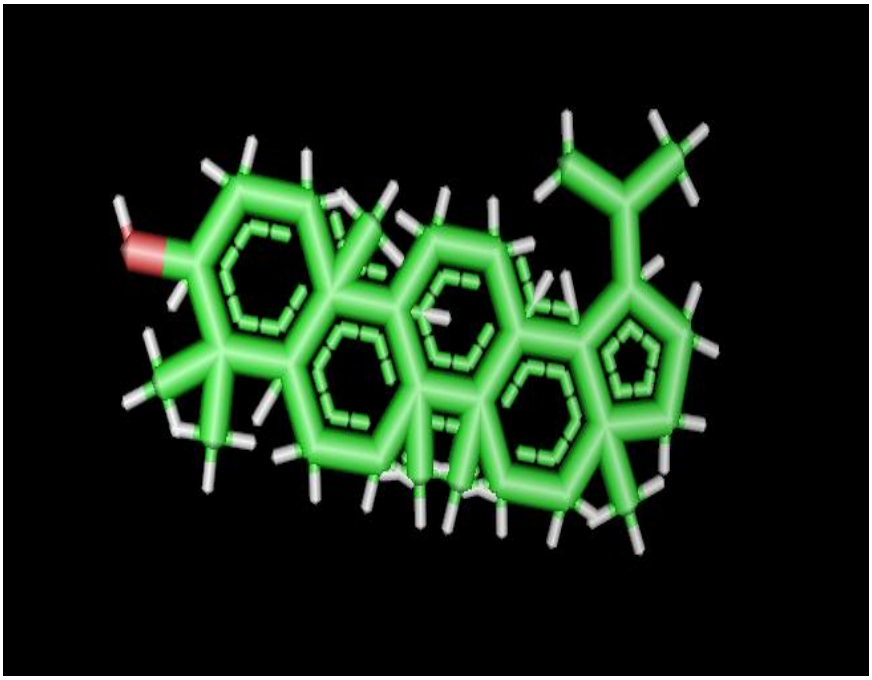


Figure 6: 3D Structure of Lupeol

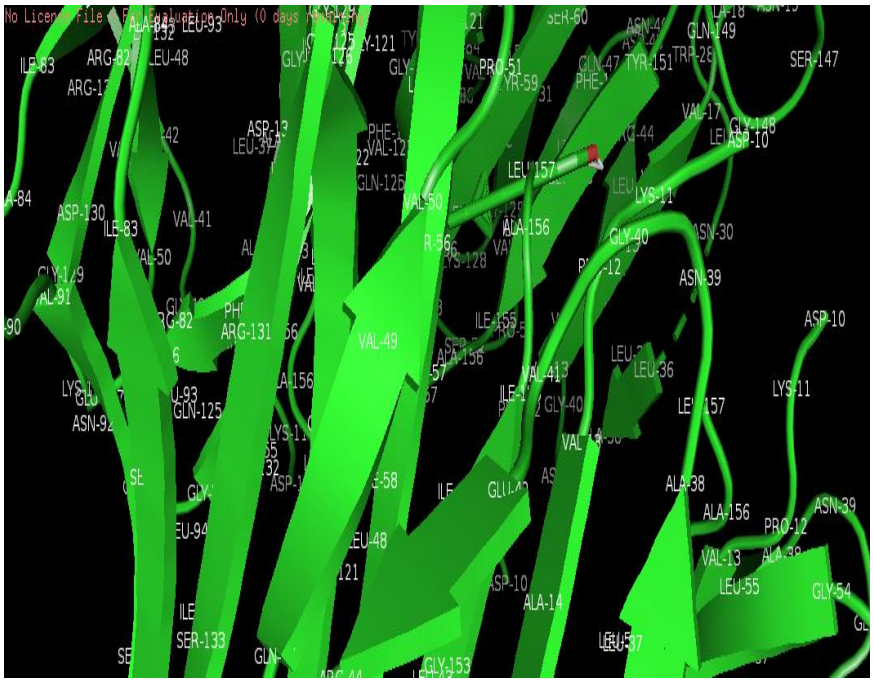


Figure 7: Docking of Lupeol with TNF-α

Table 2: Hydrogen Bonds of TNF-α lupeol complex

A T O M 1					A T O M 2				
Atom No.	**Atom Name**	**Res Name**	**Res No.**	**Chain**	**Atom No.**	**Atom Name**	**Res Name**	**Res No.**	**Distance**
976	OH	TYR	151	A	4451	O	UNL	1	2.70

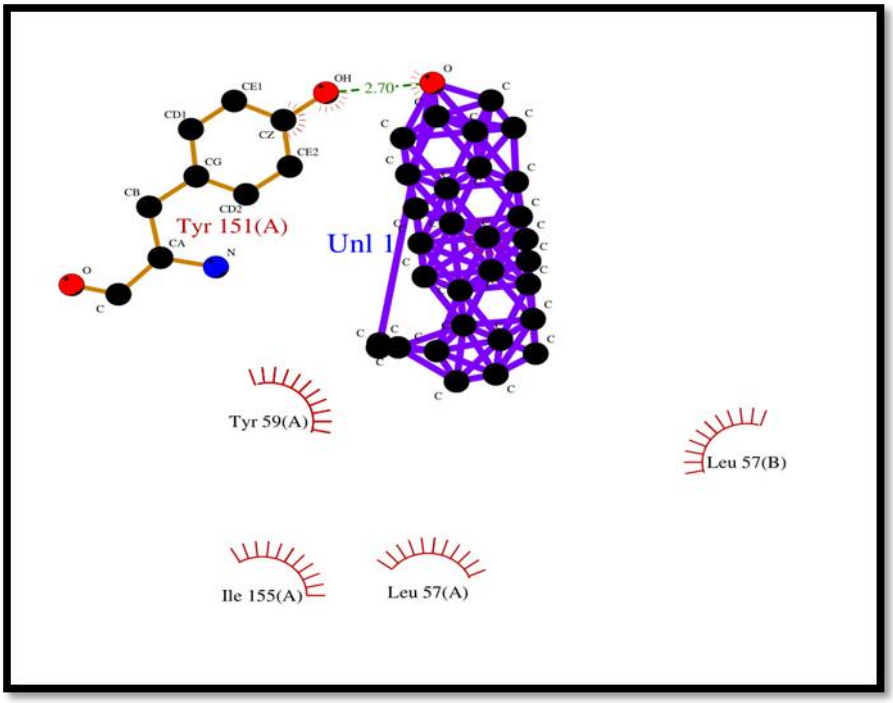


Figure 8: Ligplot figure of interaction between Lupeol 1 and TNF-α

Discussion

The overall structure of the docking of Lupeol 1 and TNF-α is given in the figure 7 ,8 where Lupeol bind with active site 151 with the hydrogen bonding. The number of hydrogen bonding formed is 1 with residue TYR with the Lupeol 1 at the distance of 2.70 with chain A of the protein .The docking figure is given in Pymol Viewer.

Nine different conformations with poses and binding energy were generated but the top ranked complex with the binding score is given in figure. Daisy et al reported that Lupeol with energy value of 92.765 with one hydrogen bond formed with autolysin with bond length 1.9505 (Daisy et al, 2011). Hyun et al Et al reported that Lupeol bind with BACE 1 with the binding energy -8.2 (kcal/mol) and the number of hydrogen bonds formed are 2 in number. Fernandez et al reported that the anti-inflammatory activity of Lupeol is linked with the neutrophils with gets reduced in the inflamed tissues.

TNF-α Boswellic Acids Complex

Binding Energy: 9.2 (kcal/mol)

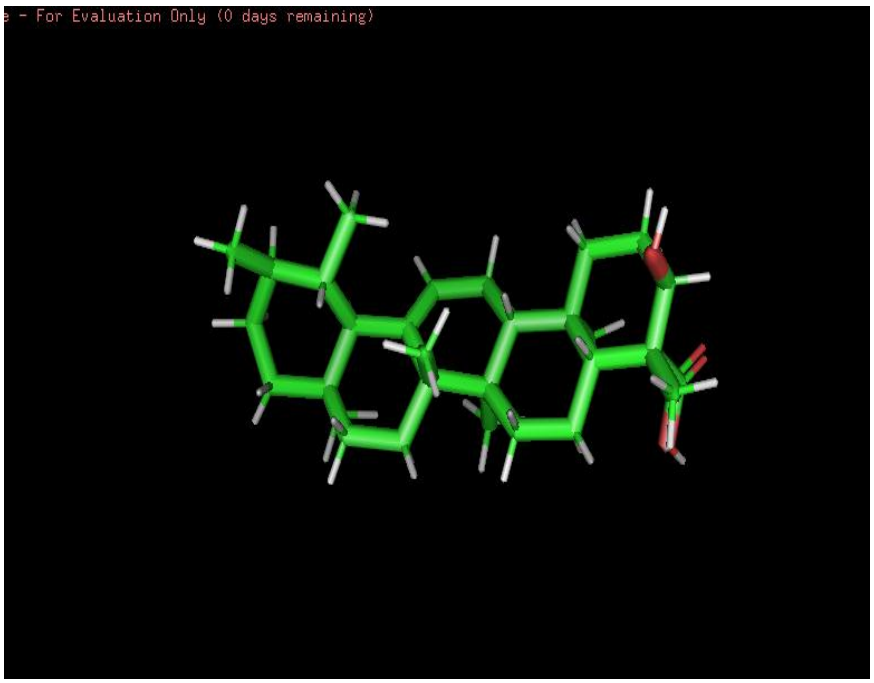


Figure 9: 3D Structure of Boswellic Acids

Figure 10: Docking of Boswellic acids with TNF-α

Table 3: Hydrogen Bonds of TNF-α Boswellic Acids Complex

A T O M 1					A T O M 2				
Atom No.	**Atom Name**	**Res Name**	**Res No.**	**Chain**	**Atom No.**	**Atom Name**	**Res Name**	**Res No.**	**Distance**
956	NEG2	GLN	149	A	4451	O	UNL	1	3.28

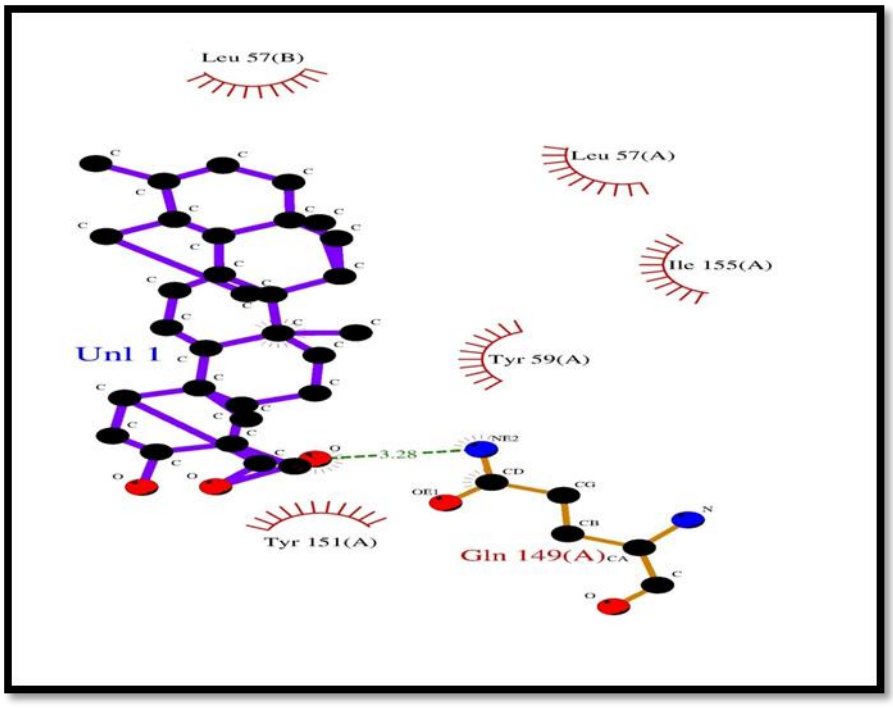


Figure 11: Ligplot figure of interaction between Boswellic acids and TNF-α

The overall structure of the docking of Boswellic acid and TNF-α is given in the figure 10,11 where Boswellic acids bind with active site 149 with the hydrogen bonding. The number of hydrogen bonding formed is 1 with residue Gln with the Boswellic acid at the distance of 3.28 with chain A of the protein .The docking figure is given in Pymol Viewer.

