CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Identification of Bioactive Compounds of Postbiotics Against Neonatal Meningitis Caused by Group B Streptococcus

by

Rabbia Altaf

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Every challenging work needs self-efforts as well as the guidance of elders. I dedicate this thesis to my mother and father whose affection makes me able to get such success and to my teachers whose encouragement has always been my source of inspiration

CERTIFICATE OF APPROVAL

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Abstract

Neonatal meningitis is continuously marked as a calamitous infection which causes morbidity and mortality. Its prevalence fluctuates across countries especially in developing countries. It is the inflammation of the meninges during the first 28 days of a neonatal life, caused by group B streptococcus. They are treated by the antibiotics but neonates can become resistance by their excessive use and causes disruption of the body's micro biome which having an important function treating against bad bugs and support function of immune system and its disruption is directly linked with high risk of infections. To overcome this we have to identify new drugs with no side effects. One of the proposal is that we can used Postbiotics components against this infectious disease. Postbiotics are the substances produced by the metabolic activity of microorganism and exerts a beneficial effects on host directly or indirectly. Some of the Postbiotic components citric acid, arachidonic acid, palmitoleic acid, plamitic acid, oleic acid, malic acid , lauric acid and linoleic acid were taken as ligands. And docked with the serine rich repeat protein Srr2 which is the virulence factor of group B streptococcus. The best ligand was selected on the basis of docking scor , ADMET properties and Lipinski rule. Then it is concluded as citric acid, arachidonic acid and palmitoleic acid are considered on the basis of high docking score and will prove itself as a future therapeutics. By considering all the parameters citric acid was seen obeying all the parameters with docking score -7.3 against serine rich repeat protein Srr2. To check the further effectiveness of citric acid it compared with the commercially available antibiotic drug cefotaxime. A comparison of drug like properties and docking score shows citric acid is better than cefotaxime. It is concluded that citric acid can prove itself as a drug candidate in a future antibiotic therapeutics.

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Abbreviations

GBS	Group B streptococcus
Hib	Haemophilus Influenza Type B
E.coli	Escherichia Coli
NCIU	Neonatal Intensive Care Unit
EOM	Early Onset Meningitis
LOM	Late Onset Meninigitis
Srr	Serine Rich Repeats
Fbs	Fibrinogen Binding Protein
PbsP	Plasminogen Binding Surface Protein
ACP	Alpha C Protein
MVs	Membrane Vesicles
FAQ-WHO	Food and Agriculture Organization and World Health Organization
ISAPP	International Scientific Association of Probiotics and Prebiotics
LTA	Lipotechoic Acid
GRAVY	Grand Average of Hydropathicity
NR	Negative Residue
PR	Positive Residue
PDB	Protein Database
I-TASSER	Iterative Threading Assembly Renement
ADMET	Absorption Distribution Metabolism Excretion and Toxicity
BBB	Blood Brain Barrier
CNS	Central Nervous System
MRTD	Maximum Tolerated Dose
HERG	Human Ether A-Go-Go-Related Gene

II	Instability Index
VDss	Volume of Distribution At Steady State
KEGG	Kyoto Encyclopedia of Genes and Genomes
Des-CTX	Desacetylcefotaxime

Chapter 1

Introduction

Neonatal bacterial meningitis is a very serious, harmful and life-threatening disease. It is causing morbidity and mortality globally. It is characterized by the swelling of the meninges, the protective membrane surrounding the brain and spinal cord during the first 28 days of life. Bacterial meningitis is a bacterial infection of meninges. It has two further two terms, Early onset meningitis which means it occurs during the start days of life i.e. 3 to 9 days and Late onset meningitis which means that it occurs during the first 20 to 28 days of child birth. So it require a medical emergency, we need rapid diagnosis and the treatment to alleviate its potentially devastating consequences. There are no.of pathogens which are responsible for the bacterial meningitis include group B streptococcus (GBS)' streptococcus pneumoniae, Neisseria meningitides, haemophilus influenza type b (Hib) and listeria monocytogenes among others [1]. There are some bacterial. fungal and viral etiologies of neonatal meningitis. Risk factor for bacterial meningitis include the preterm birth, colonization of the GBS, rupture of membrane for long term or very low birthweight [2]. Group B Streptococcus (GBS) is most common cause of the neonatal meningitis in the first week of the childbirth. The pregnant women keep the GBS bacteria in their gastro intestinal system or in genitalia which transferred from mother to the fetus during the childbirth [3]. Escherichia Coli (E. coli) Certain strains of E. coli cause the newborn meningitis during the first few days of life. This infection is developed in newborns during birth or from other food sources for example tainted food or water [4].

Listeria Monocytogenes is a food borne pathogens which infect the neonates and cause severe neurological and developmental sequelae and also the immune compromised individuals [5].

Pathophysiology of neonatal meningitis is as the bacteria occupy or infect the newborn central nervous system through various routes which originates from the respiratory or through blood streaming [6]. The early onset of meningitis is maternal during the pregnancy and during the childbirth fetus exposed to so many pathogens which transmitted through the vagina to puncture the amniotic membrane or can also through the skin of neonate during passage through the birth canal [2]. Symptoms of neonatal meningitis include the irritation or crying frequently, high fever, lack of energy, loss of appetite, soft spot on the baby's head. Trouble in breathing ,and rashes on the skin [6]. Treatment for neonatal meningitis is necessary as it is a medical emergency and to avoid the further complication it is necessary to begin its treatment. It includes

• Hospitalize in the NCIU Neonatal intensive care unit, which is also known as the neonate's nursery which is for the premature babies or in case of illness of newborn infants, it is a mutual treatment for all newborn.

• Newborn having the symptoms of seizures, trouble in breathing or high intercranial pressure which is the more pressure on brain than normal needs supportive care.

• Neonates treated with antibiotic most frequently the intravenous antibiotics such as the ampicillin, cefotaxime or ceftriaxone [7].

Group B Streptococcus virulence factors like serine rich repeat protein Srr2 that enable the cell invasion [8].

1.1 Postbiotics and Their Role

Postbiotics are fairly new concept in the field of microbiology and healthcare. Postbiotics are the bioactive compounds or the metabolic byproduct which are

produced by the microorganism during the process of fermentation and it include a vast variety of substances like enzymes, organic acids, peptides, and the cell wall components. Probiotics are live microorganism and prebiotics are substances which encourage the growth of these probiotic bacteria. Postbiotics have a various beneficial effect on health when they are consumed as they are the non-viable or non-feasible microbial product. Newborns having the diseases of gastrointestinal tract or immune system can pose contrary effect on newborn health and development. Postbiotics gained attention for their potential role in treating neonatal diseases from the recent years. There are some key ways in which postbiotics are beneficial for newborn care

1.1.1 Gut Health:

they develop healthy gut microbiota in newborns and promote the growth of healthy bacteria and inhibit the growth of pathogens, they are important for preterm infants often having underdeveloped digestive system.

1.1.2 Immune Support:

they are having immunomodulatory effects, and can help in regulating the immune response in neonates. They can be crucial for preventing infectious and inflammatory conditions in newborn, who have developing immune system.

1.1.3 Reducing Gastrointestinal Disorders:

they are crucial in treating against gastrointestinal issues like colic, diarrhea or necrotizing enterocolitis.

1.1.4 Nutrient Absorption:

postbiotics enhance the nutrient absorption in the gut and ensure that newborns receive all necessary nutrient for growth and development.

1.1.5 Antioxidant Activity:

for protecting the neonatal cells and tissues from oxidative stress, postbiotic exhibit oxidative properties.

1.1.6 Allergy Prevention:

postbiotic can modulate the immune response by reducing the risk of allergic reaction and atopic diseases and also promote the immune response [9]. There is a recent study to find the naturally occurring medication by the postbiotics for the management of newborn Group B streptococcus (GBS). They focused on the bioactive substances that are produced by the bifidobacterial, especially the short chain fatty acid, which are known for their advantageous effects on people, nine ligands are chosed which are lactacte, formate, isobutyrate, valerate, caproate, acetate, octanoic acid, propionate and butyrate which are produced by bifidobacteria, this bifidobacterial is in relation with several streptococcus protein. Docking scores, drug like characteristics, and safety factors are considered for the selection of optimal ligand [10].

1.2 Problem Statement

Neonates should be avoided from the excessive use of antibiotics. Use of the postbiotics have been proven to be exceptionally successful for prevention against disease establishment proactively. But in neonates affectively of multiple living probiotics could cause uncertain reactions.

1.3 Aim

This study aim is to identify a bioactive compounds or metabolite with postbiotic which will have potential to compete with disease neonatal meningitis cause by the GBS.

1.4 Objective

1. To screen the postbiotics involved in inhibiting neonatal meningitis caused by Group B Streptococcus.

2. To identify the target protein and identification of a ligand of postbiotic which will suppress the virulence factor.

3. To perform a molecular docking to find a optimal binding of protein and ligand

Chapter 2

Literature Review

In most of the Asian countries death of neonates occurs, which is round about 65%, Pakistan is in third rank among the top ten countries.

According to the most up to date data, the mortality rate is 42 per 1000 lives, while the neighboring country like india has the 20 and Afghanistan has the 35 percent rate, Pakistan needs a sustainable method for the improvement of this mortality rate [11].

Neonatal meningitis is one of them which is a life-threatening disease, 24th April is a world meningitis day which is intended for the awareness of this dreadful disease. The infants which are younger than 3 months are more susceptible to developed bacterial meningitis.

According to universal mortality rate for the neonatal meningitis there are about 190,000 cases per year [12]. Neonatal meningitis is causing morbidity and mortality worldwide, it is basically the inflammation of spinal cord and meninges during the first 28 days of a child. There are the two terms which are related to this, Early onset Meningitis (EOM) and Late onset Meningitis (LOM).

In the early stage of meningitis, it occurs in the start days of child birth, while in the late onset it occurs in almost from second week or in within the 28 days of life. It occurs in 25% neonates with bacteremia. In the developed countries it is caused by the GBS, well it accounts for 60% while by E.Coli there are 20% chances.

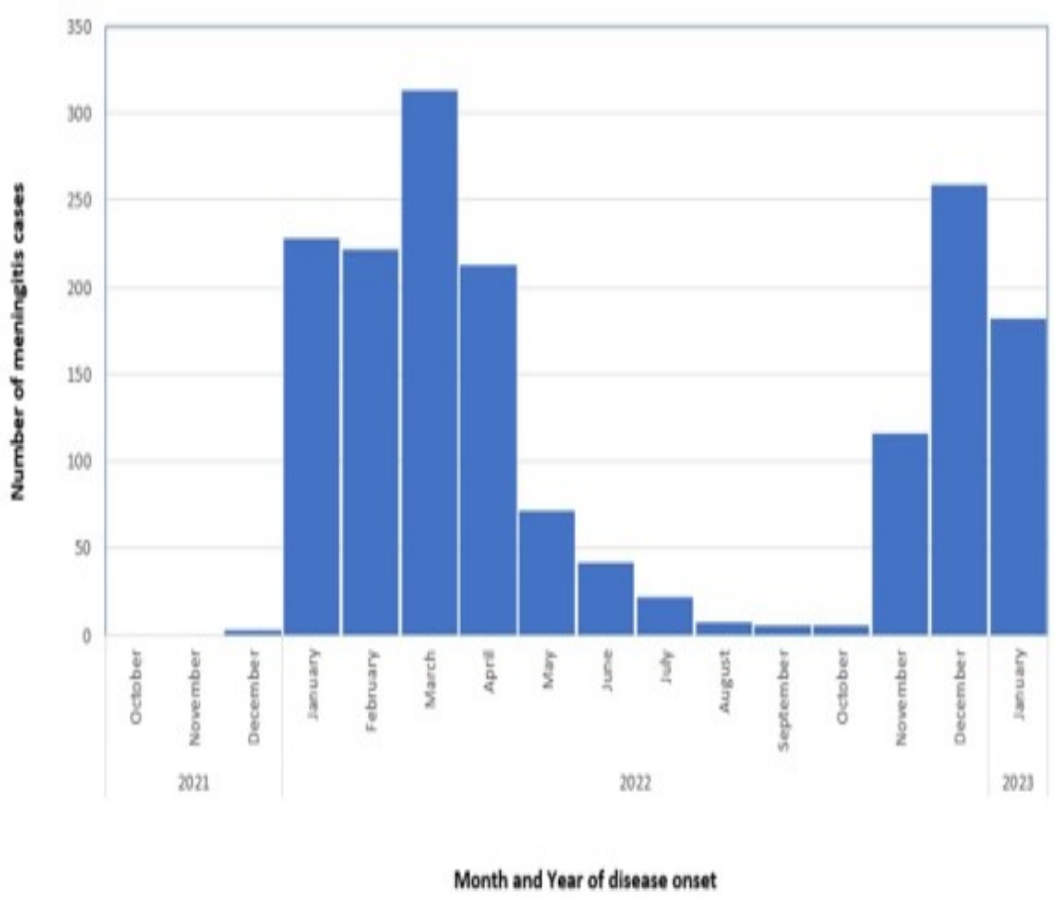


FIGURE 2.1: No of Meningitis Cases

In the developing countries it is caused by the gram-negative bacilli that is klebseilla and Ecoli.

Brain sonograpgy is done for the evaluation of the of patients with meningitis. The brain sonographies reveal the brain abnormalities if it's there any neurological complications [13].

2.1 Symptoms

The symptoms of meningitis include the

• Bulging fontanel, which is due to the increased fluid in the brain

• Fever, basically rise in temperature is a direct indication of a infection but it mostly do not occurs under the age of the 3 month child.

• Cold hand and chills, it includes the shivering

• A stiff neck and irritability and crying, It is due to the sore or stiff neck and the body ache.

• Rapid breathing and vomiting, refuse to feed and red or dark rash on the body [14].

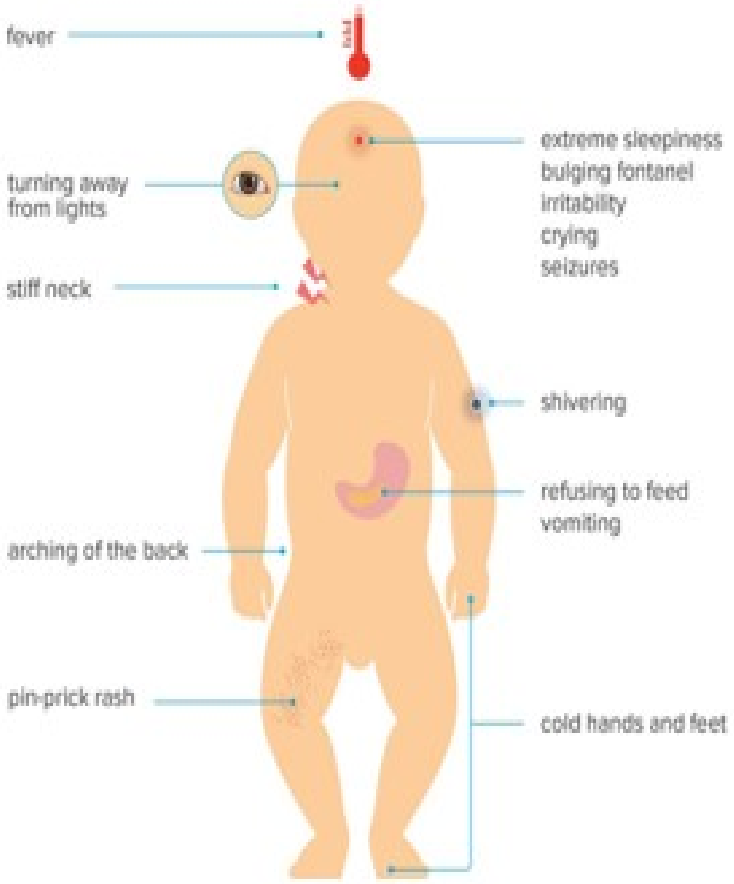


FIGURE 2.2: Symptoms of Meningitis

2.2 Causes

The causes of the neonatal meningitis are bacterial and viruses. There are no of viruses which causes neonatal meningitis which are as follows

• Non polio enteroviruses, the most common cause of viral meningitis and it spreads through the contact with infected person stools, saliva, secretion from eyes and nose.

• Influenza, it can be very common in babies, it spreads through coughing or sneezing

• Herpes simplex viruses, according to WHO, 65% of the world have this virus, a person can spread this to a baby through kissing

• Measles and mumps, well it is very rare these days because number of vaccines are developed

Now the bacterial meningitis caused by the different types of bacteria, the most common type of them are as follows

• Group B streptococcus, it is known as the group B strep. Now basically is passes from mother to the child, if the mother is infected during the labor pain and childbirth.

• Escherichia coli (E. coli) this is also spread through the labor pain, or during the childbirth, or through the contaminated food

• Streptococcus pneumoniae and haemophillus influenza type b (Hib) coughing and sneezing are the main cause of its spreadness.

• Listeria monocytogenes, during the pregnancy if mother consume the food contaminated with the listeria, then fetus will also infected.

• Neisseria meningitidis, saliva is the main reason of its spreadness. [14].

We will focus on the Group B streptococcus, which is the major cause of the neonatal mortality.

Group B streptococcus is a beta hemolytic, gram negative bacterium, it is having a specialty of the capsular polysaccharides and due to which it is divided into the 10 serotypes. These bacteria colonize a women intestine, urethra, or vagina during pregnancy.

The newborn directly gets the infection and passes through the maternal genital tract during the delivery and causes a no of diseases still birth, include the late onset and early onset meningitis and sepsis.

2.3 Mechanism of Action of GBS

Group B streptococcus is a foremost cause of newborn illnesses such as meningitis, sepsis and pneumonia. Number of processes are involve in the process by which GBS cause infection including colonization of the mother vaginal tract, transmission to the newborn, and immune system evasion. Mechanism of action of GBS is given below [15].

2.3.1 Maternal Colonization

GBS colonizes healthy humans gastrointestinal and genitourinary system specially those of expectant mothers. GBS can invade the amniotic fluid during pregnancy and ascend from mother's vaginal tract, which can cause an intrauterine infection or transmit the illness to the unborn child during delivery.

2.3.2 Vertical Transmission During Delivery

The primary mode of vertical transmission of GBS from the contaminated mother to the newborn is during childbirth. During labor and delivery the newborn may aspirate contaminated amniotic fluid or be exposed to infected vaginal secretions both of which might colonization them with GBS.

2.3.3 Invasion and Dissemination with Immune Evasion

Once the newborn has been colonized, GBS can enter and spread through the bloodstream, central nervous system and respiratory tract. Invading and adhering to host epithelial cells, the bacteria can break through mucosal barrier and spread to deeper tissues and organs.

GBS uses a number of techniques to get around the host immune system. Which lets it survive and spread infection. These consist of the expression of the surface proteins, the synthesis of capsular polysaccharides and the control of host

immunological reactions, adhesins and invasions two GB surface protein help the bacteria attach to the host cells and avoid being phagocytized by immune cells.

2.3.4 Induction of Inflammatory Responses

Tissue injuiry and inflammation are the results of GBS inducing inflammatory responses in the host. Meningitis, sepsis and pneuomonia are among the clinical signs of neonatal GBS infections that are influenced by this inflammatory cascade [16].

2.4 Virulence Factors of GBS

The virulence factors which are associated with interaction with the vagina are as follows because vagina is considered as the main reservoirs of the GBS [17].

• Adhesins

• It is the first step for the colonization of GBS in vagina, its adhesions to the epithelial cells through many surface associated adhesins. Now these factors will enable the GBS to bind with the components of the extracellular matrix so that it penetrates the host mucosal barrier and infiltrate the tissue of host.

• Srr 1/ Srr2

• The N-terminal extended signal sequence or Srr1/Srr2, contains one non-repeat binding region and the two highly glycosylated purine rich repetitions. The loci encoding for Srr1 and Srr2 are suitated at distinct loci and share a similar genomic structure. Through a lock dock and genomic mechanism, the GBS Srr family of glycoproteins bind to the single tandem repeat region of human fibrinogen. As a result alterations occur in the Srr family which subsequently leads to adhesion to the target cells. In addition to producing Srr2 with great virulence, Clonal complex strains (CC)-17 strains and are the source of bone and joint infection (BJI). Certain strains of GBS express Srr1, which mediates adherence to the cervical and

veginal epithelium by binding with keratin and fibrinogen. Research indicates that eliminating the complete Srrglycoprotein reduces the GBS colonization. Srr plays a key role in the pathogenic pathway. Srr 1 depends on the protein encoded by the Rga gene and Srr expression is decreased when Rga gene is deleted.

• The fibrinogen binding protein members FbsA, FbsB, and FbsC, which are encoded by GBS, bind to epithelial cells and facilitate vaginal colonization. Pregnant women who are infected often have FbsA and FbsB, but FbsC has the cell wall enchoring motif and the two bacterial immunoglobulin tendem repeat domains that mediate biofilm formation and encourage GBS colonization.

• PbsP is a plasminogen binding surface protein that plays a role in the pathophysiology of GBS. Adhesion proteins with rich regions of methionine and lysine have the capacity to infiltrate and colonize host tissues.

• Pili The GBS encoded pilus is a cell wall-anchored appendage with three structural subunits protein, the capillary axis backbone protein (PilB), the capillary tip protein(PilA), and the capillary base protein (PilC).

The pilus is known as the essential factor which increases the pathogenicity of GBS. The housekeeping sorting enzyme A is present in GBS and is involved in the attachment of pilli on cell walls.

• ScpB, also known as C5a peptidase is a surface associated serine protease that helps prevent complement activation by cleaving the neutrophil chelator C5a, it also aids in the bacterial attachment of fibrinorectin which facilitates the invasion of human epithelial cells by GBS. ScpB is responsible for the sepsis that newborns contract from GBS infections.

• HyiB, stands for hyaluronidase , the exolytic enzyme that the GBS release. The mother-fetal barrier broken by the HyiB cleavage of hyaluronic acid which allows GBS to pass from the vagina to the fetus and induce lethal infection and damage. By binding to toll-like receotors 2 and 4, this cleavage of HA prevents the imflammatory cascade that the GBS component cause. Thus the amniotic cavity is invaded by a strong virulence factor that results in fetal bacteremia. By cleaving the HA , the HyiB inhibit reactive oxygen species (ROS)

• Hemolytic pigment known also as Granadaene , ornithine rhamnolipid pigment is the source of hemolytic activity and is a significant virulence factor in GBS. This granadaene resists the removal of mast cells, neutrophils, and macrophages in addition to processing hemolytic and pigmentary activities. It penetrates the placenta in humans enters the amniotic sac and destroys platelets.

• Alpha C Protein designed to specifically target glycosaminoglycans (GAG) , it is the protype of a family of gram negative bacterial surface proteins, by binding GAG, it makes it easier for GBS to enter cervical epithelial cells and move across the cell layer.

The mechanism of virulence in GBS is a deficiency in the tandem repeat of ACP, which is caused via the rec-A independent route and affects immunogenicity and protective efficacy.

• CAPSULE: This operon encodes the capsule polysaccrhides synthase gene, which is essential for the production of biofilms. The capsule also contain alpha 2 and 3 linked sialic acid residues, the primarily target siglecs, an Ig suprfamily of lectins.

It successfully block the activation of neutrophils and macrophages as well as the development of platelets resistance. It also inhibit the death of GBS and resist platelets derived antimicrobial component.

• Membrane vesicles (MVs) these are extracelleular vesicles that infect the fetal membrane. They include lipids, nucleic acid, and virulence factors such as sialidases, hyaluronate lyases and the C5a peptidase lineage. Collagen is its target location and its merthod involves neutrophil and lymphocyte infiltration as well as a reduction in the integrity of the choriodecidual membrane [17].

2.5 Serine Rich Repeat Protein 2 (Srr2)

A family of cell surface proteins known as serine-rich repeat proteins (Srr2) is present in a variety of bacterial infection including Group B streptococcus. Meningitis, sepsis and pneumonia are among the many illnesses that newborns might

contract due to GBS. The Srr2 Protein is the one specific GBS virulence factor. The repetitive serine-rich domains that define Srr proteins are what give them their adhesive qualities and enable them to attach to extracellular matrix elements and host cells. In particular Srr2 has been linked to the pathophysiology of GBS as well as the bacteria capacity to infilterate and colonize host tissues [18]. Its mechanism of causing infection is as follows

2.5.1 Adherence and Colonization

Srr2 contributes to GBS early adherence to the host cells and tissues. Maternal vaginal tract and neonatal respiratory tract are two habitats where GBS colonizes more easily when Srr2 binds to certain host receptors or extracellular matrix proteins.

2.5.2 Immune Envasion

Srr2 might potentially help GBS evade the immune system. Srr2 may disrupt host immune identification and clearance processes by attaching to host proteins or altering the bacterial surface, enabling GBS to proliferate and spread infection.

2.5.3 Biofilm Formation

According to certain research Srr protein specially Srr2 help GBS develop biofilms. Biofilms are organized bacterial population that are shielded from drugs and host immunological responses by an extracellular polymeric matrix.

2.5.4 Host Cell Signaling

Srr2 may change the host cell responses or function through interfering with host cell signaling pathways. This can possibly exacerbate inflammation or tissue damage during a GBS infection [19].

2.6 Gut Microbiota

The group of microorganisms which inhabit the human body, their genome, their metabolite and the environment in which they are living are known as the microbiota. Our relation with microbiota develop during the first three years of life. Microbiota is basically composed of the different bacteria almost 160 species of bacteria. The microbial colonization in the gut of infant play a very important role in the health of human, include the immunological and metabolic pathway. When this colony got disturbed than it causes the disease. During the breastfeeding and vaginal delivery a no of inoculum of microbiota transferred to offspring during the prenatal time, and also a large no of diversity of microbes colonize with infant during the postnatal environment. Infants delivered vaginally are colonized by the bacteria present in their mothers feces and vagina as well as by species of lactobacillus and bifidobacterium, infants delivered by C-section are colonized by skin associated germs and environmental factors [20]. As there are no of factors which will effect the configuration of the microbes, including the mode of delivery, the food mother take, healing by antibiotics and also the stress which causes a no of disease include allergy, autoimmune diseases, cancer and psychiatric disorders [21].

2.7 Probiotics

According to 2001 Food and Agriculture Organization in the United Nations-World Health Organization FAQ-WHO expert group described probiotics as "live microorganism" and they exert a beneficial health effects on the host [22]. Probiotics influenced gut microbiota by suppress or inhibit adhesion and establishment of the pathogens.

Probiotics develops human immune system, synthesize nutritional elements like vitamins, also involve in enhancement of intestinal barrier by changing the genes involved in the tight junction signaling [23]. Probiotics contains the microbial taxa of lactic acid bacteria for instance Lactobacillus species and bifidobacterium

spp. Probiotics contain a promising application in modulating the immune system particularly documented in infants and shows promising result in cure of allergies, gut and respiratory infections, irritable bowel syndrome [24].

2.8 Prebiotics

International scientific Association of probiotics and prebiotics (ISAPP) prepared a statement about prebiotics that they are the substrate used by the microbes and provide health benefits in return [25]. Prebiotics variate the microbiota makeup, confers health benefits. Prebiotics are basically the organic and inorganic compounds stimulate the growth of digestive tract. intestinal tract , skin and vagina microbial growth.

Prebiotics have Short chain fatty acids, long chain fatty acids, polyphenols, organic acids, polyphenols, oligosachharides, micro algae, natural plants [26]. Prebiotics promotes the activity of probiotics i.e Bifidobacterium species and it is mostly consumed by the infants and breastfed infants, in infants formula it contains oligosaccharides which help in improving metabolism, mineral absorption, also improved the gastrointestinal tract. Prebiotics consumption play an important role in decreasing intestinal infections [27].

2.9 Postbiotics

Probiotics are the group of bacteria that are having benefical health effects on human life, they modify the immunological response, antiginize the pathagenic microbes and compete with adhesins site of the pathogenic microorganism.

Many diseases like infections of urinary tract and digestive tract, lactose intolerant, irritable bowel syndrome, cystic fibrosis and various cancers all are prevented and treated by the probiotics. The probiotics bacteria includes the Bifidobacterium, B.breve , B.lactis, Lactobacillus acidophillus, L.case, L. Plantarum 299v. Live bacteria and yeast are probiotics. Probiotic bacteria are benefical and found all

over the body and also found in the fermented foods like yogurt and kimchi [28]. In 1995, term probiotics Introduced as a group of nutrients degraded by gut microbiota by Glem Gibson and Morcel Roberfroid. Prebiotics are feeding the intestinal microbes and their degraded product is short term fatty acid which then circulate into the blood stream and affects the gastrointestinal tract and many other distant organs. The two important group of the prebiotics that are fructo-oligosaccharides and galacto-oligosacchrides having beneficial effects on human health [29].

Post biotic are the substances released by or formed by the metabolic activity of the microorganism and having directly or indirectly beneficial effects on host. Postbiotics don't contain the live microorganism. Probiotics are the byproduct of probiotic action in the gut i.e fermentation Some postbiotics drugs include [30].

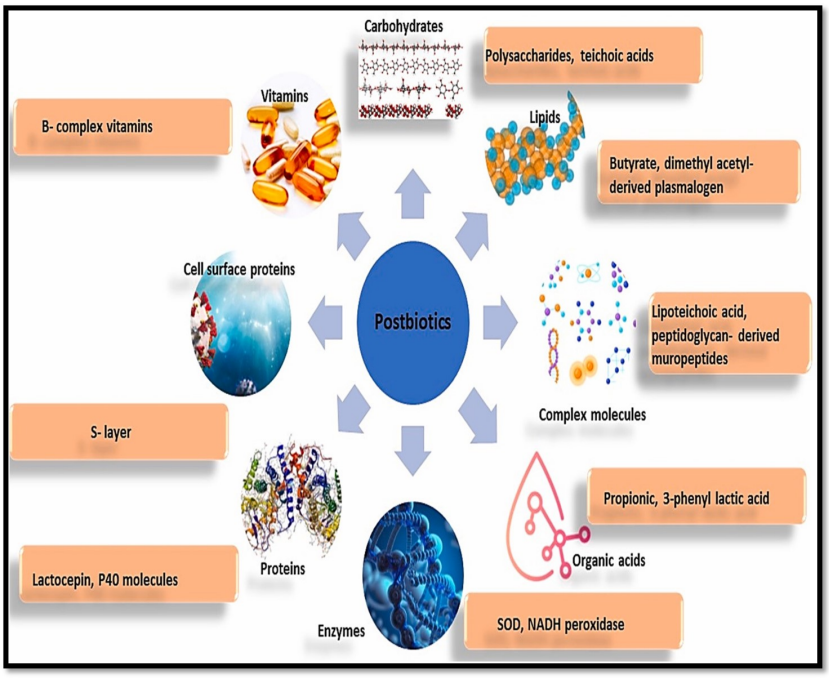


FIGURE 2.3: Main Postbiotics with Functions

1. Cell free supernatants: it contains the metabolites that are straight from cell culture and released by bacteria and yeast into the surrounding liquid. The lactobacillus, acidophilus and lactobacillus casei supernatants exhibit distinct actions such as suppressing the release of tumor necrosis factor and having anti-inflmmatory and anti-oxidant effects on intestinal of epithelial cells, neutrophils, and macrophages.

2. Expo polysaccharides: Microorganism generate exoploysacchrides which are a diverse set of chemicals that are produced outside of bacterial cells by the release of polymer. The lactobacillus strains expopolysacchrides have antioxidant and antimicrobial qualities.