Nine different conformations with poses and binding energy were generated but the top ranked complex with the binding score is given in figure. Ragunath et al reported the binding energy of -9.67, -9.5and -9.36 (kcal/mol) with the number of hydrogen bonds formed are two in number. Satpathy.et al reported the lowest free energy of -7.49 (kcal/mol) from the four different derivatives.

TNF-α EGCG complex

Binding Energy: 7 (kcal/mol)

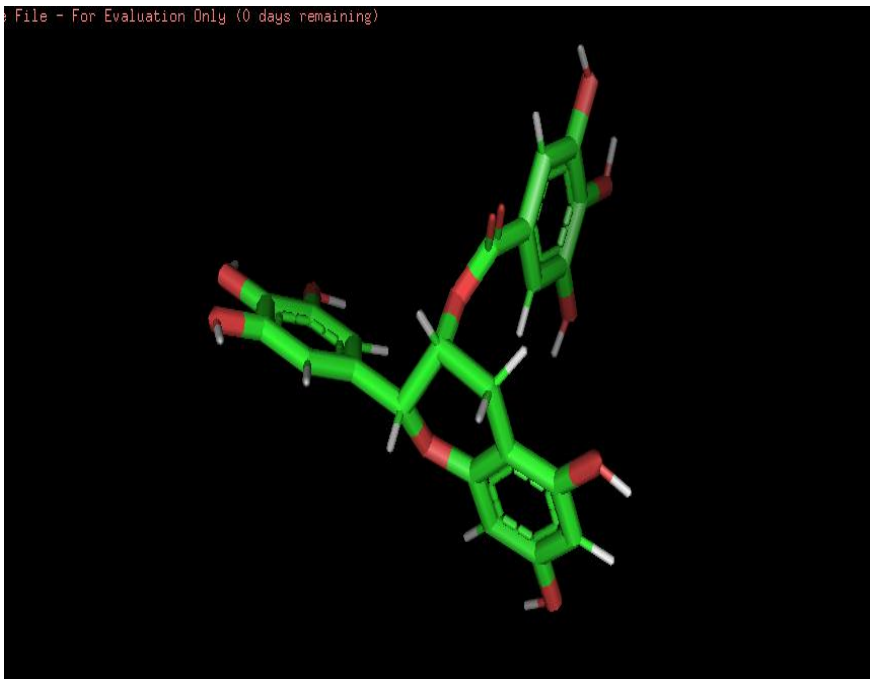


Figure 12: 3D Structure of EGCG

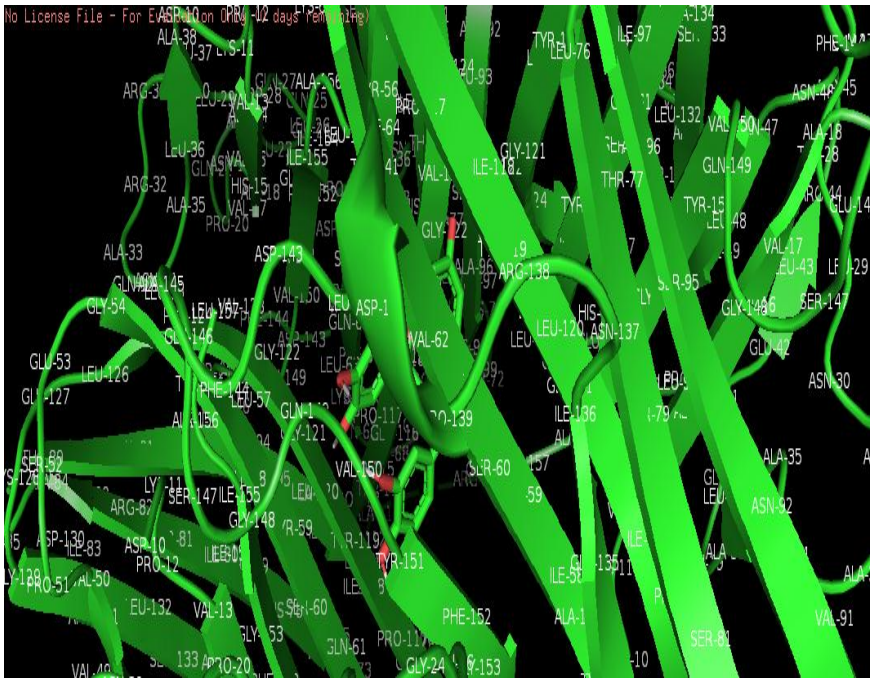


Figure 13: Docking of EGCG with TNF-α

Table 4: Hydrogen Bonds of TNF-α EGCG complex

A T O M 1					A T O M 2				
Atom No.	**Atom Name**	**Res Name**	**Res No.**	**Chain**	**Atom No.**	**Atom Name**	**Res Name**	**Res No.**	**Distance**
976	OH	TYR	151	A	4434	O	UNL	1	2.82

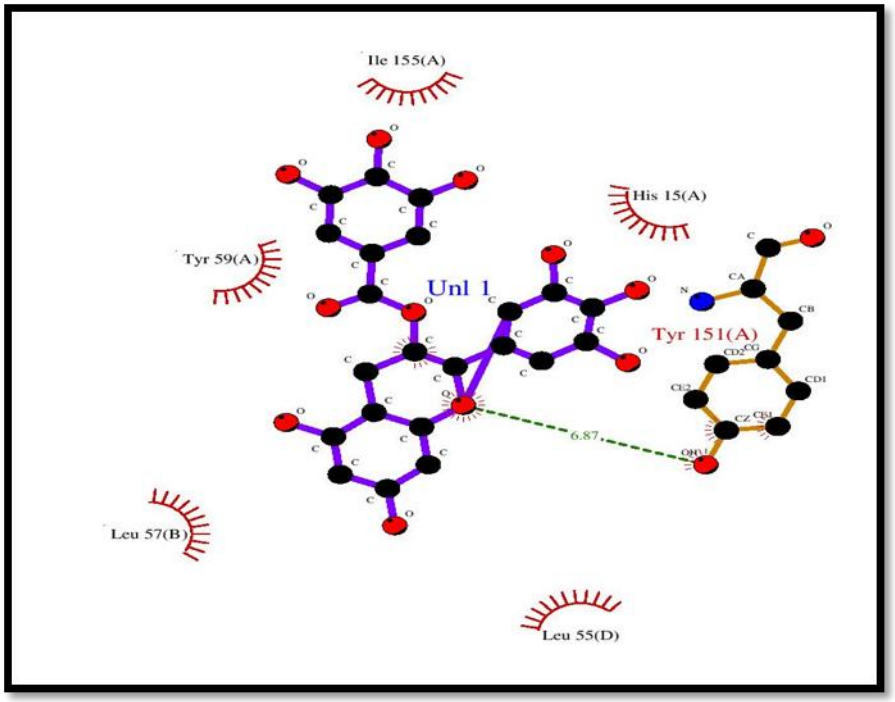


Figure 14: Ligplot figure of interaction between EGCG and TNF-α

The overall structure of the docking EGCG and TNF-α is given in the figure13,14 where EGCG with active site 151 with the hydrogen bonding. The number of hydrogen bonding formed is 1 with residue Tyr with the EGCG at the distance of 6.87 with chain A of the protein .The docking figure is given in Pymol Viewer.

Nine different conformations with poses and binding energy were generated but the top ranked complex with the binding score is given in figure. Fetcher et found that the green tea consists of cathechin which is mainly EGCG interfere with the signaling pathways interleukin 1β which is responsible for pro inflammatory response. Fechtner et al reported that green tea consumption have an anti-inflammatory effect. This EGCG is considered to be the most active substance according to a study (Djerir et al). Riegsecker et al reported that ECEG is an anti inflammatory compound found in the green tea. Binding of EGCG With IL 1 gives a score of -2.46 to -0.13 (kcal/mol) with the binding residues Glu 50, Asp 49, Arg 34 and Asn 47 with the interaction distance of 2.57 to 3.57.

TNF-α Arctiin complex

Binding Energy: 7.7 (kcal/mol)

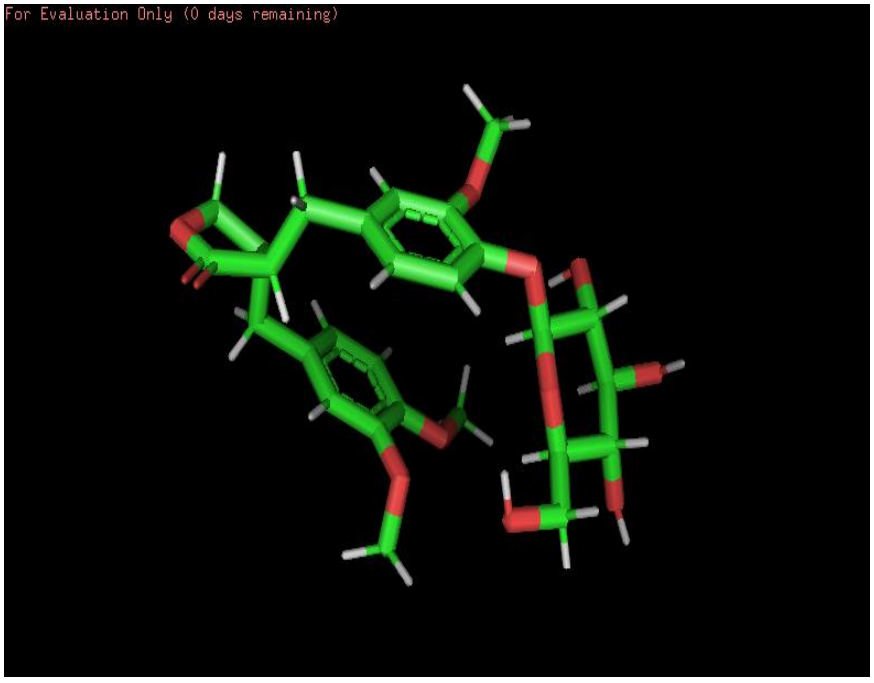


Figure 15: 3D Structure of Arctiin

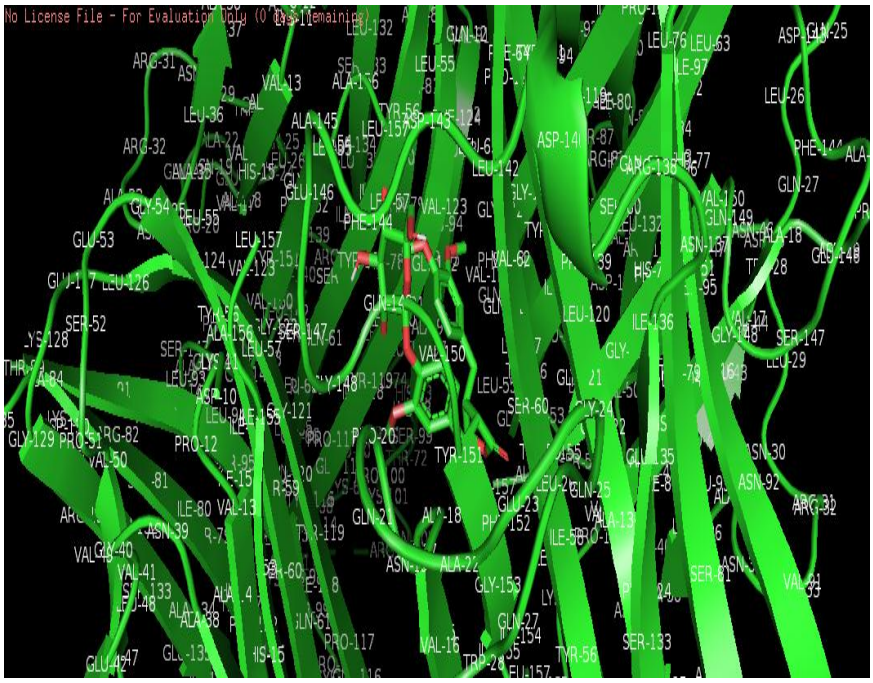


Figure16: Docking of Arctiin with TNF-α

Table 5: Hydrogen Bonds of TNF-α Arctiin complex

A T O M 1					A T O M 2				
Atom No.	**Atom Name**	**Res Name**	**Res No.**	**Chain**	**Atom No.**	**Atom Name**	**Res Name**	**Res No.**	**Distance**
363	NE2	GLN	61	A	4448	O	UNL	1	3.15
976	OH	TYR	151	A	4448	O	UNL	1	2.85
976	OH	TYR	151	A	4448	O	UNL	1	3.10

The overall structure of the docking Arctiin and TNF-α is given in the figure where arctiin with active site 151 with the hydrogen bonding. The number of hydrogen bonding formed is 3 with residue Tyr and Gln with the arctiin at the distance of 3.15, 2.85 and 3.10 with chain A of the protein .The docking figure is given in Pymol Viewer.