3. Enzymes: Microorganism has a defense mechanism against the harmful effects of the reactive oxygen species. The anti-oxidant enzyme like glutathione peroxidase, peroxidase dismutase, catalase and NADH oxidase play a key role in competing with ROS.

4. Cell wall fragments: lipoteichoic acid (LTA) is found in the cell wall of the gram-positive bacteria, leading to the secretion of anti-infectious peptides

5. Fatty acids and organic acid are produced as result of digestion of prebiotics by bacteria and fibers are fermented by enzymes and release energy. Some of the saturated fatty acid, unsaturated fatty acid and poly saturated fatty acids and organic acid are as follows with their importance in anti-bacterial activities.[31].

2.9.1 Palmitic Acid(C16:O)

Palmitic acid is a saturated fatty acid and most abundant fat in the body it is involve in the post translational modification of proteins it also contain a 16 carbon chain length. The lipid generally added to the infants formula milk is of palm oil contain palmitic acid [27].

Basically milk fat is in tryglycerides form, three fatty acids bound with glycerol at three different positions, mostly palmitic acid bound to glycerol at second position so that when it undergoes digestion, pancreatic lipase break the fatty acid from sn1 and sn2 position and leave the palmitic acid with glycerol back at position so that calcium is obsorbed properly and there is no issue of hard stools on the infants use milk with palmitic acid while the other formula milk which contain the palmitic acid at position sn1 and sn2 forms insoluble calcium soaps because it bind with intestinal lumen due to which the loss of fat(energy) and calcium occurs and also cause the formation of hard stool [32].

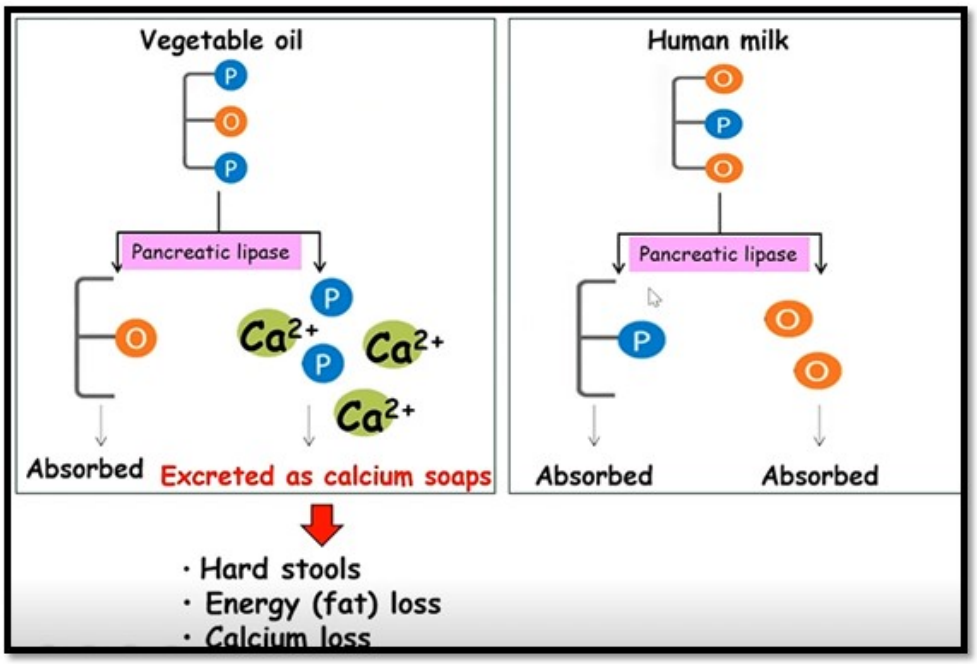


FIGURE 2.4: Absorption of Minerals, Such as Fatty Acid Depend on The Position of Palmitic Acid

2.9.2 Linoleic Acid

Linoleic acid is a fatty acid which is consumed only in the form of a diet. Conjugated linoleic acid is a isomer of a linoleic acid contain conjugated double bond.

There are many strains from various genera inclue Streptococcus, Lactobacillus, Bifidobacterium are able to produce the conjugated linoleic acid, they have a enzyme linoleic acid isomerase ehich convert linoleic acid to its isomers, and they have importance to increase the potential of health and decrease the disease.

It also have a physiological effects including anti-cancer, anti-oxidant, antiatherosclerosis anti-obesity and also have anti-microbial activity. Among the all top strains of probiotics, Lactobcillus plantarum produce the highest concentration of conjugated linoleic acid isomers [33].

2.9.3 Palmitoleic Acid

From the last few years monounsaturated fatty acids are considered as a biomarker for the improvement or regulating health benefits. Palmitoleic acid is one of the

unsaturated fatty acid which is produced by the palmitic acid stearoyl-CoA desaturase and fatty acid desturase action. It is having a benefical health effects on chronic metabolic diseases like obesity, atheroseclerosis, cardiovascular diseases, non alcoholic fatty liver disease and cancer. It is most abundant member of family having important biological actions and mod modulate metabolic responses. They are known for their anti-bacterial activity towards the gram negative and gram positive bacteria [34].

2.9.4 Arachidonic Acid

Arachidonic acid is a unsaturated fatty acid and it is formed by the biosynthesis of linoleic acid. These fatty acids play an important role in structure and functioning of the immune function , brain and retinal function , functioning of human tissues. From the last few years number of infants are not receiving breast milk, they are receiving formula milk, arachidonic acid is present in milk and a good source of nutrition [35].

2.9.5 Citric Acid

Citric acid f is a organic acid found in citrus food i.e lime and lemon. There are number of benefical functions of probiotics and citrus food as the consumption of these probiotics having natural bioactive compound maintain the blood pressure, mental health is enhanced,boost immunity ,eliminate sleep disturbance. Several citrus fruits like oranges, lemon , falvenoids are having high potential benefits include anti-microbial , anti-cancer, anti-inflammatory, anti-diabetic activities [36].

2.9.6 Lauric Acid

It is a fatty acid found in saturated fat foods like its most common source is coconut and coconut oil, they are well known for their anti-microbial activity, anti-bacterial activity, fat burning and hormone balancing properties. Lauric acid having disease fighting capabilities and inhibit growth of pathogens. Lauric acid

is a precursor of monolaurin , which is a anti-microbial and anti-bacterial agent [37].

2.9.7 Oleic Acid

Oleic acid are naturally occurred in many animal and vegetables oils and fats, they are well known for their anti-bacterial activity, specially they inhibit the growth of several species of gram positive bacteria. It is an unsaturated fatty acid contain double bond and have a potential for the anti-bacterial activity [38].

2.9.8 Malic Acid

It is an organic acid and organic acid are well known for their anti-bacterial and anti-microbial actions. They have bactetriocidal and bacteriostatic properties. Malic acid has low molecular weight and can easily enter into the bacterial cells and damage their cytoplasam. It is present in all the fruit juices [39].

2.10 Mechanism of Action of Postbiotic

In figure 2.5 Postbiotics show a pleiotropic property. The equilibrium between the two main arms of the immune system represented by the Th1 and Th2 cells, is restored by the production of T regulatory lymphocytes after infection. The balance is important for the immunoregulation and its disturbance causes many diseases. Postbiotics have Immunomodulatory effects, anti-tumor effects, infection prevention, anti-atherosclerosis effects and autophagy mechanism [40]. Bioactive substances which are known as Postbiotics are formed when probiotics are fermented or when breakdown of microbial cells take place. There is a wide range of materials in it include polysacchrides, enzymes, fatty acids, organic acid, peptides and some other metabolites, a few of them I explain earlier which I used as a ligand. Postbiotics work in a number of ways and their mechanism of action is given below

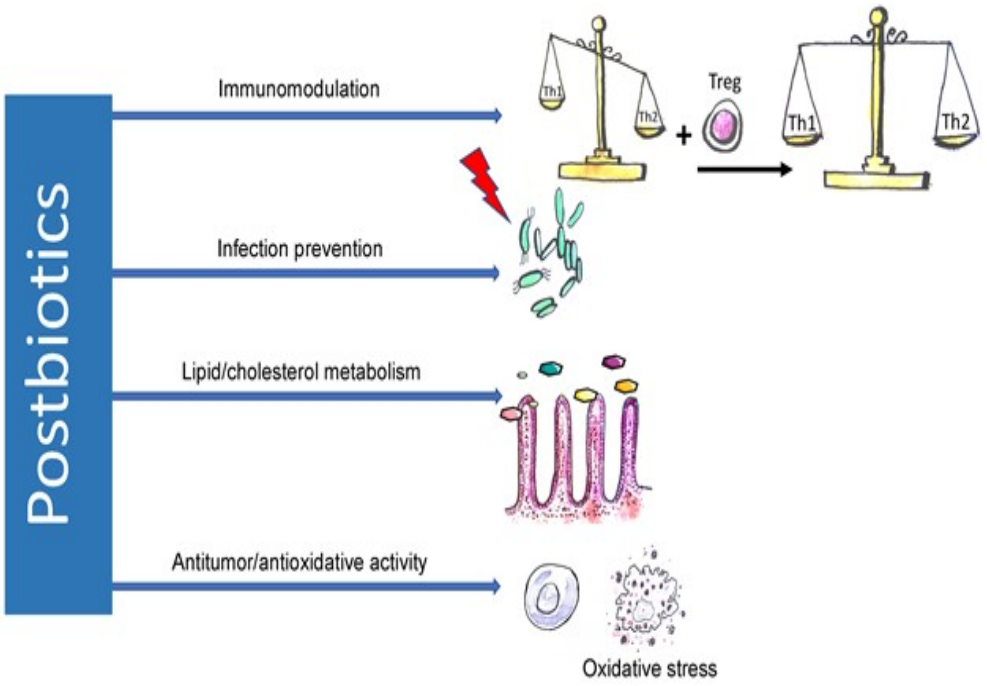


FIGURE 2.5: Mechansim of action of Postbiotics

2.10.1 Immunomodulation:

by increasing the activity of the immune cells including macrophages, dendritic cells and lymphocytes Postbiotics can modify the immune cells. They support immunological homeostasis by increasing the production of anti-inflammatory cytokines and decline the production of pro-inflammatory cytokines [41].

2.10.2 Anti-Inflammatory Activity:

by preventing the synthesis of inflammatory mediators such as prostaglandins, nitric oxide and cytokines, Postbiotics can have anti-inflammatory effects. Additionally, by preserving the integrity of the gut barrier, they can lessen the amount of inflammatory chemicals that enter the bloodstream [42].

2.10.3 Anti-Oxidant Properties:

antioxidant activity in many Postbiotics enable them to scavenge free redicals and lessen oxidative stress. This can support general health and longevity by shielding

cells and tissues from harm from reactive oxygen species [43].

2.10.4 Enhancement of Gut Barrier Function:

postbiotics have the ability to fortify the intestinal epithelial barrier through the stimulation of the tight junction protein and mucin expression. This lower the risk of inflammatory and autoimmune illnesses by preventing the release of toxic chemicals from the gut into the bloodstream [44].

2.10.5 Metabolic Regulation:

by adjusting the activity of enzymes involved in the metabolism of fats and carbohydrates, Postbiotics can affect how their host metabolize their food. Additionally they have the ability to control the expression of genes linked to lipid homeostasis, insulin sensitivity, and energy balance, which may aid in the prevention of diabetes, obesity and other metabolic diseases [45].

2.10.6 Anti-Microbial Activity:

certain Postbiotics demonstrates direct anti-bacterial actions against viruses, fungi, and harmful bacteria. By vying for nutrients, generating antimicrobial substances and altering the microbial makeup of gut microbiota, they can prevent the development and spread of dangerous microbes [46].

2.10.7 Neuroprotective Effects:

recent research indicates that postbiotics may possess neuroprotective qualities which could aid in the prevention or treatment of neurological conditions like depression, Parkinson's disease and Alzheimer's disease. They can decrease neuroinflammation, alter the gut brain axis, and increase the synthesis of neurotropic factors and neurotransmitters [47]. These modes of action demonstrate the wide range of advantages postbiotics have for overall health and wellbeing in people.

Chapter 3

Methodology

3.1 Selection of a Problem

Neonatal infections are spreading widely and for this purpose antibodies are recommended. But due to excessive use of antibiotic.it causes resistant. Postbiotics have been exceptionally proven contributor towards neonatal infections.

3.2 Target Protein Selection

To treat the neonatal infection, there are many active metabolites of Postbiotics are involved against the group B streptococcus. These metabolites are having a vital role in inhibition of infection. The target protein which is selected on the basis of their virulence and pathogenicity factors are serine rich protein Srr2 [48].

3.3 Physiochemical Properties of Protein

Physicochemical properties are important in determining protein function. ProtParam https://web.expassy.org / protparam is used for prediction of physical and chemical properties of target protein which is alpha C protein, it includes positive charge and negative charge residue, volatility, theoretical PI, molecular weight, BP,

MP, size, Instability index, aliphatic index, and grand average of hydrophobicity [49].

3.4 3D Structural Prediction of Protein

The 3D structure prediction of the target protein done through PDB protein data base https://www.rcsb.org .we can also use an alternative database which is I-TASSER, which is Interactive Threading Assembly Refinement. These are the online server which can be used for the prediction of 3D structure [50]. Alphafold https://alphafold.com is also a protein structure database which can be used to predict the 3D structure [51].

3.5 Structure Analysis by Use of PyMOL

For the three dimensional analysis and visualization of many protein there is a platform of PyMOL. Rather than protein it is also used for the small molecules nucleic acids, densities of the different electrons and varying surfaces and also the trajectories. PyMOL is used to make animations and movies, for editing the molecules and tracing the rays. The file of protein structure uploaded on PyMOL and then the attached water molecues and ligands are removed from the structure, PyMOL is used for the removal of these extra constituents attached to the protein structure. [52].

3.6 Functional Domain Identification of a Target Proteins

Interpro http://ww.interpro.com is an online database which is used for the identification of the functional domain of the target protein which is serine rich protein Srr2 . The conserved domains are in sequence structure relationship. [53].

3.7 Retrieval of Chemical Structure of Ligands

For the retrieval of the structure of ligands, there is an easily accessible chemical information database, having world chemical information repository is PubChem. https://pubchem.ncbi.nlm.nih.gov/.

The chemical compounds which are used as a ligands selected from the PubChem database. We can get there structure, canonical smiles from this database, it contains chemical substances information and their biological activites. Ligands are selected in the basis of their inhibitory action [54].

3.8 Energy Minimization

When ligands are selected we refined them by Chem 3D pro software. This is used for the energy minimization of ligand. This step having an importance for preparing the ligand for docking. The reason behind this is that unstable ligand shows unreliable score.

3.9 Bioactivity Analysis of Ligands and Toxicity Measurement

The chemical compound we selected as a ligand through PubChem database must follow the Lipinski rule of five. The success of a active drug used in humans depends on its ADMET properties. For this we have an online database which is PkCSM https://omictools.com/pkcsm-tool. The rule is described as under

The logP value of most 'drug-like' molecules should be limited to 5
Maximum no of H-bond acceptor should be 10.
Rotateable bond should be equal to 5
Molecular weigt should be less than 500
Maximum no of H-bond donor should be 5 [55].

3.10 Molecular Docking

It is employed to forecast when a ligand is bind to a protein, 3 Dimension Structure is the aim of ligand protein docking. Molecular docking is a crucial tool in structural molecular biology and computer assisted drug designing. cbDock2 is a software used for the molecular docking.

Target protein and the candidate ligand are the two essential requirements for docking. Active metabolites of Postbiotics found as a ligand against the alpha C protein. cbDock 2 *http* : *//clab.labshare.cn/cbdock/php/blinddock.php* is an online server which identify the binding sites and used to perform docking [56].

3.11 Process of Molecular Docking

The first step is the generation of the protein and ligand file. The protein file must be in pdb form and ligand in sdf files. Then protein pdb file was given to cbDock as input file and then target protein file saved in pdbqt format [57].