Nine different conformations with poses and binding energy were generated but the top ranked complex with the binding score is given in figure. Arctiin have the docking score of -96.59 with the TNF-α hydrogen bond having the value of -8.78 (kcal/mol) and score of -135.2 (kcal/mol) with the IL 1 with hydrogen bond having the value of -4 (Xu et al, 2018). Arctiin reduce the inflammatory mediators (Knipping, 2008). Artciin reported for decrease in collagen which in turn decrease the concentration of IL 6 and TNF-α (Knott, 2008).

TNF-α Gingerol complex

Binding Energy: 6.4 (kcal/mol)

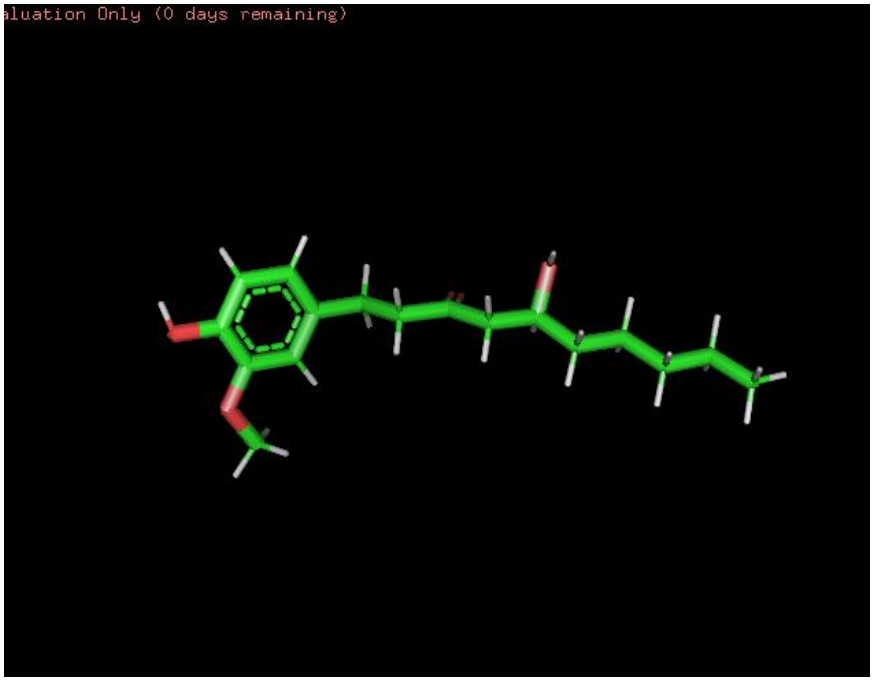


Figure 17: 3D Structure of Gingerol

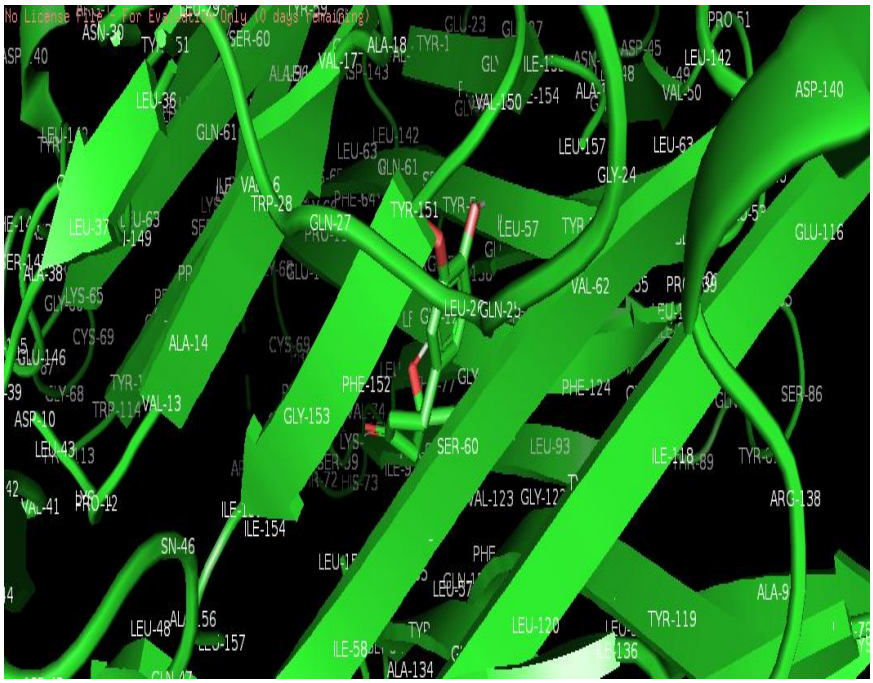


Figure 18: Docking of Gingerol with TNF-α

Table 6: Hydrogen Bonds of TNF-α Gingerol complex

A T O M 1					A T O M 2				
Atom No.	**Atom Name**	**Res Name**	**Res No.**	**Chain**	**Atom No.**	**Atom Name**	**Res Name**	**Res No.**	**Distance**
976	OH	TYR	151	A	4198	O	UNL	1	3.04

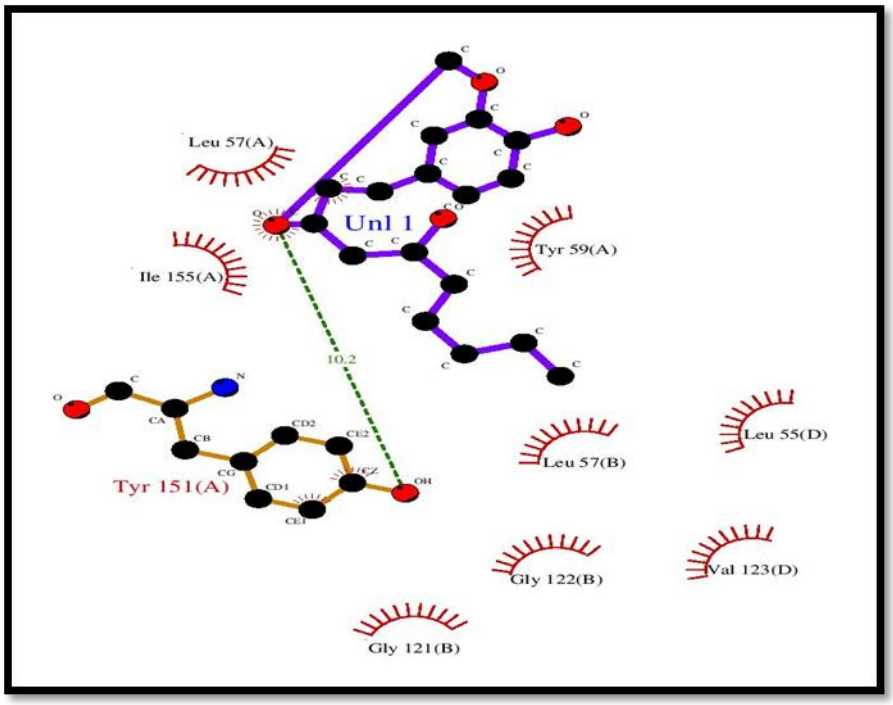


Figure 19: Ligplot figure of interaction between Gingerol and TNF-α

The overall structure of the docking Gingerol and TNF-α is given in the figure18,19 where Gingerol with active site 151 with the hydrogen bonding. The number of hydrogen bonding formed is 3 with residue Tyr with the Gingerol at the distance of 10.2 with chain A of the protein .The docking figure is given in Pymol Viewer.

Nine different conformations with poses and binding energy were generated but the top ranked complex with the binding score is given in figure. Gingerol have an anti-inflammatory effects is good for arthritis as have an additive joint protective effects in the arthritis.(Funk et al, 2016) all the types of gingerols have an anti-inflammatory effects (Jolad et al, 2004). The binding energy of -7.92 (kcal/mol) explains that this compound is good for docking and binded with some other protein. (Rampogu et al, 2018) ginger has active compound for arthritis which is Gingerol.

TNF-α bicalin complex

Binding Energy: -8.4 (kcal/mol)