Then ligands file are uploaded in the same way one by one and then saved in pdbqt format, then grid box setting applied to the protein ligand structure, for this purpose macromolecules option selected from grid and pdqt files was opened and then set map type option. Docking file were created for the choosen data set after completion of this step and then results are saved in pdbqt format [58].

3.12 Active Site Identification

Ligands show highest or lowest interaction with the protein, where the target protein has their active site, amino acid are exceedingly involved in the establishment of the ligand and protein complex.

CASTp software https://sts.bioe.uic.edu/edu/castp is used for the identification of protein binding pockets [59].

3.13 Protein Ligand Interaction

For the interpretation of docking results the interaction of protein and ligands are calculated by using Ligplot plus. This software generates diagram of protein ligand interaction in PDB files. It shows both hydrogen bonding and hydrophobic bonding type interaction [60].

3.14 Lead Compound Identification

When a detail analysis of protein and interactions are done, then according to their docking scores and toxicity studies the compound show highest score is selected as lead compound.

3.15 Reference Anti-Bacterial Drug Identification and Selection

This step is performed to identify the drug used as an anti-bacterial its ADMET properties and physiochemical properties are identified and then compared with the ligand and same docking scores are also compared.

3.16 MD Simulations

Then in the last step MD simulation is done. Molecular dynamic simulations are a computational method to evaluate macromolecular structure-to-function relationships. The main objective of the MD simulations was to evaluate the complexes dynamics in a fixed period. The MD simulations were done using the AMBERv22 package. The preprocessing was done using an antechamber module. To parameterize the proteins, the ff19SB force field was applied while the ligands parameters were generated using the GAFF2 force field. The initial preprocessing was done in

different phases such as all atoms energy minimization, water box minimization, and heavy atoms energy minimization with a variable set of restraints on the carbon alpha atoms. The heating of each system was achieved gradually to 310 K for a time scale of 2 fs. This was followed by pressure equilibrium at 1 atm. The production run was performed at 100 ns. To apply constrain on the temperature, the Langevin algorithm was applied while the hydrogen bonds were constrained using the SHAKE algorithm. The simulation trajectories were investigated using the CPPTRAJ module.

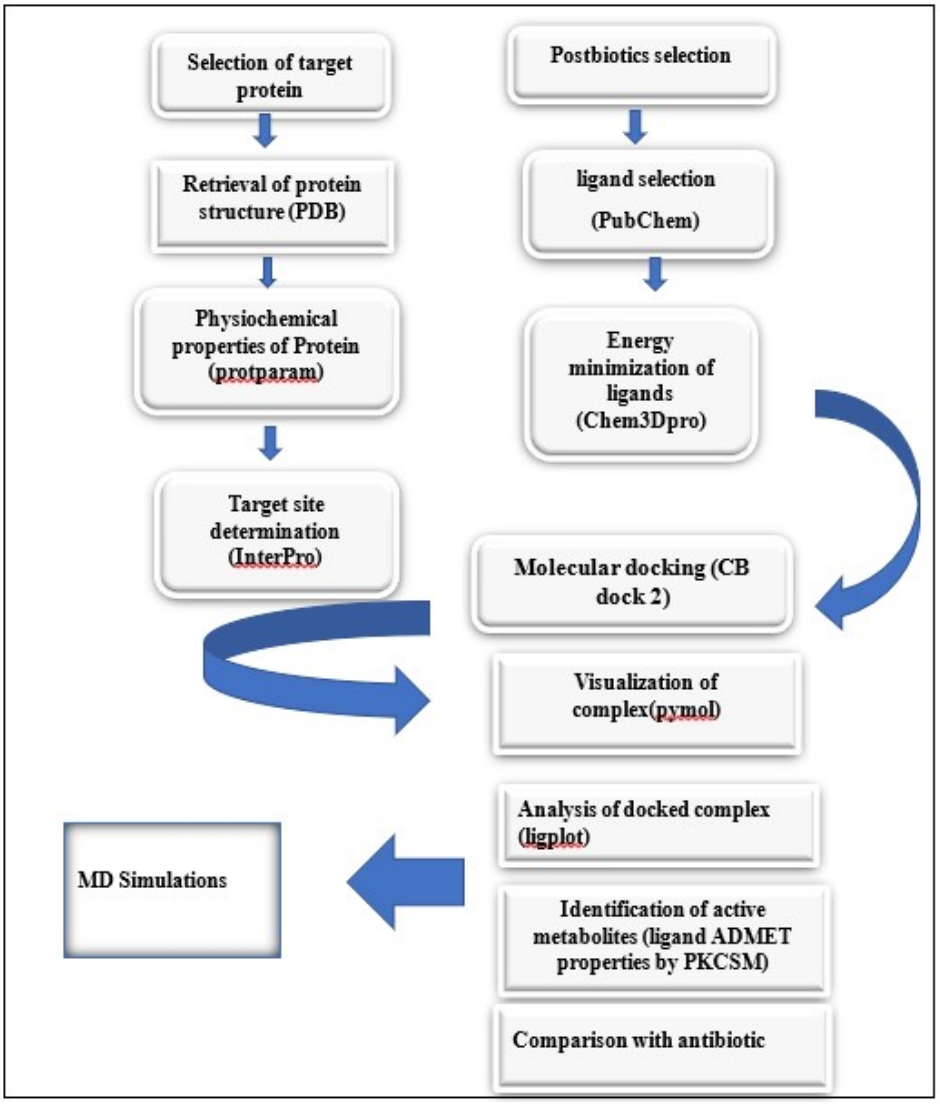


FIGURE 3.1: Flow Chart

Chapter 4

Results and Discussion

Now this chapter will explain the results and discussion by following our methodological steps. 3D structure of proteins and ligands taken as input, after examining their physiochemical properties the protein was docked against the selected ligand which was refined by minimizing their energy. ADMET properties and Lipinski rule helped in the prediction of the drug like features of the compounds. All these step are described below.

4.1 Structure Modeling

It includes the primary sequence retrieval, physiochemical properties prediction, 3D structure prediction, and functional domain identification of proteins.

4.1.1 Primary Sequence Retrieval

FASTA sequence of selected protein was retrieved through NCBI, under the accession number of $4MBR_AGI$: 557129588.This protein is selected on the basis its pathogenicity and virulence causing factor. FASTA sequence of Serine rich repeat protein 2 was downloaded from NCBI.

```
>pdb|4MBR|A Chain A, Serine-rich repeat protein 2
METLPAALISGEGDVTTVQGQDVTDKLQNLDIKLSGGVQAKAGVINMDKSESM
HMSLKFTIDSVNRGDTF
EIKLSDNIDTNGASNYSIVEPIKSPTGEVYATGIYDSQKKSIVYSFTDFAASK
NNINGILDIPLWPDDTT
VQNTKEDVLFSVKIKDQEATIKETVKYDPPVRIDFAGGVSVDSRITNIDDVGK
KMTYISQINVDGKSLYN
YNGLYTRIYNYSKESTADLKNSTIKIYKTTSDNIVESMVQDYSSMEDVTSKFA
NSYPEKGWYDIYWGQFI
ASNETYVIVVETPFTNAVTLNTTLSDYNENNGVEHNHTYSSESGYSDVNAQER
KILLEHHHHHH
```

FIGURE 4.1: Fasta Sequence of Srr2

Comparative investigations are made possible by the sequence retrieval of serine rich repeat protein 2 from genomic databases, which reveals both functional divergence and evolutionary conservation among species (chen et al.,2019;) [61]. Finding conserved motifs and putative post translational modification sites that are essential for protein function is made easier by the integration of like BLAST and HAMMER (Altschul et al., 1990; Eddy, 2011) [62][63]. Now we retrieve these sequence to check the virulence cause by these in causing neonatal infection.

4.1.2 Physicochemical Characterization of Protein

An online tool called protparam can be used to forecast the chemical and physical characteristics of a given protein. Molecular weight, amino acid composition, theoretical protein index value, atomic protein composition, extinction coefficient estimated half life of protein instability , aliphatic index, and grand average of hydrophillicity abbreviated GRAVY are among the parameters that can be identified using software.

Proteins with a computed PI of greater than 7 are considered basic, whilest those with a PI of less thyan 7 are considered acidic. A light absorption is shown by the extinction coefficient . protein stability is indicated by an instability index of less than 40, whereas protein instability is indicated by an index greater than 40 . serine rich repeat protein factor 2 physiochemical characterization are as follows [64].

TABLE 4.1: Physicochemical Properties of Protein

Target Protein	**Serine Rich Repeat Protein Srr2**
Molecular weight	38442.52
No of amino acids	344
Theoretical PI	4.80
Negatively charged residue (NR)	49
Positively charged residue (PR)	30
Extinction coefficient	46300
Abs 0.1 %	1.204
Instability index	25.64
Aliphatic index	78.14
GRAVY	-0.522

The protein's aliphatic composition is indicated by the aliphatic index. The protein's thermostability is indicated by the high aliphatic index value. Protein residues are positively and negatively charged are included in molecular weight. Better interaction with water molecule is seen at low GRAVY. MM denotes molecular weight, while PI represents the hypothesized isoelectric point at which a protein is charge free and neutral.

PR stand for the total amount of positively charged residues (Arg + Lys), and NR stands for negatively charged residues (Asp + Glu). When all cys residues are assumed to be reduced. The extinction coefficient is expressed as Ext Co2 and the grand average of hydropathicity as GRAVY.

4.1.3 3D Structure Prediction of Proteins

A target protein 3D Structure can be downloaded in PDB format from the RCSB PDB. A three dimensional database including complex compounds from living things, such as proteins and nucleic acids is called as the protein database. I-TASSER iterative threading Assembly Renement is also a special procedure in order to predict the structure of the protein and also the function of the protein.3D Structure of alpha C protein is downloaded from the PDB [65].

The 3D structure of purine rich repeat protein 2 Srr2 taken from the PDB under ID 4MBR

Using PyMOL, the protein structure was created by eliminating any ligands and water molecules that might have been present. The missing polar hydrogens were added after the ligands and other atoms were eliminated. In order to achieve stable conformation by avoiding overlaps, the energy reduction for the structure was carried out and the amended file was saved in PDB format.

The refined structure of a protein is given in figure 4.2.

FIGURE 4.2: Refined Structure of Srr2

Serine rich repeat protein 2 Srr2 function in cellular processes is aided by the 3D structure prediction which provides important insights into the protein's functional architecture and possible protein-protein interaction. We can produce structural

models that clarify important structural elements and binding interfaces by using computational techniques like homology modeling and molecular dynamics simulations. This make mechanistic study easier to conduct. (Doe et al.,2021 ;Smith et al.,2020) [66][67].

4.1.4 Functional Domain Identification of Protein

The domains and functional location of a choosen protein are identified using the Inter Pro database. Protein sequences can be functionally analyzed using interpro as a resource. The connection between sequencing and structure involves conserved domains. A protein may have a multiple functional domains, each of which carries out a distict task. The portion of a protein that is actively involved in how other substances interact with proteins is known as its functional domain. Purine rich repeat protein factor 2 is a 344aa long protein having two domains. One is SDR-Ig starting from residue 47 and ending on 145, while the other one is the $adhesion_Fg - bd - dom_2$. It starts from the residue 179 and ends at 320 [68].

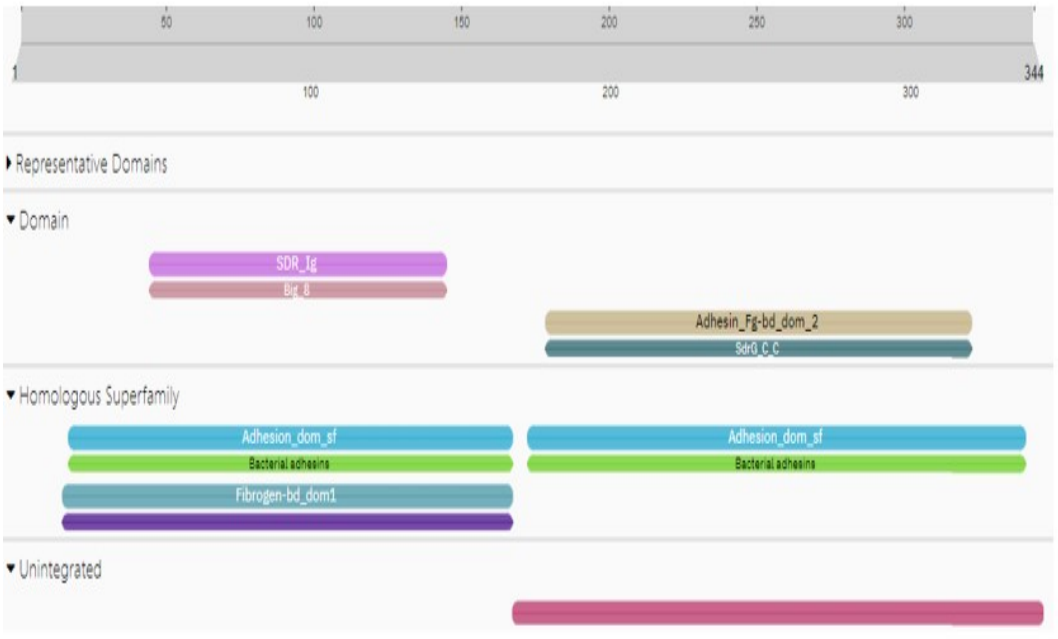


FIGURE 4.3: Scan Result of Domain of Srr2

The structure of a Srr2 protein with domains highlighted by different colors is given in figure 4.4.

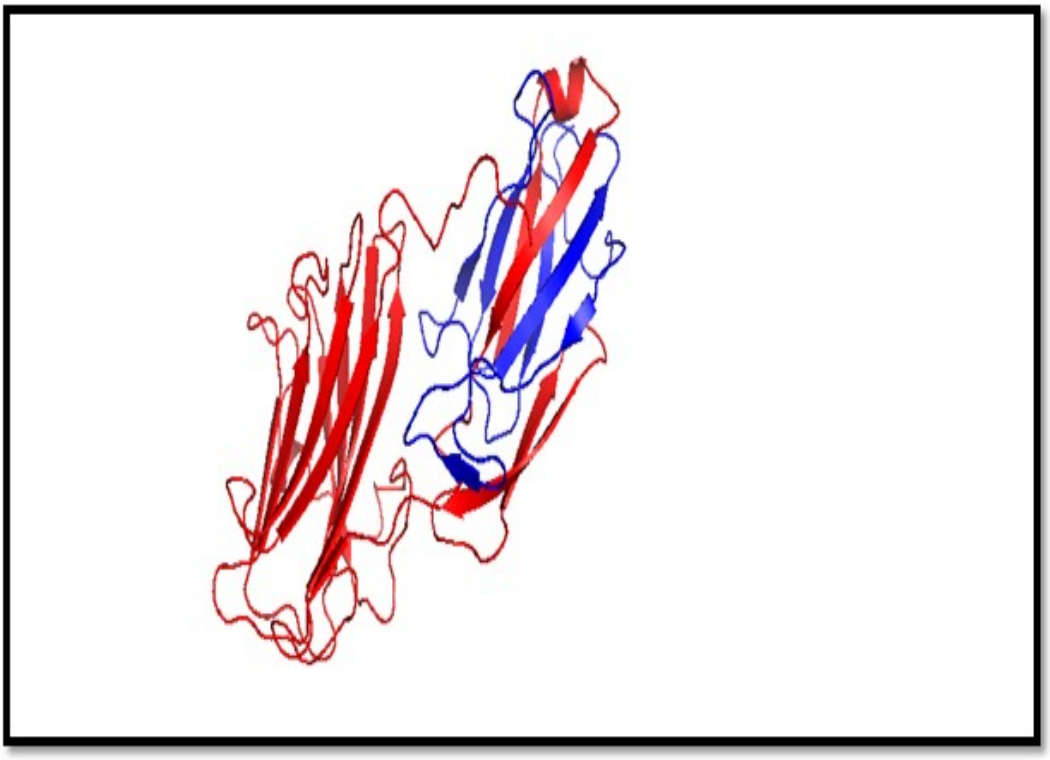


FIGURE 4.4: Shows Domain of Srr2

There is the first domain named as SDR_Ig, entry represents a bacteria Ig fold domain, found in the members of serine aspartate repeat containing protein SDR family, it starts from the 47 and ends at 145. While the blue color represents second domain named as $adhesion_Fg - bd - dom_2$. It represents the fibrinogen binding domains having similar core beta-sandwich topologies.

4.2 Ligand Selection

Protein ligand complexes, particularly for the target protein are abundant in the protein databank. As a result the best binding affinity, chemical class of the co crystal ligand attached to the protein structure, and structure resolution are taken into consideration while choosing ligands.

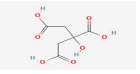
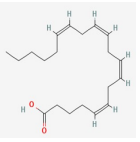
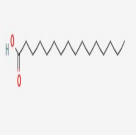
The process known as conformational selection occurs when a ligands bind to one of these conformers with preference, strengthening it and increasing its population relative to the protein's overall population, finally producing the complex that have seen. [69]. Ligands were searched out from the chemical information database

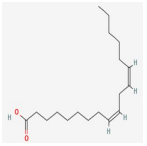
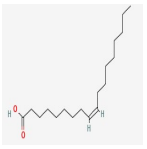
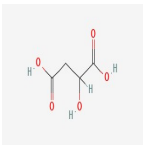
which is PubChem https://pubchem.ncbi.nlm.nih.gov from here the information can be accessed freely around the globe.3D Structures are downloaded from the PubChem in sdf format so that we will easily perform the docking. When ligands are selected, then we refined them by minimizing its energy through chem pro software (chem 3D v 12.0.2).

The lipimski rule deals with different parameters include molecular weight which should be less than equal to 500, log P less than or equal to 5, hydrogen bond acceptors is less than or equal to 10 [70]. In the prepration of the ligands for docking this is an essential step because the unstable ligands reveals the inconsistent vina scores of docking.

Bioactive compounds of postbiotic are selected as ligands for the study. The selected ligands are citric acid, lauric acid, oleic acid, palmitoleic acid, palmitic acid, arachidonic acid, malic acid and linoleic acid . All of these follow the Lipinski rule [71].

TABLE 4.2: Ligands With their Structure and Molecular Weight

Sr. No	Ligands name	Molecular formula	Molecular weight (g/mol)	Structure
1	Citric acid	$C_6H_8O_7$	192.12	
2	Arachidonic acid	$C_{20}H_{32}O_2$	304.5	
3	Palmitolic acid	$C_{16}H_{30}O_2$	254.41	
4	Palmitic acid	$C_{16}H_{32}O_2$	256.42	

Sr. No	Ligands name	Molecular formula	Molecular weight (g/mol)	Structure
5	Lauric acid	$C_{12}H_{24}O_2$	200.32	
6	Linoleic acid	$C_{18}H_{32}O_2$	280.4	
7	Oleic acid	$C_{18}H_{34}O_2$	282.5	
8	Malic acid	$C_4H_6O_5$	134.09	

4.3 Molecular Docking

Using a unique scoring function, the molecular docking technique estimates the strength of the interaction between the ligand and a target protein and establishes the proper ligand structure within the target binding site. Docking uses the three dimensional structures of the ligands and target protein as its input. It represents a frequently used approach in structure based drug design since it requires a 3D structure of a target protein.

It can be used to determine the correct structure of the ligand within the target binding site and to estimate the strength the of the binding between the ligand and the target proteins through a specific scoring function. It also helps in the recognition of new small molecular compounds, revealing the essential properties such as high interaction between binding with target protein having reasonable absorption, distribution, metabolism, and excretion which help in the selection of the lead compound for the target [72].

Srr2's molecular docking studies provide important information about possible interactions with other biomolecules and its functional involvement in cellular processes. We can estimate the binding affinities and putative binding sites of Srr2 with ligands using this computational technique of molecular docking which facilitate the investigation of its molecular processes and its therapeutics application (Grosdidier et al.,2011) [73] [74].

The docking was performed using purine rich repeats protein 2 Srr2 and ligand were citric acid, arachidonic acid, palmitoleic acid, palmitic acid, lauric acid, linoleic acid, oleic acid and malic acid . Ligands with the best binding score values with the target protein presented in a table given below. To automatically predict binding modes without information about binding sites, a user friendly blind docking web server called CB Dock was used.

CB dock uses the well known Auto dock vina docking tool to perform docking and predicts and estimate a binding site for a given protein. It also calculates the centers and sizes using a unique rotating cavity detecting algorithem. CB Dock gives five best interacting confirmations for each ligand molecule [75].

These confirmation were arranged based on binding affinity and then finest confirmation selection was done on the basis of highest affinity score of protein-ligand interaction. After docking process, the dock structures were selected for further analysis. On the basis of docking score, cavity size, grid map, binding energy one can select best docked structure.

4.4 Active Site Identification

To identify active sites of protein, CASTp software was used which forecast available pockets for binding and also tells about surface area and volume of serine rich repeat protein 2. The table below shows the area and volume of serine rich repeat Protein 2 [76].

The table 4.4 representing binding pocket IDs with area and volume of purine rich repeat protein 2 along with area and volume. It shows that there are twenty four

TABLE 4.3: Results of CB Dock with Ligands Name, Binding Score and Cavity Size

Sr No	Ligands Name	Binding Score	Cavity Size
1	Citric acid	-7.3	6126
2	Arachidonic acid	-6.3	6126
3	Palmitoleic acid	-5.8	354
4	Palmitic acid	-5.5	6126
5	Lauric acid	-5.4	6126
6	Linoleic acid	-5.4	6126
7	Oleic acid	-5.2	6126
8	Malic acid	-5.0	6126

pockets available for purine rich repeats protein 2. The largest binding pocket has surface area 1223.98 where as its volume is 876.646 while the smallest binding pocket has surface area 0. 817 and volume 0.051.

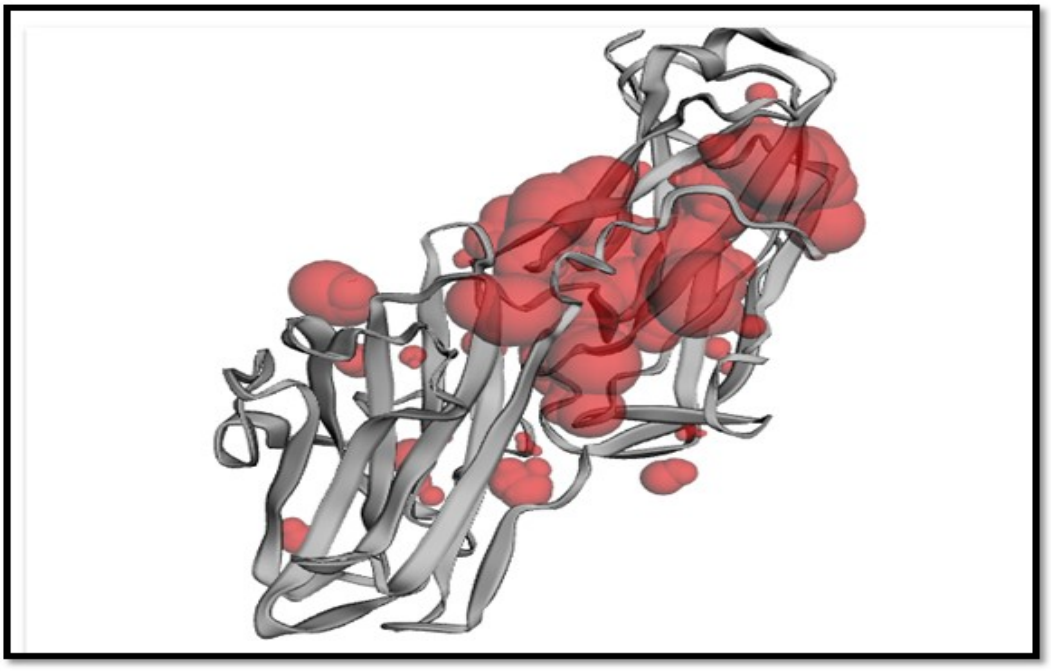


FIGURE 4.5: Shows the Packet Available for Binding

Figure 4.5 representing serine rich repeat protein srr2 . Red color showing the available binding pocket for protein. Binding pocket is the region where ligand can bind. The number of pockets with size and volume is already shown in above table.

TABLE 4.4: Area and Volume of Binding Pockets of Serine Rich Repeat Protein 2 by CASTp

Pocket id	**Area (SA)**	**Volume (SA)**
1	1223.98	876.646
2	42.387	25.232
3	55.284	22.611
4	44.117	19.709
5	17.263	12.520
6	33.307	10.352
7	18.820	5.391
8	11.205	1.645
9	8.665	1.394
10	3.276	1.391
11	5.761	1.094
12	5.916	0.966
13	14.193	0.866
14	4.190	0.571
15	6.051	0.563
16	6.296	0.513
17	3.813	0.269
18	2.526	0.230
19	2.755	0.160
20	1.876	0.111
21	1.149	0.104
22	1.962	0.069
23	1.879	0.063
24	0.817	0.051

4.5 Interaction of Ligands and Target Protein

The interaction of the active pockets of the ligand and the protein were calculated for the interpretation of docking results. Two types of the interactions were studied , hydrogen bonding and the hydrophobic bonding interaction. Using Ligplot plus

(version v. 1.4.5) the protein ligand interactions were studied [77] [78] [79]. By using Ligplot plus the interaction of the active confirmation of ligands and the target protein has been identified. The saved conformations for ligand receptor complex of each molecule were analyzed in detail [80]. This software automatically generate schematic diagrams of the protein-ligand interactions of the given ligands in the PDB file.

The docked files were uploaded in PDB format to get hydrogen and hydrophobic bonding. A significant number of hydrophobic and hydrogen bond interactions were observed between 8 ligands and a target protein. Ligand-receptor complex shows strong hydrogen bonding and hydrophobic interactions [81] [82].

Following diagrams shows ligand receptor interactions

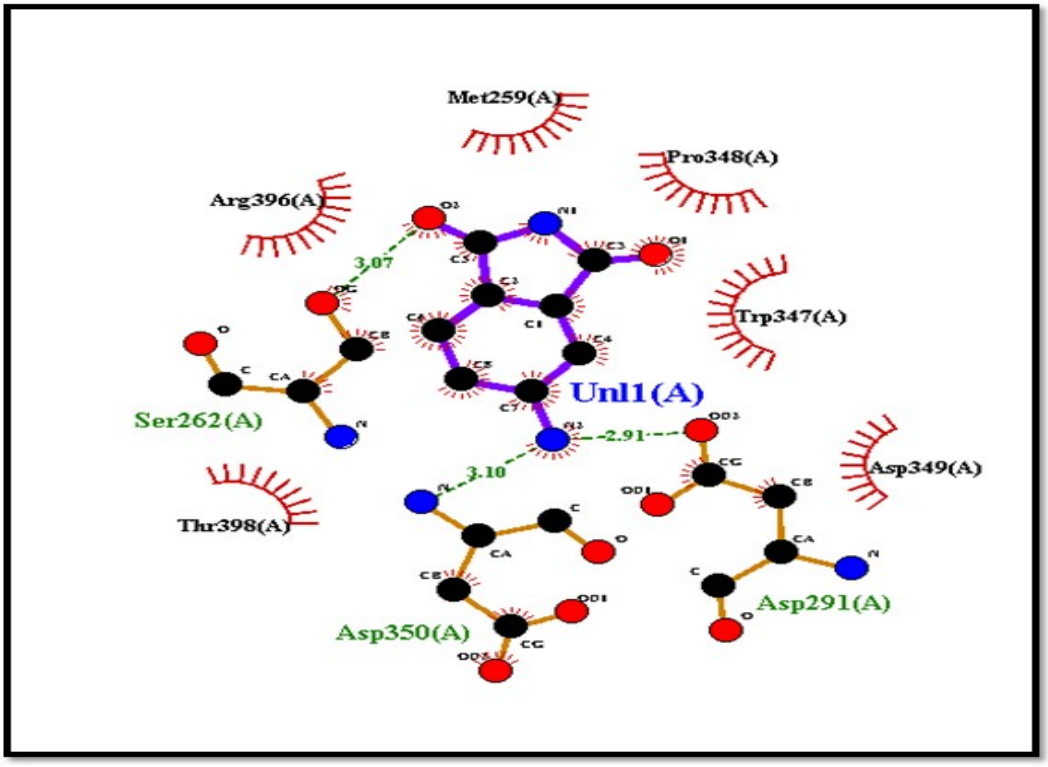


FIGURE 4.6: Interaction of Citric Acid by Ligplot

Figure 4.6 shows the interaction of citric acid with receptor protein. It shows citric acid formed three hydrogen bonds and six hydrophobic interactions.

Figure 4.7 shows the interaction of arachidonic acid with receptor protein by ligplot. It shows propionic acid formed one hydrogen bond and twelve hydrophobic interactions