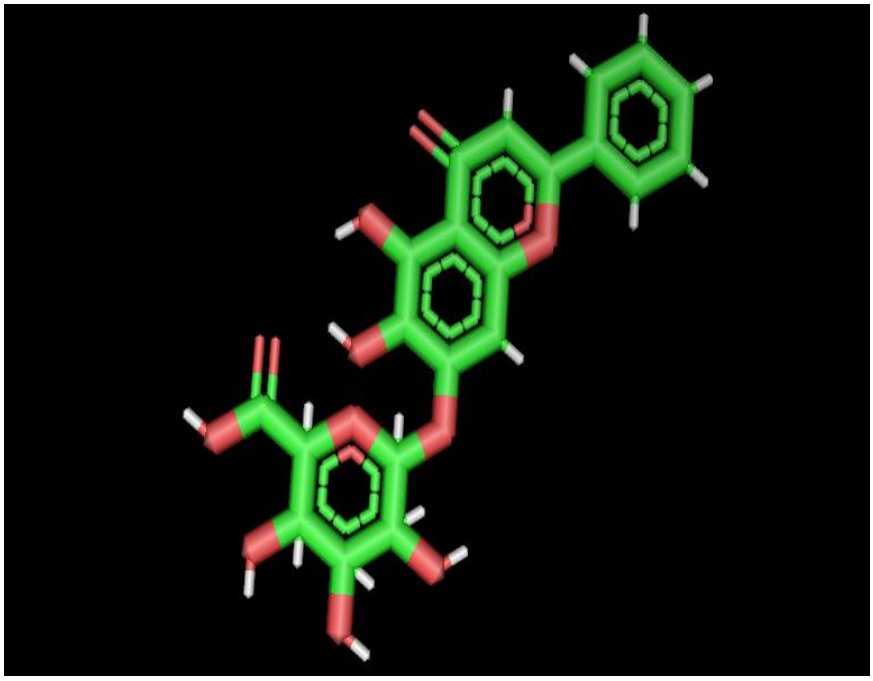


Figure 20: 3D Structure of bicalin

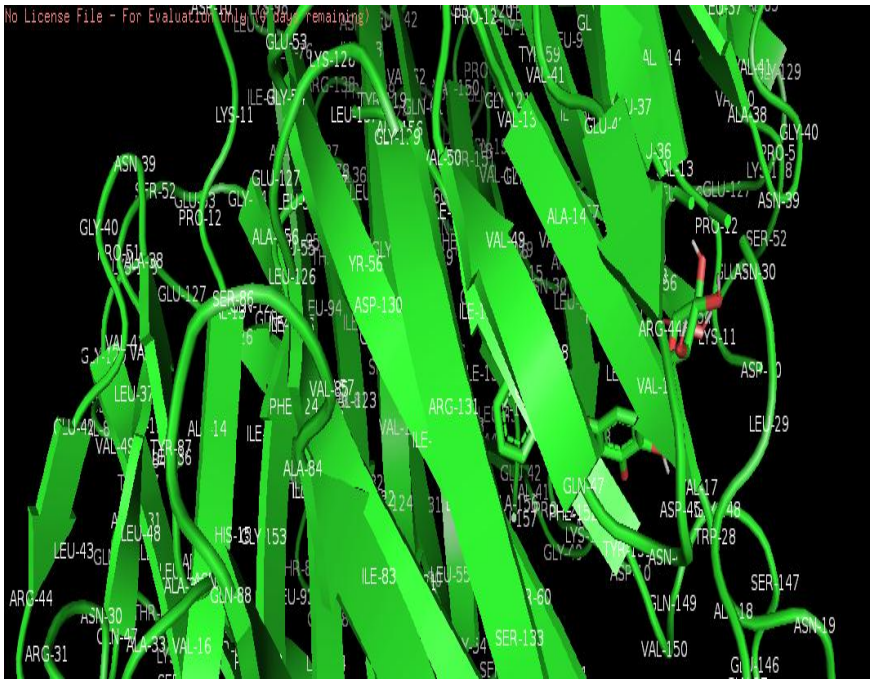


Figure 21: Docking of bicalin with TNF-α

Table 7: Hydrogen Bonds of TNF-α bicalin complex

ATOM 1					ATOM 2				
Atom No.	Atom Name	Res Name	Res No.	Chain	Atom No.	Atom Name	Res Name	Res No.	Distance
2340	N	ASP	10	C	22	O	UNL	1	3.30
2345	N	LYS	11	C	22	O	UNL	1	3.18
2527	ND2	ASN	39	C	22	O	UNL	1	3.08
2527	ND2	ASN	39	C	22	O	UNL	1	3.22
1034	NE2	GLN	149	A	22	O	UNL	1	2.97
1034	NE2	GLN	149	A	22	O	UNL	1	3.06

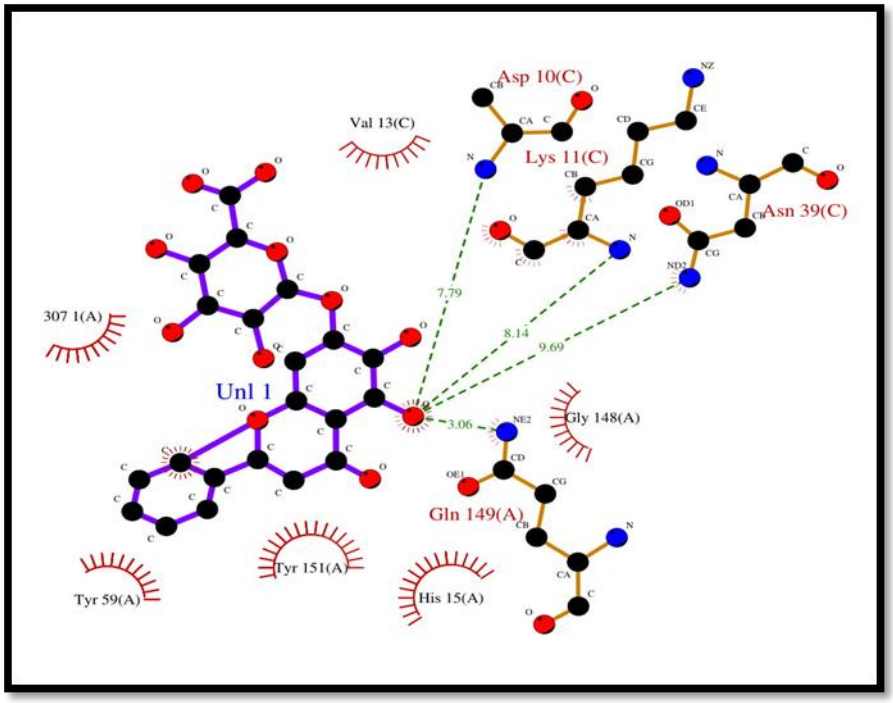


Figure 22: Ligplot figure of interaction between bicalin and TNF-α

The overall structure of the docking bicalin and TNF-α is given in the figure21,22 where bicalin with active site 10, 11, 39, 149 with the hydrogen bonding. The number of hydrogen bonding formed is 6 with residue Asp, Lys, Asn and Gln with the bicalin at the distance of 3.30 ,3.18,3.08, 3.22 2.97 and 3.06 with chain A C of the protein .The docking figure is given in Pymol Viewer.

Nine different conformations with poses and binding energy were generated but the top ranked complex with the binding score is given in figure. this is inhibiting the inflammation by of joint in arthritis and lowers the inflammation (Khosravi et al, 2014) This is an important component of FDA product known as flavocoxid which is anti-inflammatory and against arthritis (Levi et al, 2010).

TNF-α tanetin complex

Binding Energy: 6.9 (kcal/mol)

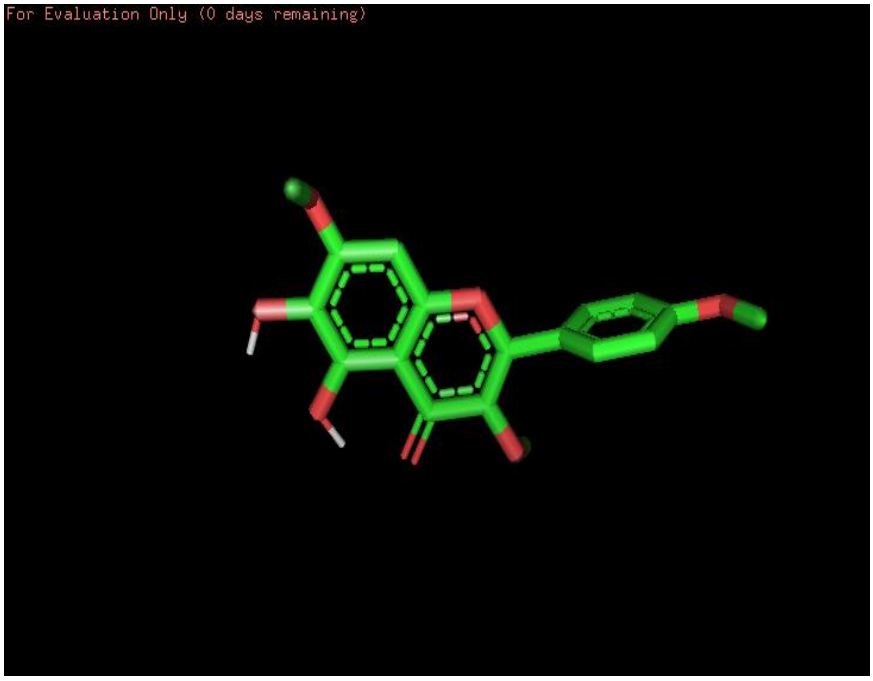


Figure 23: 3D Structure of Tanetin

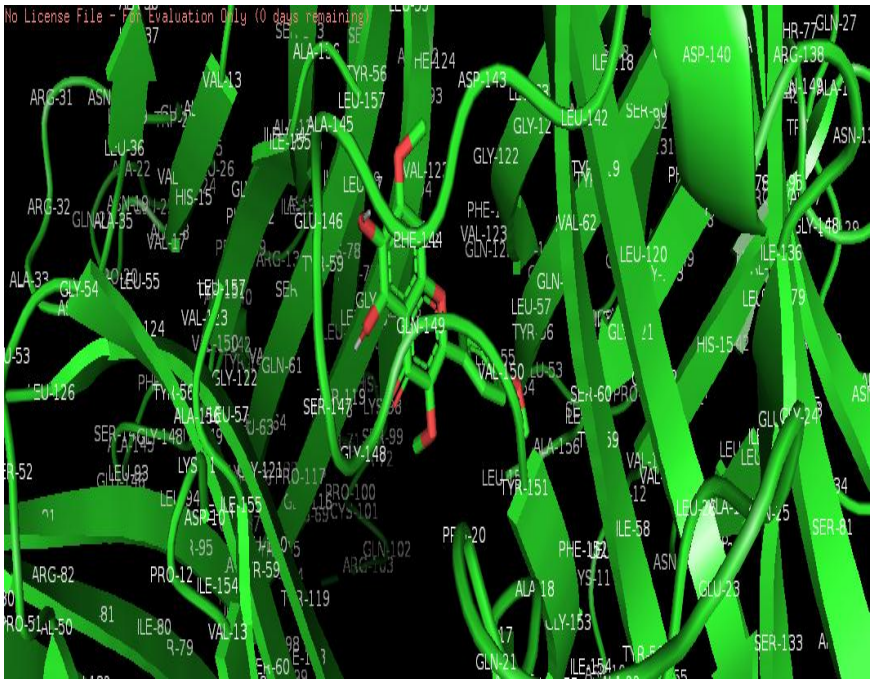


Figure 24: Docking of Tanetin with TNF-α

Table 8: Hydrogen Bonds of TNF-α tanetin complex

A T O M 1					A T O M 2				
Atom No.	**Atom Name**	**Res Name**	**Res No.**	**Chain**	**Atom No.**	**Atom Name**	**Res Name**	**Res No.**	**Distance**
956	NE2	GLN	149	A	4213	O	UNL	1	3.01
956	NE2	GLN	149	A	4213	O	UNL	1	3.21

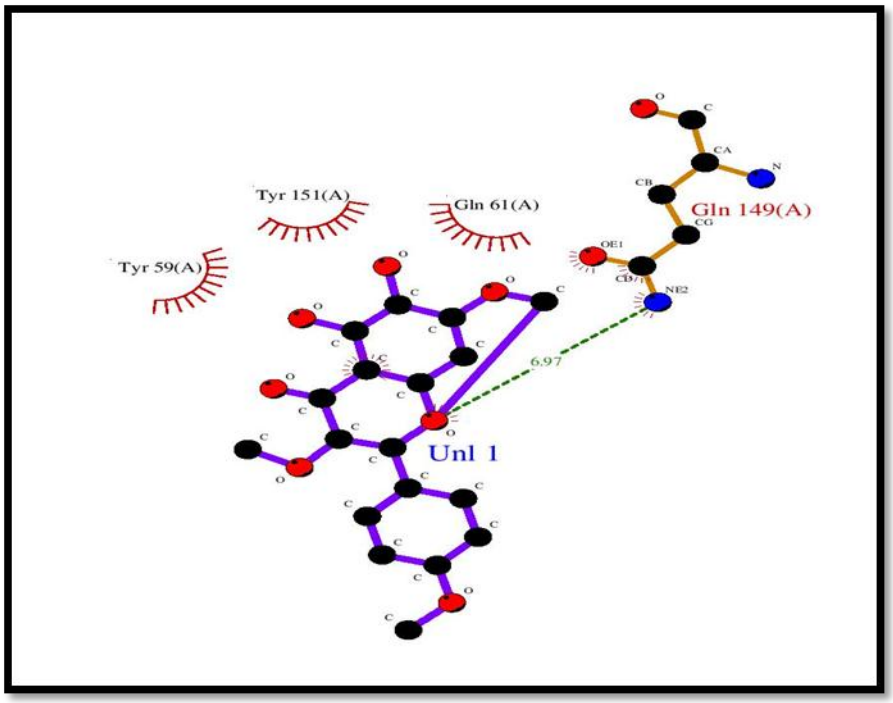


Figure 25: Ligplot figure of interaction between tanetin and TNF-α

The overall structure of the docking of tanetin and TNF-α is given in the figure 24,25 where tanetin bind with active site 149 with the hydrogen bonding. The number of hydrogen bonding formed is 1 with residue Gln with the lupeol 1 at the distance of 6.97 with chain A of the protein .The docking figure is given in Pymol Viewer. Nine different conformations with poses and binding energy were generated but the top ranked complex with the binding score is given in figure. Tanetin is used for disease such as arthritis, fever and headache (Rogers et al, 2000).

TNF-α stigmasterol complex

Binding Energy: 9.9 (kcal/mol)

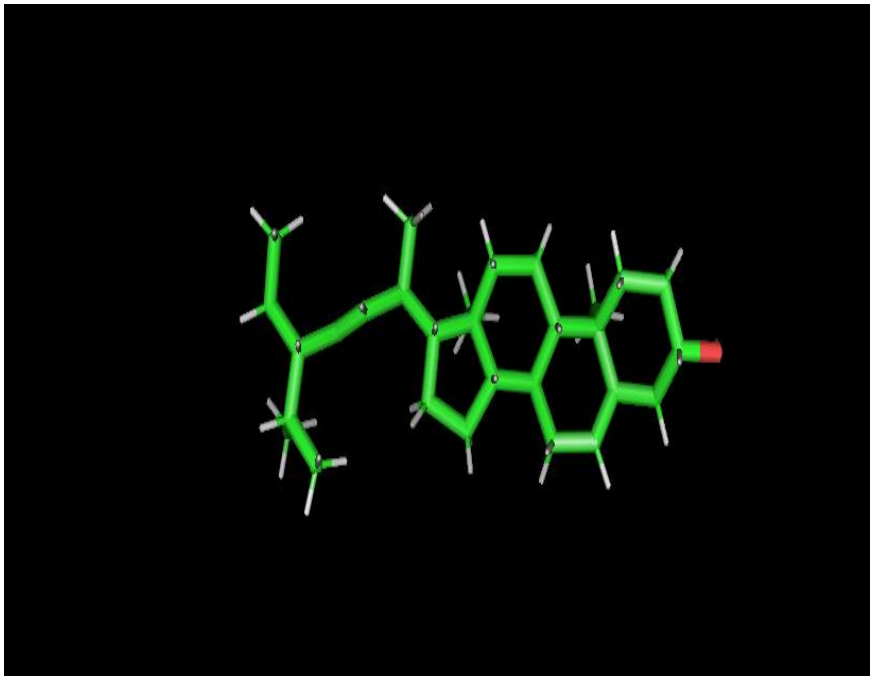


Figure 26: 3D Structure of stigmasterol

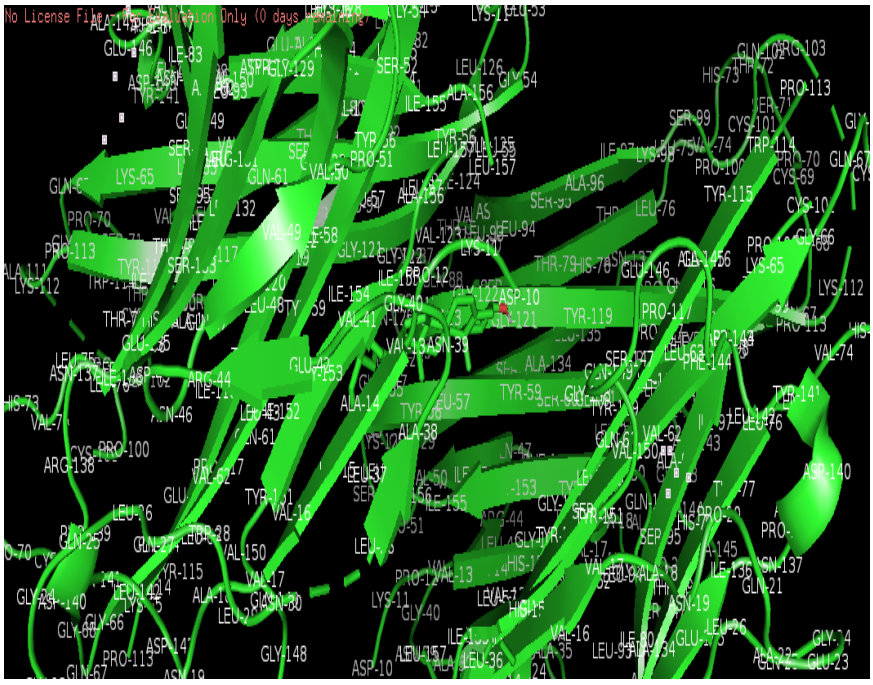


Figure 27: Docking of Stigmasterol with TNF-α

Table 9: Non-Bonded Contacts of TNF-α stigmasterol complex

ATOM 1					ATOM 2				
Atom No.	Atom Name	Res Name	Res No.	Chain	Atom No.	Atom Name	Res Name	Res No.	Distance
1413	CD1	LEU	57	B	4421	C	UNL	1	3.82
1852	CA	GLY	121	B	4450	O	UNL	1	3.37
1853	C	GLY	121	B	4421	C	UNL	1	3.83
1853	C	GLY	121	B	4421	C	UNL	1	3.88
1853	C	GLY	121	B	4450	O	UNL	1	3.23
1854	O	GLY	121	B	4450	O	UNL	1	3.33
1855	N	GLY	122	B	4421	C	UNL	1	3.55
1855	N	GLY	122	B	4450	O	UNL	1	3.82
1856	CA	GLY	122	B	4421	C	UNL	1	3.81
1865	CG2	VAL	123	B	4421	C	UNL	1	3.53
1865	CG2	VAL	123	B	4421	C	UNL	1	3.25
2564	CG	TYR	59	C	4421	C	UNL	1	3.69
2564	CG	TYR	59	C	4421	C	UNL	1	3.81
2565	CD1	TYR	59	C	4421	C	UNL	1	3.53
2566	CD2	TYR	59	C	4421	C	UNL	1	3.62
3606	CD2	LEU	55	D	4421	C	UNL	1	3.73
3606	CD2	LEU	55	D	4450	O	UNL	1	3.62
3625	CD1	LEU	57	D	4421	C	UNL	1	3.89
4071	CG2	VAL	123	D	4421	C	UNL	1	3.76
4343	CD1	LEU	157	D	4421	C	UNL	1	3.69
4343	CD1	LEU	157	D	4421	C	UNL	1	3.27

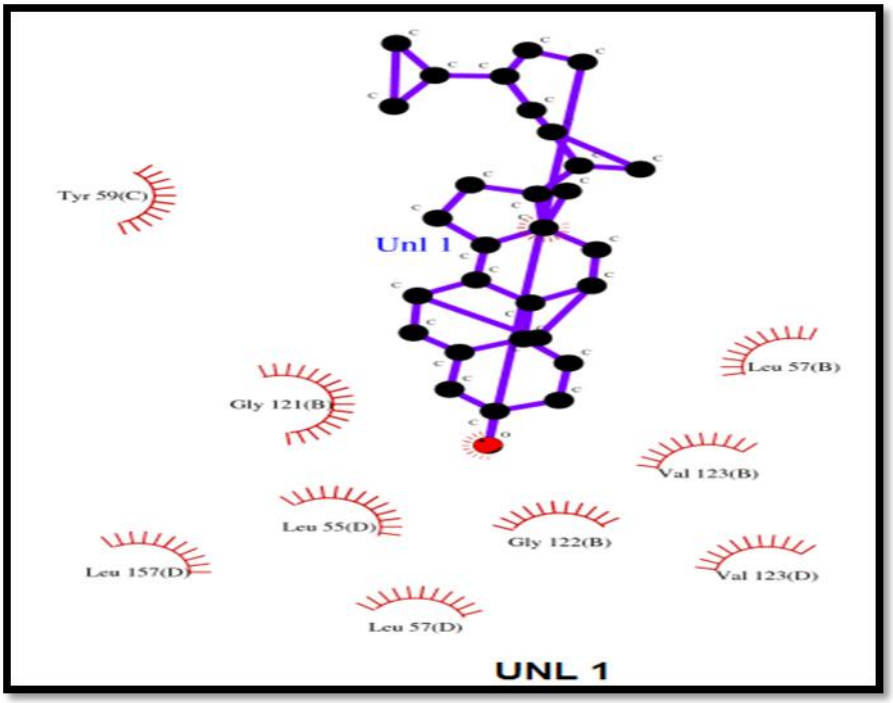


Figure 28: Ligplot figure of interaction between Stigmasterol and TNF-α

The overall structure of the docking stigmasterol and TNF-α is given in the figure 27,28 where stigmasterol with non-bonded binding. The docking figure is given in Pymol Viewer. Nine different conformations with poses and binding energy were generated but the top ranked complex with the binding score is given in figure. Stigmasterol and glucoside has highest docking energy value of -10.868 (kcal/mol). Against βlactamase. (Shasank et al, 2015).

TNF-α Ethylcinnamate complex

Binding energy -6.4 (kcal/mol)