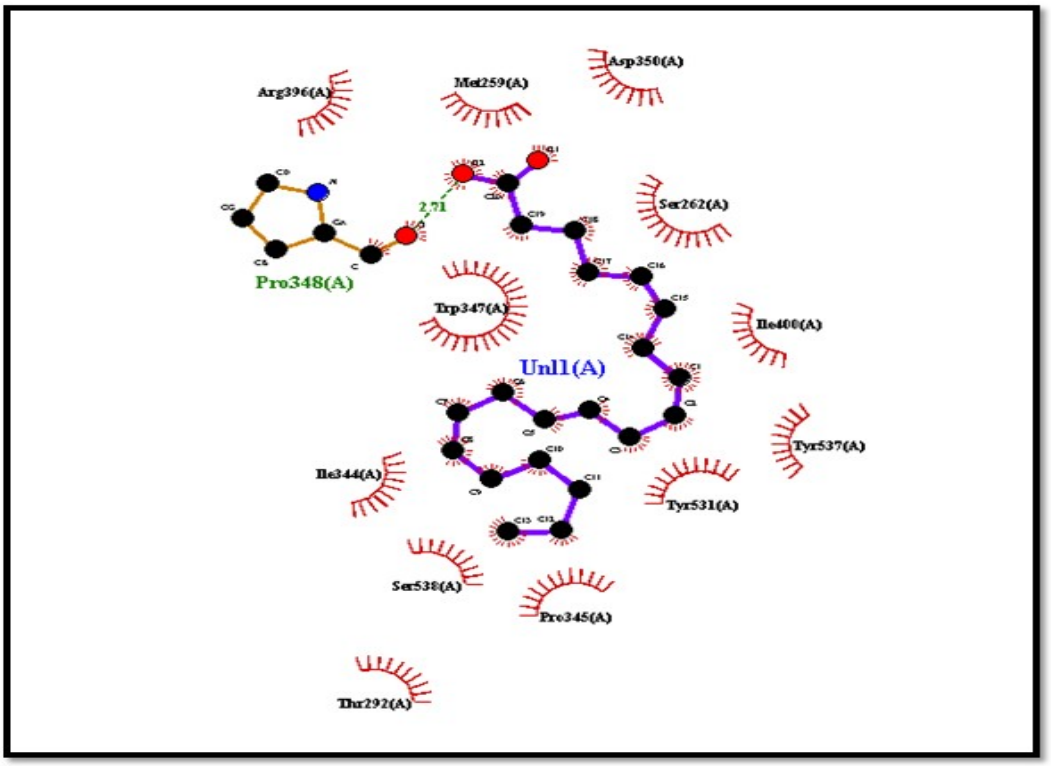


FIGURE 4.7: Interactions of Palmitoleic cAd by Ligplot

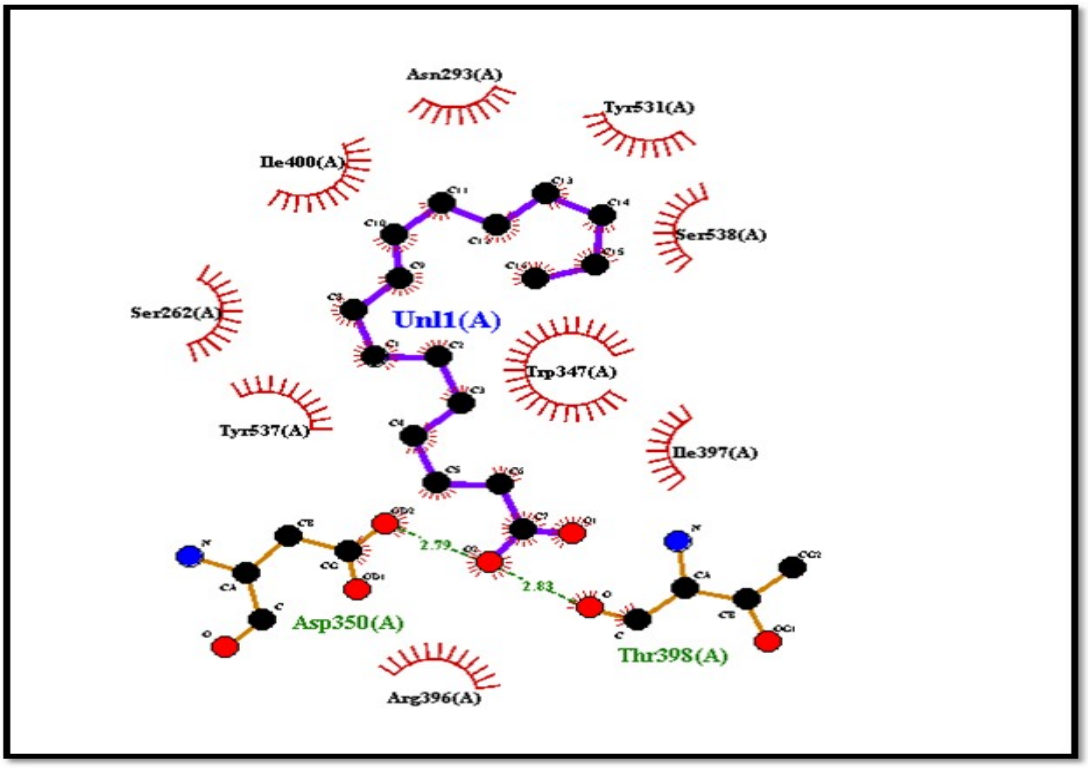


FIGURE 4.8: Interactions of Palmitoleic Acid by Ligplot

Figure 4.8 shows the interaction of palmitoleic acid with receptor protein by using ligplot. This shows palmitoleic acid formed two hydrogen bond and nine

hydrophobic interactions.

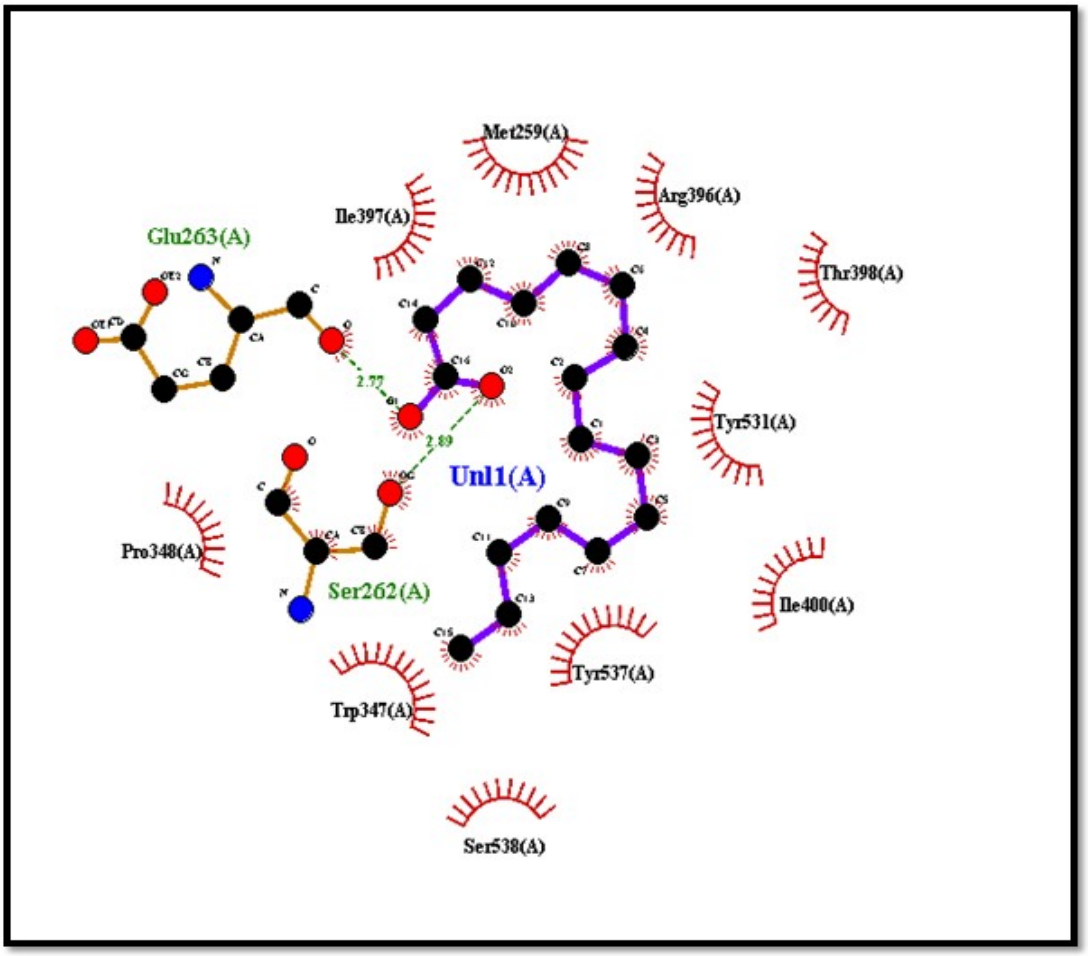


FIGURE 4.9: Shows Interaction of Palmitic Acid

Figure 4.9 shows the interaction of palmitic acid with receptor protein by using ligplot. Palmitic acid formed two hydrogen bond and have ten hydrophobic interactions.

Figure 4.10 shows the interaction of lauric acid with receptor protein by ligplot. Lauric acid formed two hydrogen bond and six hydrophobic interactions.

Figure 4.11 shows interaction of linoleic acid with receptor protein by ligplot. Linoleic acid formed three hydrogen bonds and twelve hydrophobic interactions.

Figure 4.12 shows the interaction of oleic acid with the receptor protein by using ligplot. Oleic acid shows three hydrogen bond and eleven hydrophobic interactions.

Figure 4.13 shows the interaction of malic acid with receptor protein by using ligplot. Malic acid formed four hydrogen bonds and four hydrophobic interactions.

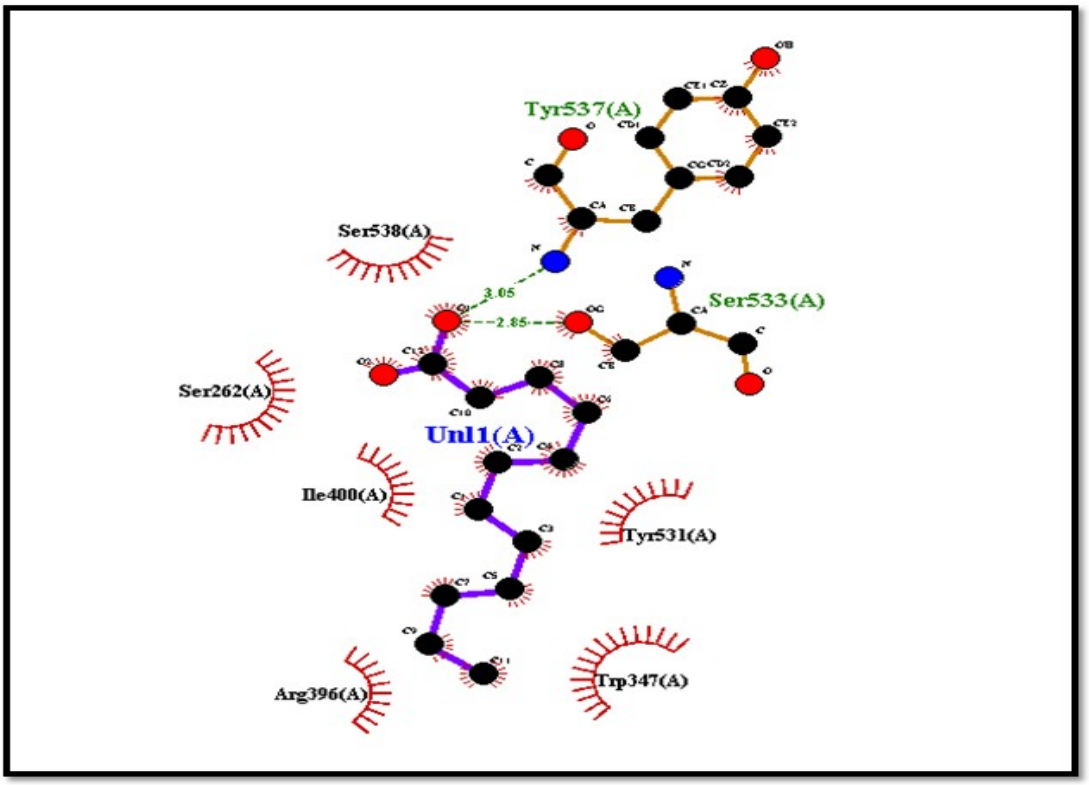


FIGURE 4.10: Shows Interaction of Lauric Acid by Ligplot

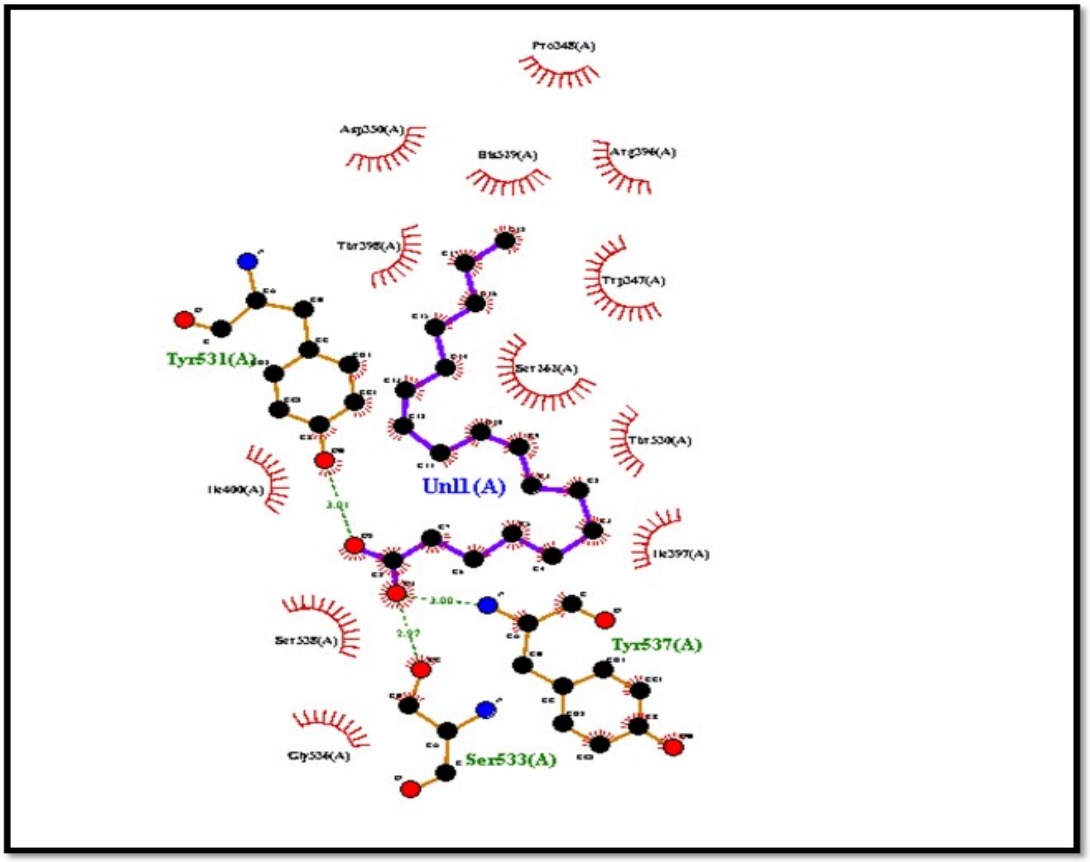


FIGURE 4.11: Interactions of Linolic Acid by Ligplot

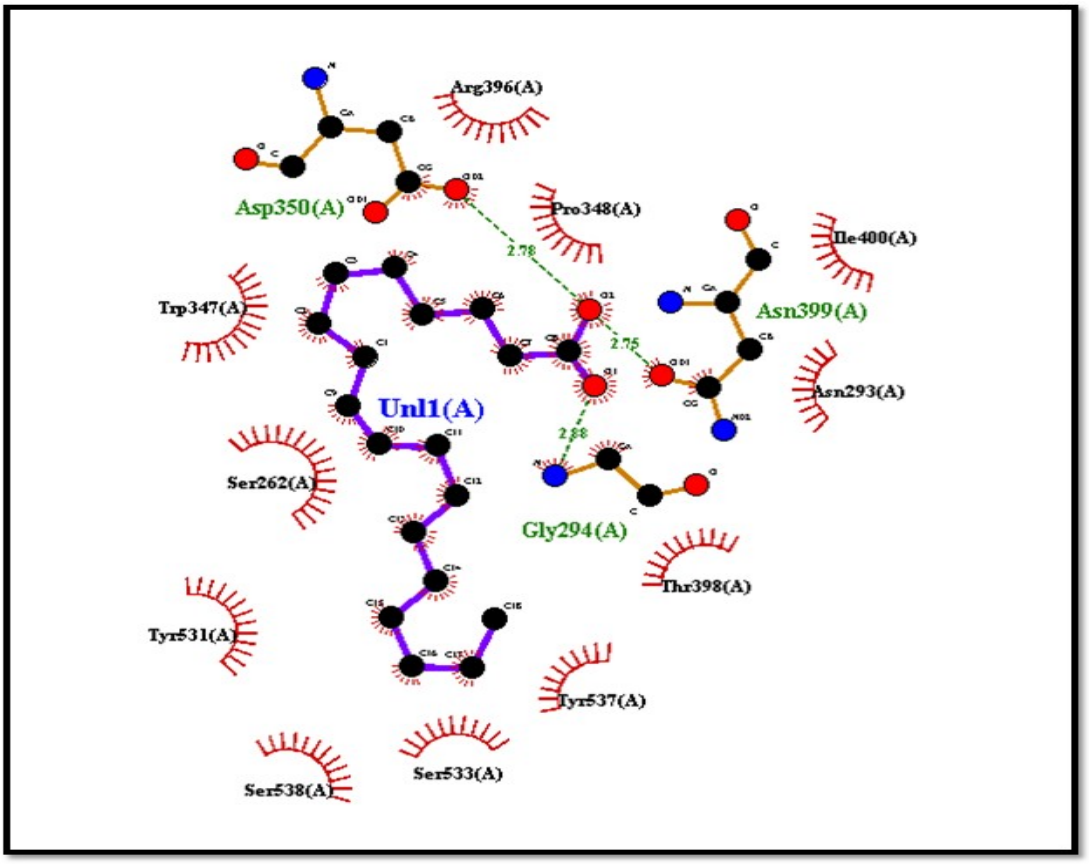


FIGURE 4.12: Interactions of Oleic Acid by Lig Plot

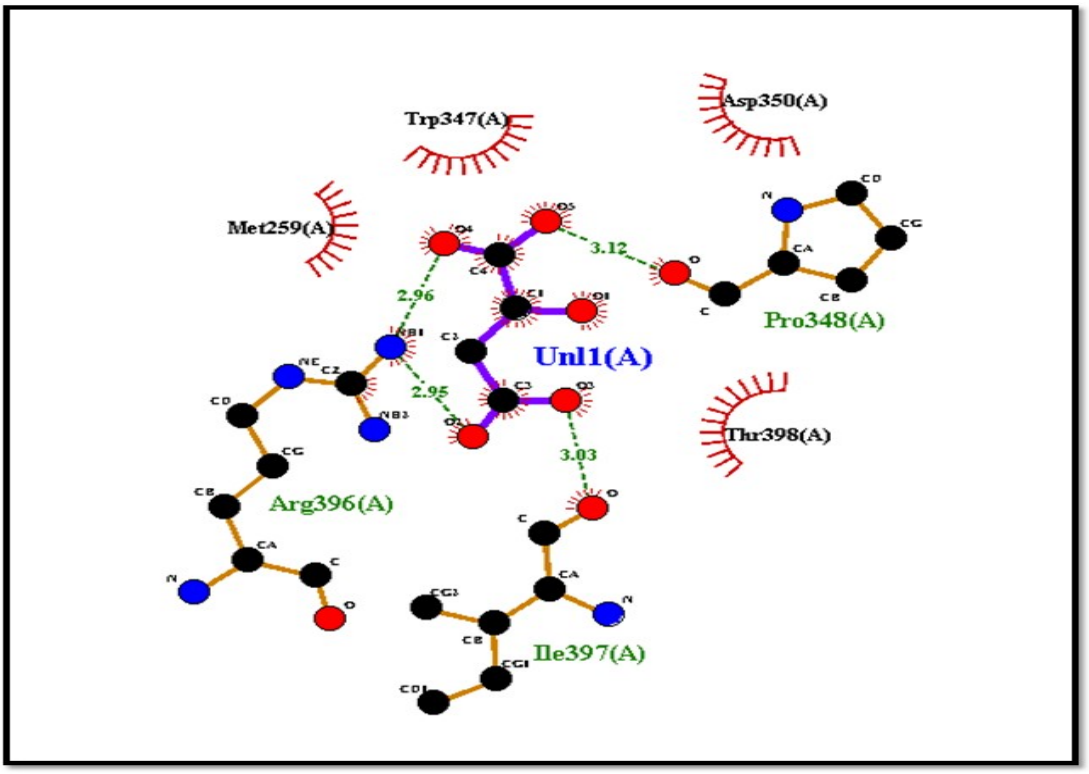


FIGURE 4.13: Interactions of Malic Acid with Receptor Protein

4.6 ADMET Properties of Ligands

To assess the verbal bioavailability and artificial availability, Lipinski first drug law is used as a first step [83].In the Second study the online program PkCSM is used to predict the ADMET properties of the ligands as a measure of pharmacokinetics. There are two general words in pharmacology, pharmacokinetics and pharmacodynamics [84].

4.6.1 Pharmacodynamics

It is a branch of pharmacology in which we study the effect of drug on the body.

4.6.2 Pharmacokinetics

In this we studied the absorption of drugs, distribution of drugs, metabolism of the drug and excretion of the drugs.

4.6.3 Absorption Properties of Ligands

In pharmacology, especially in pharmacokinetics absorption is defined as the transfer of drug from the bloodstream into the tissues .Therefore, the rate and degree of medication absorption are determined by both the chemical and makeup of the medicine and the environment in which it is injected too. A medicine must pass through cellular barriers such as epithelial or endothelial cells in order to be absorbed only a few medications pass cellular barriers in an active manner that demands the use of energy and transports the drug from a low concentration to a higher concentration.

Most medicine on the other hand pass past cellular barriers by passive diffusion in which they travel from a high concentration area to a low concentration area by diffusing through cell membranes. This sort of drug movement does not involve any energy expenditure but it is controlled by the drug size and solubility.

4.7 Absorption Properties of Ligands

Water solubility and skin absorption for all ligands in low while CACO2 permeability is normal.

TABLE 4.5: Shows Absorption Properties of Ligands

Ligands name	**Water solubility**	**CaCO2 Permeability**
Malic acid	-1.381	-0.395
Palmitic acid	-5.526	1.558
Palmitoleic acid	-5.477	1.565
Citric acid	-1.423	-0.24
Arachidonic acid	-6.042	1.582
Oleic acid	-5.924	1.563
Linoleic acid	-5.862	1.57
Lauric acid	-4.181	1.562

4.8 Absorption Properties of Ligands

TABLE 4.6: Shows Absorption Properties of Ligands

Ligands Name	**Intestinal Absorption**	**Skin Permeability**
Malic acid	13.831	-2.735
Palimitic acid	92.12	-2.217
Palmitoleic acid	92.51	-2.715
Citric acid	90.21	-2.735
Arachidonic acid	92.655	-2.728
Oleic acid	91.8823	-2.725
Linoleic acid	92.329	-2.723
Lauric acid	93.379	-2.693

Intestinal obsorption include the mechanism of active and passive transport.All the ligands have intestinal absorption more than 90%, while it is average for malic

acid. Skin permeability describe the passage of molecules in the outmost layer of the epidermal skin. Skin permeability for all the ligands is low.

4.9 Absorption Properties of Ligands

All the ligands have no p-glucoprotein substrate and inhibitor.

TABLE 4.7: Shows Absorption Properties of Ligands

Ligands	**p-glycoprotein Substrate**	**p-glycoprotein I Inhibitor**	**p-glycoprotein II Inhibitor**
Malic acid	No	No	No
Palmitic acid	No	No	No
Palmitoleic acid	No	No	No
Citric acid	No	No	No
Arachidonic acid	No	No	No
Oleic acid	No	No	No
Linoleic acid	No	No	No
Lauric acid	No	No	No

4.9.1 Distributive Properties of Ligands

Distribution is a branch of pharmacokinetics deals with the movement of drug from one location to another within the body. Volume of distribution in human contains models include fraction unbound (fu).

The permeability of the blood brain barrier (BBB) named as log BB and permeability of the central nervous system(log PS). VDss is considered low if it is less than 0.71 and higher if it is above 2.81. if it is high than its mean more of the drug is distributed to the tissues than to plasma. And if a compound show more Fu value than its mean it is effective. In table 4.8 VDss of all the ligands is low, Fu value for all the ligands is in positive numbers.

H4: Intention to use AI mediates the relationship between Employee knowledge of AI and the employee creativity

4.9.2 Distributive Properties of Ligands

Table 4.8: Shows Distributive Properties of Ligands

Ligands Name	**BBB Permeability (Human) (Log BB)**	**CNS Permeability (Log PS)**
Malic acid	-0.788	-3.523
Palmitic acid	-0.111	-1.816
Palmitoleic acid	-1.084	-1.763
Citric acid	-1.017	-3.61
Arachidonic acid	-0.172	-1.3385
Oleic acid	-0.168	-1.654
Linoleic acid	-0.142	-1.6
Lauric acid	0.057	-2.034

Blood brain barrier BBB named as log BB in human is important parameter. If the predicted value of the log BB greater than 0.3 its mean substance can cross BBB and if it is less than -1 then it causes no harm to brain. CNS permeability log PS is the product of the blood brain permeability and surface area and if its value is greater than 2 it considered to penetrate the Central nervous system and less than -3 considered as safe. BBB permeability of all the ligands is in range of negative, except lauric acid. Log PS value for malic acid and citric acid is less than -3, while for all the other ligands it is in range of more than -3.

4.9.3 Metabolic Properties of Ligands

Cytochrome P450 is an important cleansing enzyme found in the liver, it contains various isoforms of cytochrome CYP1A2, CYP2C9, CYP2D6, CYP2C19, CYP2C9, CYP2D6 and CYP3A4. First two are substrate and rest are inhibitors. The results are given below CYP-3A4 is present in palmitic acid, palmitoleic acid,

oleic acid,linoleic acid and arachidonic acid. CYP1A2 inhibitor is present in oleic acid and linoleic acid.

TABLE 4.9: Shows Metabolic Properties of Ligands

Ligands Name	**CYP-2D6 Substrate**	**CYP-3A4 Substrate**	**CYP1A2 Inhibitor**	**CYP-2C19 Inhibitor**
Malic acid	No	No	No	No
Palmitic acid	NO	yess	No	No
Palmitoleic acid	No	No	No	No
Citric acid	No	No	No	No
Arachidonic acid	No	No	No	No
Oleic acid	No	yess	Yess	No
Linoleic acid	No	Yess	Yess	No
Lauric acid	No	No	No	No

4.9.4 Metabolic Properties of Ligands

4.9.5 Excretion Properties of Ligands

Kidney is involved in the drug excretion, it plays an important role in excretion (renal excretion) and the liver (biliary excretion), lungs are also involve in the excretion of volatile or gaseous agents. Drug also excreted in the form of saliva, sweating and tears. Excretion property contains two models first one is the total clearance per day and second one is renal OCT2 substrate and its result are shown as yess/no.

4.9.6 Toxicity Properties of Ligands

PkCSM also predict the toxicity property of these ligands. The maximum tolerated dose (MRTD) provide a measure of toxic chemicals on individuals, it helps in

TABLE 4.10: Shows Metabolic Properties of Ligands

Ligands Name	**CYP2C9 Inhibitor**	**CYP2D6 Inhibitor**	**CYP3A4 Inhibitor**
Malic acid	No	No	No
Palmitic acid	No	No	No
Palmitoleic acid	No	No	No
Citric acid	No	No	No
Arachidonic acid	No	No	No
Oleic acid	No	No	No
Linoleic acid	No	No	No
Lauric acid	No	No	No

TABLE 4.11: Shows Excretion Properties of Ligands

Ligands Name	**Total clearance (ml/day)**	**Renal OCT2 substrate**
Malic acid	0.81	No
Palmitic acid	1.763	No
Palmitoleic acid	1.817	No
Citric acid	0.895	No
Arachidonic acid	2.102	No
Oleic acid	1.884	No
Linoleic acid	1.936	No
Lauric acid	1.623	No

directing the first dose of the treatment regimen in phase 1 clinical trials, it is expressed in the form of logarithms. If the maximum tolerated dose is less than 0.477 log it is considered as low and if it is more than 0.477 log than it is considered as high.

The HERG 1 and 11 Inhibitors model is reported to generate chronic QT syndrome and fatal ventricular arrhythmia by inhibiting potassium channels induced by the HERG ($humanether - a - go - g - gene$) . many pharmaceuticals have been withdraw from the market due to the inhibition of HERG channels.

TABLE 4.12: Shows Toxicity Properties of Ligands

Ligands Name	**AMES Toxicity**	**Max. tolerated dose (human)**	**HERG 1 Inhibitor**
Malic acid	No	1.212	No
Palmitic acid	No	-0.708	No
Palmitoleic acid	No	-0.713	No
Citric acid	No	0.749	No
Lactate	No	1.211	No
Arachidonic acid	No	-0.92	No
Linoleic acid	No	-0.827	No
Lauric acid	No	-0.34	No

TABLE 4.13: Shows Toxicity Properties Of Ligands

Ligands Name	**HERG 11 Inhibitor**	**Oral rate acute Toxicity (LD50)**	**Oral Rat Chronic Toxicity (LOAEL)**
Malic acid	No	1.818	3.104
Palmitic acid	No	1.44	3.181
Palmitoleic acid	No	1.449	3.109
Citric acid	No	2.148	3.698
Arachidonic acid	No	1.435	3.196
Oleic acid	No	1.417	3.259
Linoleic acid	No	1.429	3.187
Lauric acid	No	1.511	2.89

The amount of the substance that kills 50% of the experimental animals such as mice is known as the LD50. While LOAEL looks for the lowest dosage of a chemical that has a substantial negative effect, LD50 (mol/kg) forecasts the toxicity of a likely compound. Long term exposure to low to moderate level chemical exposures is crucial in medicine.

Table 4.14: Shows Toxicity Properties of Ligands

Ligands Name	**Hepatotoxicity**	**Skin Sensitization**	**T.Pyriformis Toxicity**	**Minnow Toxicity**
Malic acid	No	no	0.285	3.348
Palmitic acid	No	yess	0.84	-1.083
Palmitoleic acid	No	No	0.865	-0.956
Citric acid	No	No	0.285	4.251
Arachidonic acid	No	No	0.562	-1.538
Oleic acid	No	yes	0.676	-1.438
Linoleic acid	yes	yes	-0.701	-1.31
Lauric acid	No	No	0.954	-0.084

Hypotoxicity reveals drug-induced liver damaged and it is a major safety concern for the drug development. Skin sensitivity is a potential adverse effect of skin care and applied products. T.pyriformis is a protozoan bacterium used as a toxic endpoint and this toxin inhibit 50% growth. Its value greater than 0.5 is considered toxic. In minnow toxicity LC50 values below 0.5Mm (LC 50 less than -0.3) are regarded as high acute toxicity. Toxicity predicted values of selected ligands are given in the table above. All ligand claimed no for HERG inhibitor I and II. Maximum tolerated dose of all the ligands are high except lauric acid,. Some ligands are skin sensitive which are palimitic acid, oleic acid and linoleic acid.

4.9.7 Lipinski Rule of Five

Lipinski rule of five is as follows

- The log P value of most drug-like molecules should be less than 5
- Molecular weight should be under 500
- Maximum no of H-bond acceptor should be less than 10
- Maximum no of H-bond donor should be less than 5
- The no of rotatory bond count should be less than 5

the following rule is applied to our compounds and hence analysis of different ligands Postbiotics are checked and results are given below The table 4.16 shows the molecular weight, log p value , hydrogen bond acceptor and donor and rotatable bond counts. All the ligands follow more than three rule of Lipinski rule of 5.

TABLE 4.15: Shows Lipinski Rule of 5

Ligands	**Log p value**	**Molecular weight**	**H-bond acceptor**	**H-bond donor**	**Rotatable bound count**
Malic acid	-1.3	134.09	5	3	3
Palmitic acid	5.6	256.42	2	1	14
Palmitoleic acid	6.4	254.41	1	1	10
Citric acid	1.7	192.12	7	4	5
Arachidonic acid	4.9	304.5	2	1	14
Oleic acid	6.0	282.5	2	1	0
Linoleic acid	6.8	280.4	2	0	14
Lauric acid	4.2	200.32	2	1	10

4.10 Lead Compound Identification

The final destiny of compounds as drug or non-drug is determined by the physiochemical properties and pharmacokinetics properties. Physicochemical properties or Lipinski rule of five works as primary filter and pharmacokinetics studies as a

secondary filter in the screening of the potential compounds. Citric acid, palmitoleic acid and arachidonic acid is considered for further screening on the basis of docking score, Lipinski rule of 5 and physicochemical properties. While others are knock out in primary screening.and citric acid, is selected as the lead compound which could inhibit the target protein.

4.11 Reference Anti-Bacterial Drug Identification

Most efficient drug is selected on the basis of physiochemical, ADMET properties with mechanism of action with side effects. PubChem database is used for the physiochemical properties, pkCSM online tool is used for the identification of ADMET properties. Mechansim of action is identified by the KEGG databases and Drug Bank.

4.11.1 Cefotaxime (Beta Lactam Antibiotic)

Cefotaxime is selected as a reference drug because of its repetitive use and effectiveness against bacterial infections. Its broad spectrum anti bacterial activity is usefeull in the treatment of bacterial infections [85]. This drug is approved by the FDA in 1976 to treat the gram negative bacteria, gram positive bacteria , and anaerobic bacteria.