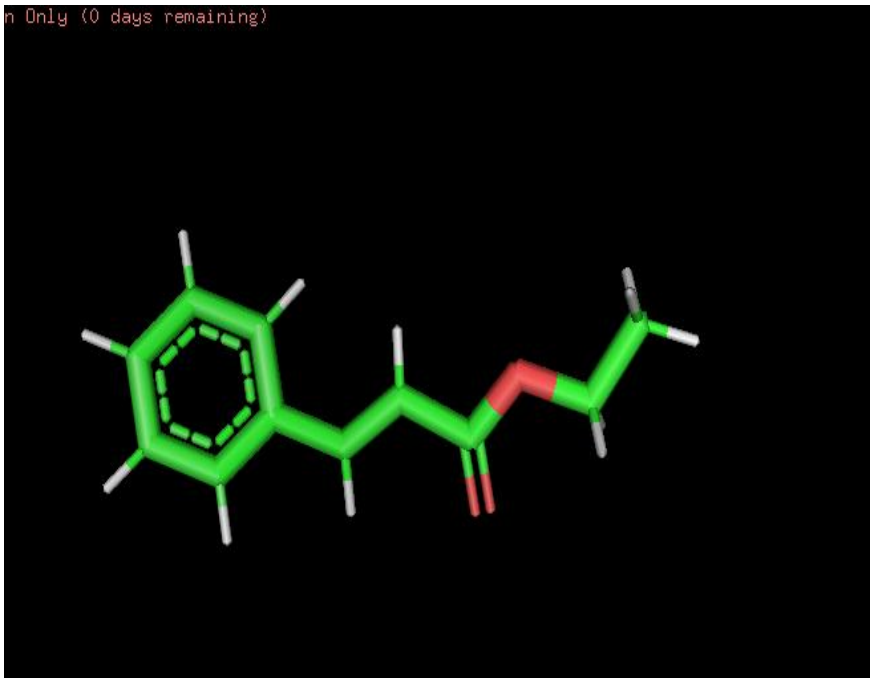


Figure 29: 3D Structure of Ethylcinnamate

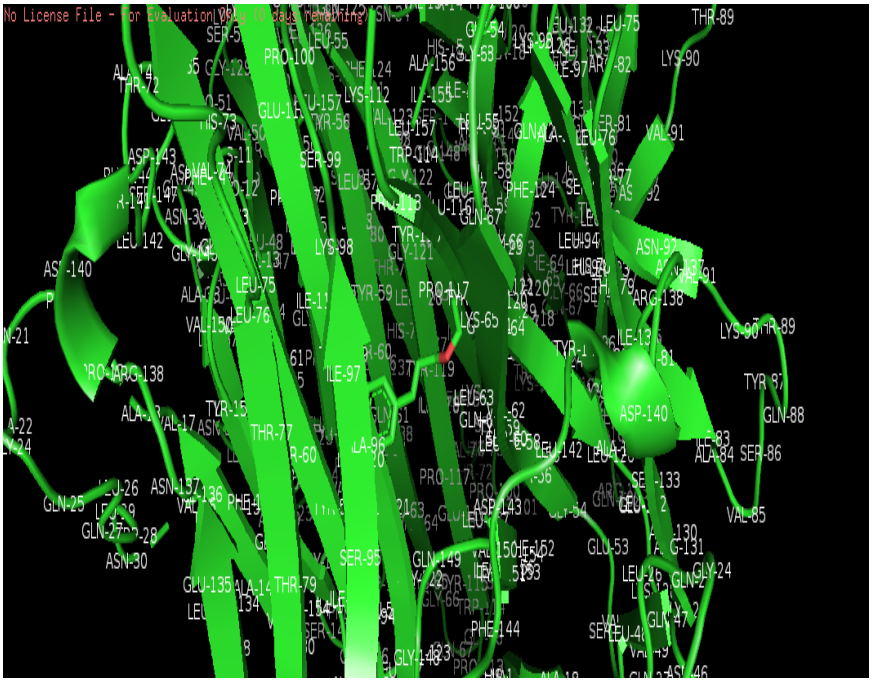


Figure 30: Docking of Ethylcinnamate with TNF-α

Table 10 : Non-Bonded Contacts of TNF-α Ethylcinnamate complex

ATOM 1					ATOM 2				
Atom No.	Atom Name	Res Name	Res No.	Chain	Atom No.	Atom Name	Res Name	Res No.	Distance
334	CB	TYR	59	A	4413	C	UNL	1	3.77
335	CG	TYR	59	A	4413	C	UNL	1	3.65
337	CD2	TYR	59	A	4413	C	UNL	1	3.75
1404	CG	LEU	57	B	4413	C	UNL	1	3.89
1404	CG	LEU	57	B	4424	O	UNL	1	3.51
1405	CD1	LEU	57	B	4413	C	UNL	1	3.59
1405	CD1	LEU	57	B	4413	C	UNL	1	3.71
1405	CD1	LEU	57	B	4424	O	UNL	1	3.75
1406	CD2	LEU	57	B	4424	O	UNL	1	3.56
1845	C	GLY	121	B	4413	C	UNL	1	3.77
1846	O	GLY	121	B	4413	C	UNL	1	3.87
1847	N	GLY	122	B	4413	C	UNL	1	3.64
1847	N	GLY	122	B	4413	C	UNL	1	3.84
1848	CA	GLY	122	B	4413	C	UNL	1	3.74
1849	C	GLY	122	B	4413	C	UNL	1	3.89
1857	CG2	VAL	123	B	4413	C	UNL	1	3.78
1857	CG2	VAL	123	B	4424	O	UNL	1	3.77
3598	CD2	LEU	55	D	4413	C	UNL	1	3.79
4062	CG1	VAL	123	D	4413	C	UNL	1	3.80

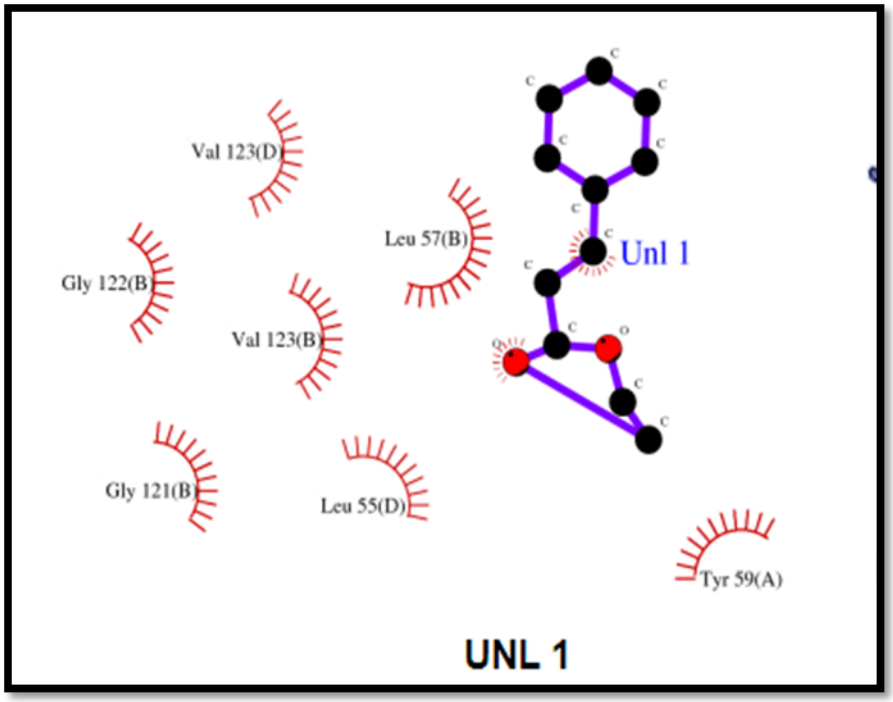


Figure 31: Ligplot figure of interaction between Ethylcinnamate and TNF-α

The overall structure of the docking penin and TNF-α is given in the figure 30,31 there is non-bonded interactions. The docking figure is given in Pymol Viewer. Nine different conformations with poses and binding energy were generated but the top ranked complex with the binding score is given in figure.

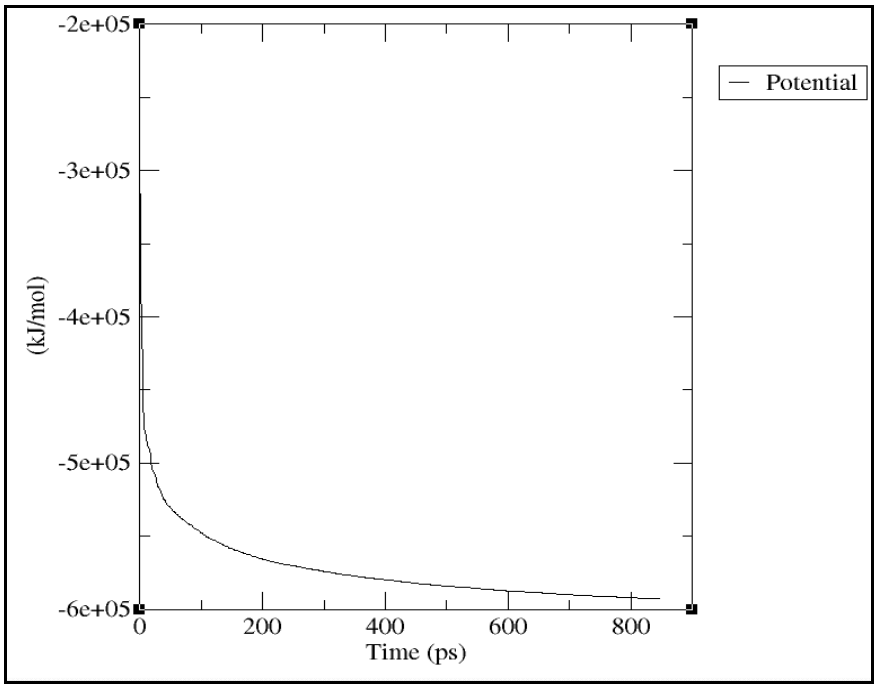
Table 11: Binding affinity of TNF-α with the ligands

Ligands	Binding energy (affinity) (kcal/mol)	Number of hydrogen bond
Lupeol	15.6	1
Boswellic acids	9.2	1
EGCG	7	1
Arctiin	7.7	3
Gingerol	6.4	1
Tanetin	6.9	2
Stigmastreol	9.9	0
Bicalin	8.4	6
Ethylcinnamate	6.4	0

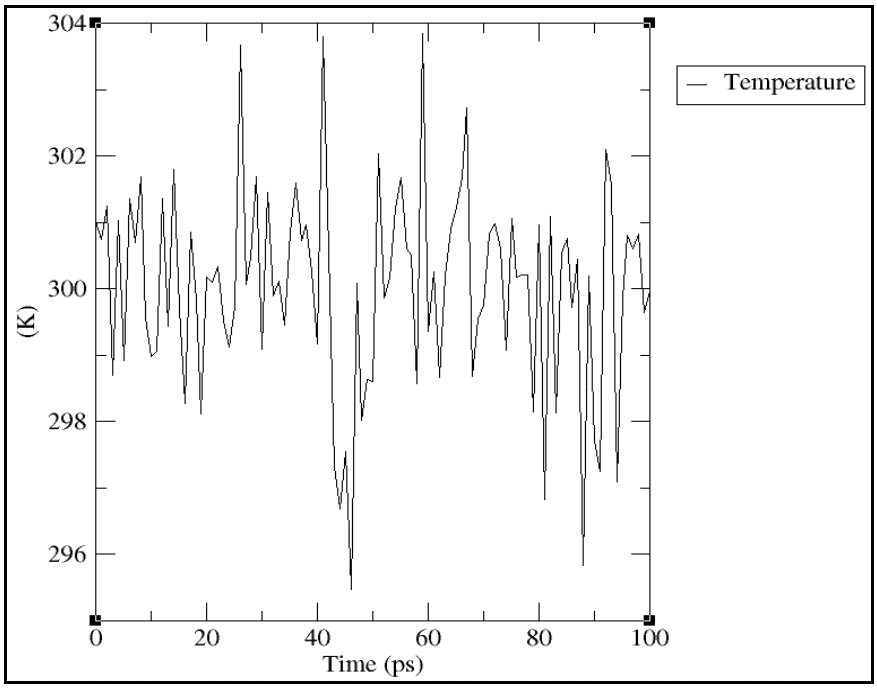
From the table it is conclude that best binding energy is of lupeol which is 15.6 (kcal/mol), the second best energy is of stigmasterol but with no hydrogen bonding. The red colour is the oxygen, green is carbon and the white hydrogen. Hydrogen bond is six in number from bicalin; the next is Arctiin with three hydrogen bond. The best binding structure complex is of bicalin, the next is Arctiin, the third is Tanetin. This complex with good binding energy is best complex for simulation.

Simulation:

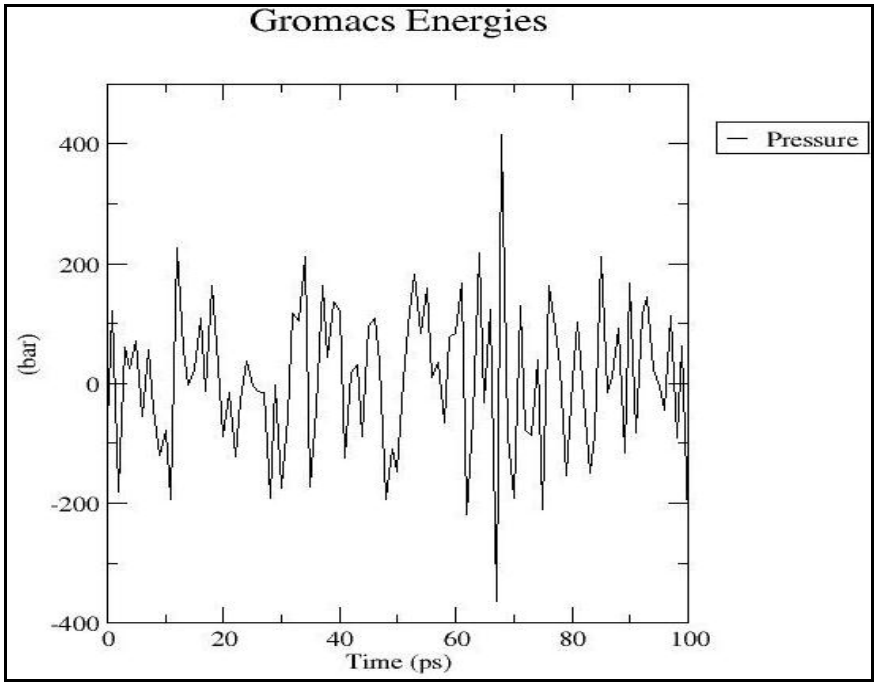
Results:



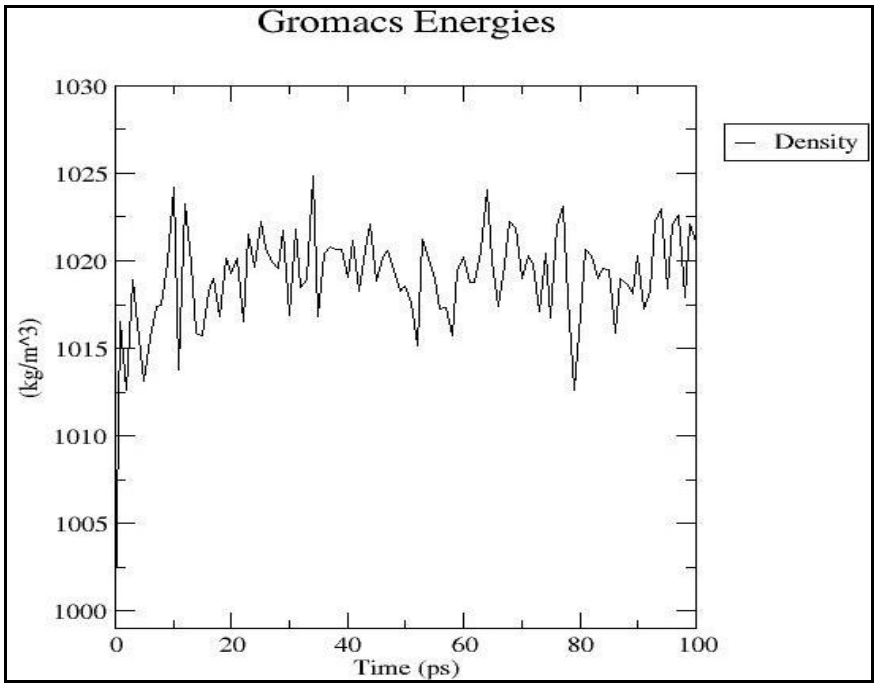
Graph 1: Potential energy



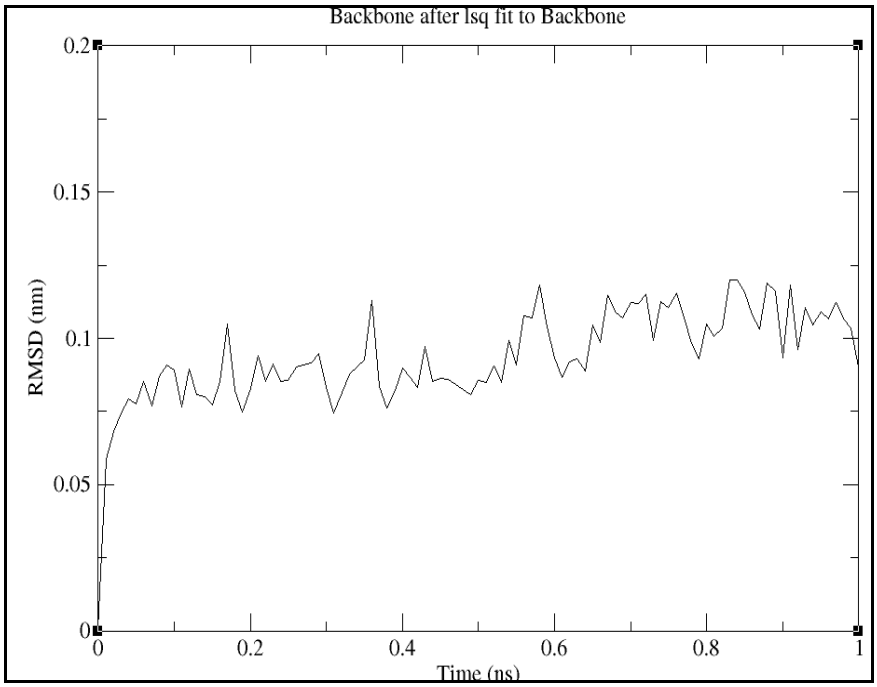
Graph 2: Temperature



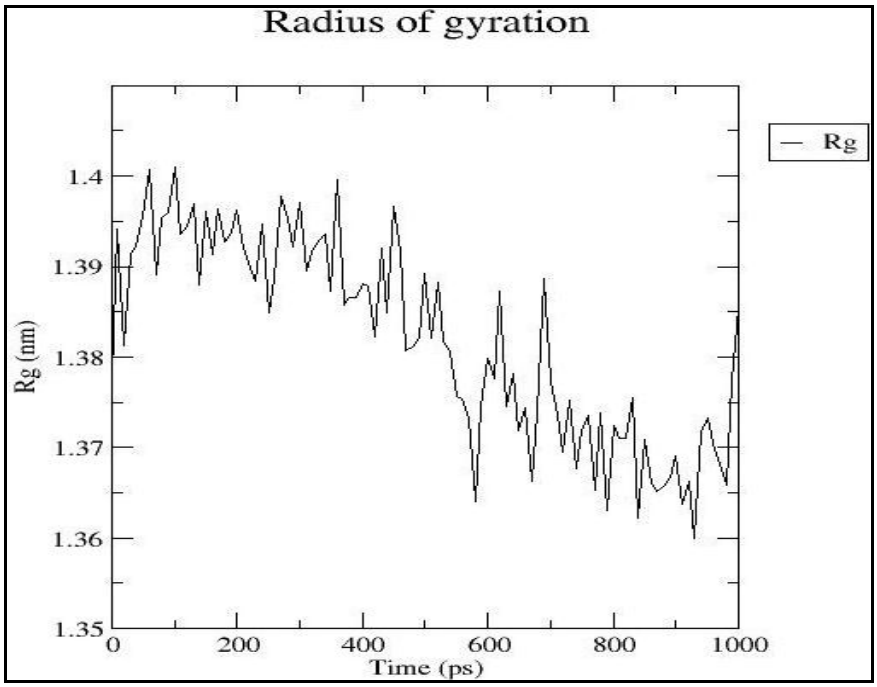
Graph 3: Pressure



Graph 4: Density



Graph 5: RMSD



Graph 6: Radius of Gyration

The simulation of protein lysozyme in water with PDB ID 1aki.

The resulting plot of energy minimization looks like the figure with the nice steady convergence of Epot. This figure gives the result that the energy is minimum so simulation begins. When the energy of the simulation is balanced, this give the result that all the forces of the atoms are in balanced state. The stability of the molecule is given by this that the molecule is in stable state. The potential energy is negative for simple protein in water and the value of the potential energy is in the order of the 10^5 to 10^6 which mainly depends on the six and the number of water molecules. Maximum force is also one of the important features of simulation. This graph is plotted by the help of a tool known as Xmgrace.

For beginning of the real simulation, solvent and ions should be equilibrated around the protein for optimization of the solvent with the protein temperature is brought. Right temperature is followed by the right pressure and lastly the density of the complex. If the temperature of the system is not in stable state, so this takes more time for stabilization.i.e 50-10 ps. This simulation took 1.5 hours to simulate the results of the temperature graph. The graph of temperature is given in the figure. This graph tells that the temperature reaches the value of 300K and is stable for the remains of the equilibrium.

The graph of the pressure is given in the figure. This result tells that the pressure fluctuation for a time of 100 ps. The graph of the density is given in the figure. This results tells that the density fluctuation for the time of 100 ps. The graph also tells that the value of the density is stable for the of 100ps. The simulation is 1ns. This took 3 days to get the simulation results which mainly depend on the performance of the CPU. The output is RMSD graph given in the figure which tells that the structure is in the minimization state. The RMSD value is given in the time of the 1 ns. The value of RMSD tells that the simulation of the system is in stable state. The graph of the radius of gyration is given n the figure. The graph fluctuation for a time of 1000ps tells that the protein is stable.

Pruzanski et al reported that comparing the samples of serum and synovial fluids, from Rheumatoid arthritis patient and the one who is fit, gives the results that lysozyme activity was higher in 35% of serum and 55% of synovial fluid samples which suggested the lysozyme as a contributor to intra-articular inflammation. Robert et al reported that increase in lysozyme found in rheumatoid arthritis and osteoarthritis.

CHAPTER 5

CONCLUSION

Arthritis is a joint pain and inflammation which is also known as inflammatory disease. People are unable to walk due to pain in knee. This disease is inhibited by the docking and simulation results by the drug designing.

The docking of the ligand and the target protein gives the energy or affinity of the binding complex. The best the binding energy in negative in higher energy the best the binding complex is. This binding complex tells that the ligand is in stable binding state. Hydrogen bonding is the most important features in docking which bind the two binding complex of ligand and the target.

The best binding structure complex is further simulated to get the stability of the protein in body like conditions. If the protein is stable in the simulation results, the protein is be stable in body like condition. The stability of the complex is given by the graph of simulation which is run for time of different nano seconds. This stable complex is best for drug designing. This complex is further taken for drug designing.

CHAPTER 6

FUTURE PROSPECTS

Docking allows predicting the ligand with and identifying the drug receptor complex with lowest free energy. The lesser the free binding energy, the greater the stability of the ligand and target molecule binding. The greater the stability, then better it will work for the treatment of arthritis.

Molecular dynamics simulation is a method which is helpful for the study of physical movement of atoms and molecules and hence is called as a type of body simulation. The atoms and molecules are allowed to interact for a fixed period, giving a view of the dynamic evolution of the system. If the docking molecule after molecular simulation gives better result, then it will be used for preclinical trial, then clinical trial and then be commercialized for treatment of arthritis.

Going to the practical side, a computer experiment seems to be used in discovering and designing of new molecules. Computer modeling is a faster and less expensive for the testing of the molecule than in a real experiment. Pharmaceutical industry are most commonly taking the help of computer for drug designing.

REFERENCES

Allen, M.P., Tildesley, D.J. Computer Simulation of Liquids, Clarendon, Oxford. (1987)

Berendsen, H., van der Spoel, D., van Drunen, R. "GROMACS: A messagepassing parallel molecular dynamics implementation". *Comp. Phys. Comm.* (1995) 91, pp.43–56.

Braunstein, E.M., White, S.J., Russell, W., Harris, J.E." Paleoradiologic evaluation of the Egyptian royal mummies*". Skeletal radiology.* (1988) 17, pp.348-52

Bremner, J.M., Lawrence, J.S., Miall, W.E. "Degenerative joint disease in a Jamaican rural population". *Annals of the rheumatic diseases.* (1968) 4, pp 326-32.

Brooks, B., Bruccoleri, R., Olafson, D., States, D., Swaminathan ,S., Karplus, M. "CHARMM: A Program for Macromolecular Energy, Minimization, and Dynamics Calculations". *J Comp Chem,* (1983) 4, pp.187–217.

Centers for Disease Control and Prevention: Prevalence and impact of arthritis by race and ethnicity—United States, 1989-1991. MMWR Morb Mortal Wkly Rep (1996) 45, pp 373-378.

Chabib, L., Awaluddin, R., Ikawati, Z., Ismail, H., "molecular docking, pharmacophore modelling, and adme-toxicity prediction of curcumin analog compounds as inflammatory inhibitor on rheumatoid arthritis"*International Journal of Pharmacy and Pharmaceutical Sciences.* **(**2017) 9, pp.16.

Daisy, P., Suveena, S., Sr. Lilly., V. "Molecular docking of medicinal compound Lupeol with autolysin and potential drug target of UTI*" J. Chem. Pharm. Res.* (2011) 3, pp 557-562.

Djerir, D., Iddir, M., Bourgault, S., Lamy, S., Annabi, B. "Biophysical evidence for differential gallated green tea catechins binding to membrane type-1 matrix metalloproteinase and its interactors". *Biophys Chem.* (2018) 234, pp 34-41.

Dodson, G.G., Lane, D.P., Verma, C.S. "Molecular simulations of protein dynamics: New windows on mechanisms in biology" *Embo Reports* (2008) 9, pp.144-150.

Dror, R.O., Pan, A.C., Arlow, D.H., Borhani, D.W., Maragakis, P., Shan, Y.B., Xu, H.F., Shaw, D.E. “Pathway and mechanism of drug binding to G-protein-coupled receptors*”. Proceedings of the National Academy of Sciences of the United States of America* (2011) 108, pp.13118-13123.

Farnsworth, N.R., Akerlee, A.S., Bingel, Guo, Z.G. Soejarto, D.D. “MedicinalPlants in Therapy. “ *Bull World Health Organ*. (1985) 63, pp 965-981.

*Fechtner, S., Singh, A., Chourasia, M., Ahmed, S. “*Molecular insights into the differences in anti-inflammatory activities of green tea catechins on IL-1β signaling in rheumatoid arthritis synovial fibroblasts*”. Toxicol Appl Pharmacol.* (2017) 329, pp 112-120.

Felson, D.T. “An update on the pathogenesis and epidemiology of osteoarthritis”. *Radiol.Clin.North Am*. (2004) 42, pp 1-9.

Fernández, M.A., Álvarez, A., García, M.D., Sáenz, M.T.” Anti-inflam- matory effect of Pimenta racemosa var. ozua and isolation of the triterpene lupeol”. *Farmaco* (2001a) 6, pp 335-338.

Funk, J.L., Frye, J.B., Oyarzo, J.N., Chen, J., Zhang, H., Timmermann BN “. Anti-Inflammatory Effects of the Essential Oils of Ginger (Zingiber officinale Roscoe) in Experimental Rheumatoid Arthritis”. *PharmaNutrition* (2016) 4, pp 123-131.

Gabriel, S.E., “The epidemiology of rheumatoid arthritis”. *RheumDis Clin North Am* (2001) 27, pp.269-281.

Grossfield, A. “Recent progress in the study of G protein-coupled receptors with molecular dynamics computer simulations”. *Biochimica Et Biophysica ActaBiomembranes* (2011) 1808, pp 1868-1878.

Gumbart J, Chipot C, Schulten K: Free-energy cost for translocon-assisted insertion of membrane proteins. Proceedings of the National Academy of Sciences of the United States of America (2011) 108, pp 3596-3601.

Hashim, H., Kamali, E.L., Mohammed, Y. "Antibacterial activity and phytochemical screening of ethanolic extracts obtained from selected Sudanese medicinal plants". *Current Research Journal of Biological Science*, (2010) 2, 143-146.

Jena, J., Gupta, A. "Ricinus communis linn: A phytopharmacological review" *International Journal of Pharmacy and Pharmaceutical Sciences.* (2012) 4, pp.25-29.

Jolad, S.D., Lantz, R.C., Solyon, A.M., Chen, G.J., Bates, R.B., Timmermann, B.N. Fresh organically grown ginger (Zingiber officinale): composition and effects on LPS-induced PGE2 production. *Phytochemistry* (2004) pp 65.

Kallubai, M., Rachamallu, A., Daniel Pushparaju Yeggonia, D.P., Subramanyam, R. "Comparative binding mechanism of lupeol compounds with plasma proteins and its pharmacological importance" *Mol Biosyst.* **(**2015**)** 11, pp.1172-83.

Khosravi, I., Sahihi, M. "Computational Studies on the Interaction of Arctiin and Liquiritin With β-lactoglobulin" *Journal of Macromolecular Science* (2014) pp 1591-1600.

Knipping, K., van Esch, E.C., Wijering, S.C., van der Heide, S., Dubois, A.E., Garssen. J " In vitro and in vivo anti-allergic effects of Arctium lappa L". *Exp Biol Med (Maywood)*. (2008) 233, pp 1469-77.

Knott, A., Reuschlein, K., Mielke, H., Wensorra, U., Mummert, C., Koop, U., Kausch, M., Kolbe, L., Peters, N., Stäb, F., Wenck, H., Gallinat, S. "Natural Arctium lappa fruit extract improves the clinical signs of aging skin". *J Cosmet Dermatol*. (2008) 7, pp 281-9.

Koirala, P., Seong, S. H., Jung, S. H. A., Choi, J. S., "Comparative molecular docking studies of lupeol and lupenone isolated from *Pueraria lobata* that inhibits BACE1: Probable remedies for Alzheimer's disease," *Asian Pacific Journal of Tropical Medicine*,(2017) 10, pp 1117-1122.

Leigh, J.P., Seavey, W., Leistikow, B. "Estimating the costs of job related arthritis". *J.Rheumatol*. (2001) 28, pp 1647-54.

Levi, R.M., Khohlov, A., Kopenkin, S., Bart, B., Ermolova, T., Kantemirova, R., Mazurov, V., Bell, M., Caldron, P., Pillai, L. Efficacy and safety of flavocoxid, a novel therapeutic, compared with naproxen: A randomized multicenter controlled trial in subjects with osteoarthritisof the knee. *Adv. Ther*. (2010) 27, pp 731–742.

Lindorff-Larsen K, Piana S, Dror RO, Shaw DE: How Fast-Folding Proteins Fold. Science (2011) 334, pp 517-520.

McCammon ,J. A., Gelin ,B. R., Karplus, M. "Dynamics of folded proteins". *Nature*, 267 (1977).

Mcgeagh, J.D., Ranaghan, K.E., Mulholland, A.J. "Protein dynamics and enzyme catalysis: Insights from simulations". *Biochimica Et Biophysica Acta-Proteins and Proteomics* (2011) 1814, pp 1077-1092.

Mikkelsen, W. M., Dodge, H. J., Duff I. F., Kato, H. "Estimates of the prevalence of rheumatic diseases in the population of Tecumseh, Michigan, 1959-60," *Journal of Chronic Diseases*. (1967) 6, pp. 351–369.

Muskel og skeletsygdomme. "Folkesundhedsrapporten. København". *Statens Institut for Folkesundhed* (2007) pp 101-16.

Nelson, M., Humphrey ,W., Gursoy, A., Dalke A., Kalé, L., Skeel, R. D., Schulten, K. "NAMD—A parallel, object-oriented molecular dynamics program". *International Journal of Supercomputer Applications and High Performance Computing*. (1996) 10, pp 251–268.

Oanh, T. P. K., Manh, D. Le., Hoang, X. T., Hai, V. N. "*In silico* studies for the interaction of tumor necrosis factor-alpha (TNF-α) with different saponins from Vietnamese ginseng (*Panax vietnamesis*)" Biophys Physicobiol. (2016) 13, pp. 207.

Pedersen., J.K., Svendsen, A.J., Horslev-Petersen ,K." Prevalence of rheumatoid arthritis in the southern part of Denmark". *Open Rheumatol J* (2011) 5, pp 91-7.

Ponder, J.W., Case,D.A. "Force fields for protein simulations". *Adv. Prot. Chem.* (2003) 66, pp 27-85.

Pruzanski, W., Saito,S., Ogryzlo, M. A. "The Significance of Lysozyme (Muramidase) in Rheumatoid Arthritis". *Arthritis and Rheumatism*. (1970) 13 pp 339-399.

Rahman,A. "Correlations in the Motion of Atoms in Liquid Argon". *Phys Rev*, 136 (1964) 136, pp.405–411.

Ramakrishnappa, K. "Impact of Cultivation and Gathering of Medicinal Plants on Biodiversity: Case Studies from India". *Forestry and Fisheries*.(2002) pp 1-21.

Rampogu, S. , Baek, A., Gajula, R. G., Zeb, A, Bavi, R. S. Kim, Y ., Kwon,Y. J., Lee, K. W. "Ginger (*Zingiber officinale*) phytochemicals—gingerenone-A and shogaol inhibit SaHPPK: molecular docking, molecular dynamics simulations and in vitro approaches" *Ann Clin Microbiol Antimicrobial*. (2018) pp 16.

Ricci, J.A., Stewart, W.F., Chee, E., Leotta, C., Foley, K., Hochberg, M.C. "Pain exacerbation as a major source of lost productive time in US workers with arthritis". *Arthritis Rheum*. (2005) 5, pp 673-81.

Riegsecker, S., Wiczynski, D., Kaplan, M.J., Ahmed, S. "Potential benefits of green tea polyphenol EGCG in the prevention and treatment of vascular inflammation in rheumatoid arthritis". *Life Sci*. (2013) 93, pp 307-12.

Roberts, J., Burch, T.A."Osteoarthritis prevalence in adults by age, sex, race, and geographic area".*Vital and health statistics*.(1966) 15, pp.1-27.

Robert, M.B., John, L. S. "Lactoferrin and lysozyme levels in synovial fluid" *Arthritis and Rheumatism*. (1977) 20, pp 84-90.

Rogers, K. L., Grice, I. D., Griffiths, L. R. Inhibition of platelet aggregation and 5-HT release by extracts of Australian plants used traditionally as headache treatments. *Eur. J. Pharm. Sci.* (2000) 9, pp 355–363.

Satpathy,R., Guru, R., Behera, R., Nanda, S .S., "Computational Study of Some Bowsellic Acid and Curcumin Derivatives As Potential Anti-Arthritis Compounds" IJBR (2013) , pp.52-59.

Sgourakis, N.G., Garcia, A.E. "The Membrane Complex between Transducin and DarkState Rhodopsin Exhibits Large-Amplitude Interface Dynamics on the

SubMicrosecond Timescale: Insights from All-Atom MD Simulations*". Journal of Molecular Biology* (2010) 398, pp.161-173.

Shasank, S. S., Rabindra, N. P. "In vitro antibacterial efficacy of plants used by an Indian aboriginal tribe against pathogenic bacteria isolated from clinical samples" *Journal of Taibah University Medical Sciences.* (2015) pp 379–390.

Shaw, D.E., Maragakis, P., Lindorff-Larsen, K., Piana, S., Dror, R.O., Eastwood, M.P., Bank, J.A., Jumper, J.M., Salmon, J.K., Shan, Y.B., et al. " Atomic-Level Characterization of the Structural Dynamics of Proteins". *Science.* (2010) 330, pp 341-346.

Stansfeld, P.J., Sansom, M.S.P. "Molecular Simulation Approaches to Membrane Proteins". *Structure* (2011) 19, pp.1562-1572.

Symmons, D., Turner, G., Webb, R., Asten, P., Barrett, E., Lunt, M., et al. "The prevalence of rheumatoid arthritis in the United Kingdom: new estimates for a new century". *Rheumatology (Oxford)* (2002) 41, pp 793-800.

Trott, O., Olson ,A. J., "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading", *Journal of Computational Chemistry 31 (2010)* pp*.455-461*

Vaidya, A.D.B., Devasagayam ,T.P.A. "Current status of Herbal Drugs in India: An Overview". *J. Clin. Biochem. Nutr.* (2007) 41, pp 1-11

van der Ploeg,P. and Berendsen ,H. J. C. "Molecular dynamics simulation of a bilayer membrane". *J. Chem. Phys.* (1982) 76, pp. 3271.

van Gunsteren ,W.F., Berendsen, H.J.C. "A leap-frog algorithm for stochastic dynamics". *Mol. Sim.* (1988) 1, pp.173–185.

Verlet, L. "Computer experiments on classical fluids. I. Thermodynamical properties of Lennard-Jones molecules". *Phys. Rev.* (1967) 159, pp.98–103.

Verstappen, S.M.M.: Outcomes of early rheumatoid arthritis – The WHO ICF framework". *Best Pract Res Clin Rheumatol.* (2013) 27, pp.555-570.

Wakdikar S. "Global health care challenge: Indian experiences and new prescriptions". *Electron J Biotechnol* . (2004) 7, pp 214–220.

Wang, R., Lu, Y., Wang, S. "Comparative evaluation of 11 scoring functions for molecular docking*".J Med Chem.* **(**2003**)** 4, pp 2287-303.

Weiner, P. K., Kollman, P. A. "Assisted model building with energy refinement. A general program for modeling molecules and their interactions". *J Comput Chem,* (1981) 2, pp 287–303.

Wu,J., Qu ,Y., Deng, J.X., Yu, Q.H. "Molecular docking studies of kirenol a traditional Chinese medicinal compound against rheumatoid arthritis cytokine drug targets (TNF-α, IL-1 and IL-6)".(2017) 28, pp 1992-1995.

Xu, S., Peng, H., Wang, Na., Zhao, M." Inhibition of TNF-α and IL-1 by compounds from selected plants for rheumatoid arthritis therapy: In vivo and in silico studies" *Tropical Journal of Pharmaceutical Research (*2018**)** 17, pp 277-285

Xuli, W., Raja, Dey., Hui, Wu., Zhigang, Liu., Qingqing, He., Xiaojuan Zeng. "Studies on the interaction of -epigallocatechin-3-gallate from green tea with bovine β-lactoglobulin by spectroscopic methods and docking". *International Journal of Dairy Technology* (2013) 66, pp 7-13.