4.11.2 Cefotaxime Mechanism of Action

It is a bactericidal drug that works by binding penicillin-binding proteins (PBPs) through beta lactum ring. It also prevents the sensitive bacterial organism from synthesizing peptidoglycan cell walls by blocking the final step of transpeptidation, once cefotaxime administered, it undergoes metabolism. Its metabolism occur in liver and it excreted renally, and convert into desacetylcefotaxime which again

converted into desacetylcefotaxime lactone and then to M metabolites. 80% is recovered in the urine with one third desacetylcefotaxime (des-CTX) although its activity is eight fold weaker than cefotaxime [86].

4.11.3 Drug ADMET Properties

PkCSM online tool is used for the ADMET properties prediction which are absorption, distribution, metabolism, excretion, and toxicity.

4.11.3.1 Absorption Properties

The absorption properties of selected drug cefotaxime are shown

TABLE 4.16: Shows Absorptive Properties of Cefotaxime

Properties	**Predicted values**
Water solubility	-2.576
CaCo2 permeability	-0.461
Intestinal absorption	37.939
Skin permeability	-2.735
p-glycoprotein substrate	yess
p-glycoprotein I inhibitor	No
p-glycoprotein II inhibitor	No

4.11.3.2 Distribution Properties

The distribution properties of cefotaxime are shown

4.11.3.3 Metabolism Properties

The metabolism properties of cefotaxime are shown

Table 4.17: Shows Distribution Properties of Cefotaxime

Properties	**Predicted Value**
VDss (human)	-1.744
Fraction unbound (human)	0.563
BBB permeability	-1.713
CNS permeability	-3.967

Table 4.18: Shows Metabolism Properties

Properties	**Predicted values**
CYP2D6 substrate	No
CYP3A4 substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP3A4 inhibitor	No

4.11.3.4 Excretion Properties

Excretion properties of drug are given below

Table 4.19: Shows Excretion Properties of Cefotaxime

Properties	**Predicted value**
Total clearance	0.015
Renal OCT2 substrate	No

4.11.3.5 Toxicity Properties

Toxicity properties of drug are given below

Table 4.20: Shows Toxicity Properties of Cefotaxime

Properties	**Predicted values**
AMES toxicity	No
Max. tolerated dose (human)	1.608
HERG I Inhibitor	No
HERG II inhibitor	No
Oral rat acute toxicity (LD50)	1.933
Oral rat Chronic toxicity (LOAEL)	2.359
Hepatotoxicity	Yess
Skin sensitization	No
T. pyriformis toxicity	0.285
Minnow toxicity	4.653

4.11.3.6 Lipinski Rule of Five

The table below shows the properties of selected drug cefotaxime according to Lipinski rule of five.

Table 4.21: Shows Lipinski Rule Of 5 of Cefotaxime

Ligand	**Log P value**	**Molecular Weight**	**H-bond acceptor**	**H-bond donor**	**Rotatory bound count**
Cefotaxime	-1.4	455.5	12	3	8

4.11.3.7 Cefotaximine Docking

CB Dock 2 online tool is used for the docking. First of all the 3D structure of cefotaxime is downloaded from the PubChem, and then in chem 3D Pro it is

refined by minimizing its energy, and then in cbDOCK 2 its docking is done with alpha C protein.

Table 4.22: Shows Docking of Cefotaxime

Drug Name	**Binding Score**	**Cavity Size**
Cefotaxime	-5.5	58

4.12 Cefotaximine and Lead Compound Comparison

The comparison amongst the cefotaximine and the lead compound citric acid helps in understanding the well treatment for infectious diseases. The comparison parameters are ADMET properties and physiochemical properties of both compounds.

4.12.1 ADMET Properties Comparison

ADMET Properties include the adsorption, distribution, metabolism, excretion and toxicity. We compare the citric acid properties and cefotaximine to determine the drug activity and efficiency

4.12.1.1 Absorption Properties Comparison

Cefotaximine and citric acid properties are given in table 4.24. It is cleared from the result that intestinal absorption of citric acid is greater than cefotaximine. And it is permeable to skin.

The absorption properties of selected drug cefotaxime are shown

Table 4.23: Shows Comparison of Absorption Properties

Properties	**Cefotaximine**	**Citric acid**
Water solubility	-2.576	-1.423
CaCo2 permeability	-0.461	-0.24
Intestinal absorption	37.939	90.21
Skin permeability	-2.735	-2.735
p-glycoprotein substrate	yes	No
p-glycoprotein I inhibitor	No	No
p-glycoprotein II inhibitor	No	No

4.12.1.2 Distributive Properties Comparison

The distributive properties of cefotaxime and citric acid is given below. Distributive properties of citric acid is better than cefotaximine.

Table 4.24: Shows Comparison of Distributive Properties

Properties	**Cefotaximine**	**Citric acid**
VDss (human)	-1.744	0.418
Fraction unbound (human)	0.563	0.104
BBB permeability	-1.713	-1.017
CNS permeability	-3.967	-3.61

4.12.1.3 Excretion Properties Comparison

The total clearance value of citric acid is greater that helps in the emission of drug from the body.

Table 4.25: Shows Comparison Of Excretion Properties

Properties	**Cefotaximine**	**Citric Acid**
Total clearance	0.015	0.895
Renal OCT2 substrate	No	No

4.12.1.4 Metabolic Properties Comparison

The metabolic properties is same in both

Table 4.26: Shows Comparison of Metabolic Properties

Properties	**Cefotaximine**	**Citric Acid**
CYP2D6 substrate	No	No
CYP3A4 substrate	No	No
CYP1A2 inhibitor	No	No
CYP2C19 inhibitor	No	No
CYP2C9 inhibitor	No	No
CYP3A4 inhibitor	No	No

4.12.1.5 Toxicity Properties Comparison

Toxicity properties of drug are given below, Cefotaximine shows hepatotoxicity, it exposes drug-induced liver damage and a major safety concern for drug development. And its tolerated dose is high

Table 4.27: Shows Comparison of Toixicity Properties

Properties	**Cefotaximine**	**Citric acid**
AMES toxicity	No	No
Max. tolerated dose (human)	1.608	0.749
HERG I Inhibitor	No	No
HERG II inhibitor	No	No
Oral rat acute toxicity (LD50)	1.933	2.148
Oral rat Chronic toxicity (LOAEL)	2.359	3.698
Hepatotoxicity	Yess	No
Skin sensitization	No	No
T. pyriformis toxicity	0.285	0.285
Minnow toxicity	4.653	4.251

4.12.1.6 Lipinski Rule of Five

Citric acid acid and cefotaximine Lipinski rule of five is given in table 4.29. It is cleared that citric acid show better result over cefotaximine with respect to log P value, H-Bond acceptor and rotatory bond count.

Table 4.28: Shows Comparison of Lipinski Rule of Five

Ligand	**Log P value**	**Molecular Weight**	**H-bond acceptor**	**H-bond donor**	**Rotatory bond count**
Cefotaxime	-1.4	455.5	12	3	8
citric acid	1.7	192.12	7	4	5

4.12.2 Docking Score Comparison

The standard and the lead compound docked against the target protein and docking result gives us the best binding score than that of standard drug

Table 4.29: Comparison of Docking Score

Drug name	**Binding score**	**Cavity size**
Cefotaxime	-5.5	58
Citric acid	-7.3	6128

4.13 MD Simulations

The MD simulations were conducted in order to investigate the complexes intermolecular dynamics and determines the interactions strength over the course of simulation time. The different simulation trajectories based structure statistics are given in Table while the plots are presented in Fig. The first analysis done was root mean square deviation (RMSD) that tells about the average distance of superimposed molecules. The lower RMSD value describes less deviations and vice

versa. All the three complexes revealed very stable RMSD plots with no major deviations seen.

The mean RMSD of Srr2-Citric acid, Srr2-Arachidonic acid and Srr2-Palmitoleic acid is 2.64 Å, 2.43 Å and 2.40 Å, respectively. The minor structure adjustments were due to flexible loops that deviate upon ligand binding. The ligands binding conformation the protein was seen stable as can be depicted by lower ligand RMSD value as given in Table.

The citric acid was found to show the most stable conformation with value of 0.13 Å. Similarly, the root mean square fluctuation (RMSF) was analyzed for the complexes that tells about the local residue level changes. The mean RMSF of Srr2-Citric acid, Srr2-Arachidonic acid and Srr2-Palmitoleic acid is 5.72 Å, 9.61 Å and 28.30 Å, respectively.

The first complex revealed to show stable residues level interactions with the ligands. Similarly, all the complexes were reported to show high compact nature due to stable intermolecular conformation.

Table 4.30: Different Structure Analyses Statistics

Parameter	**Srr2-Citric acid**	**Srr2-Arachidonic acid**	**Srr2-Palmitoleic acid**
RMSD Mean	2.64 Å	2.43 Å	2.40 Å
RMSD Maximum	3.72 Å	3.38 Å	3.61 v
Ligand RMSD Mean	0.13 Å	1.95 Å	1.13 Å
Ligand RMSD Maximum	0.54 Å	3.32 Å	2.15 Å
RMSF Mean	5.72 Å	9.61 Å	28.30 Å
RMSF Maximum	11.36 Å	18.93 Å	53.05 Å
Rg Mean	44.26 Å	44.16 Å	23.85 Å
Ligand Rg Mean	23.21 Å	36.67 Å	23.31 Å

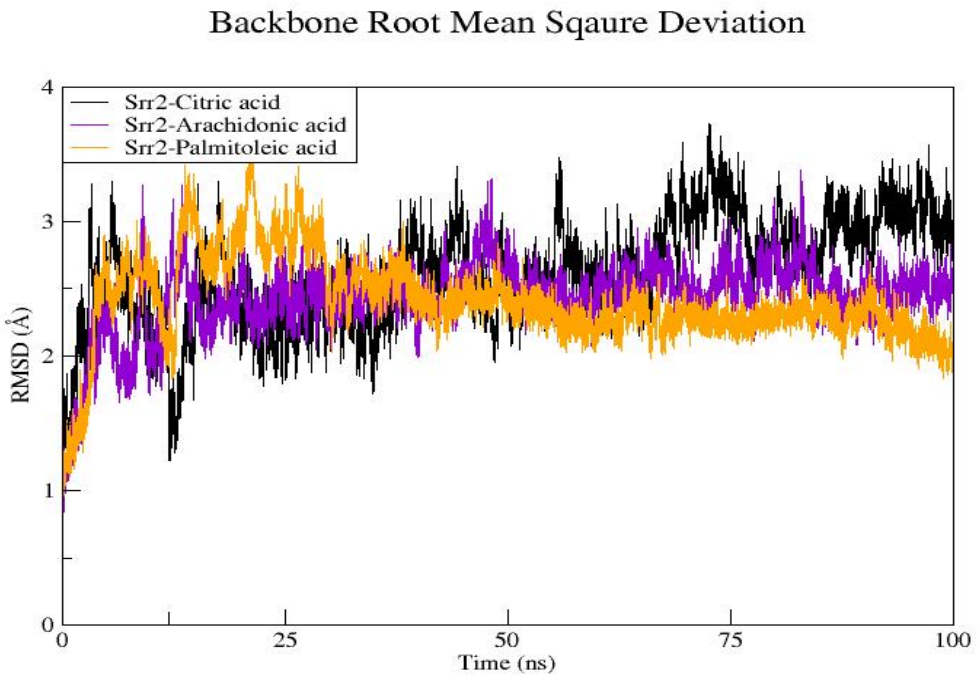


FIGURE 4.14: Simulation trajectories based different structure analyses. A. RMSD for complexes

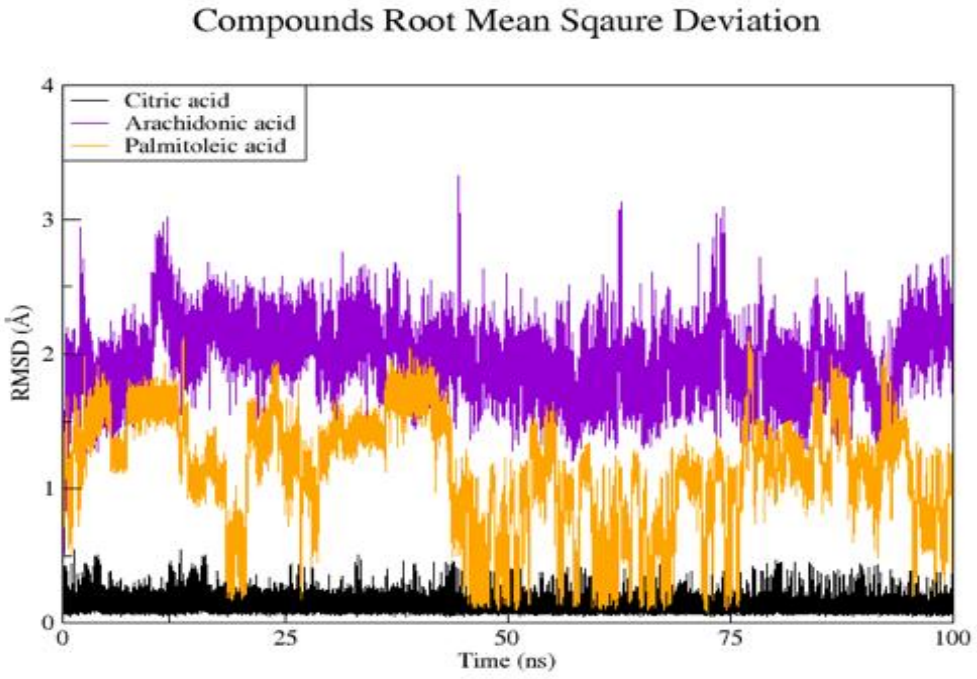


FIGURE 4.15: Simulation trajectories based different structure analyses. B. RMSD for docked ligands

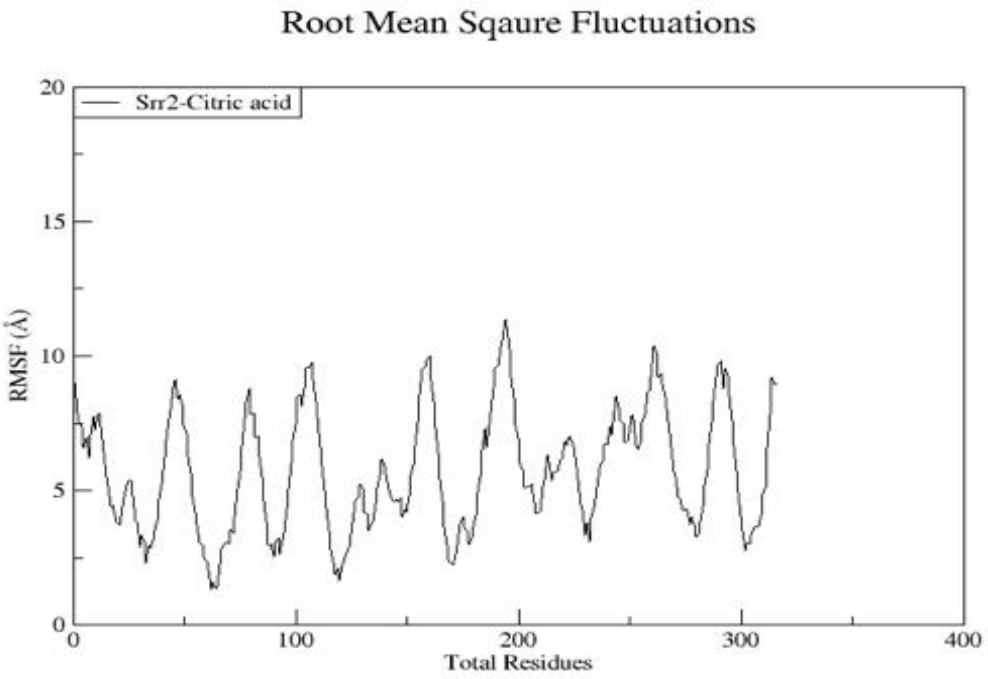


FIGURE 4.16: Simulation trajectories based different structure analyses. C. RMSF

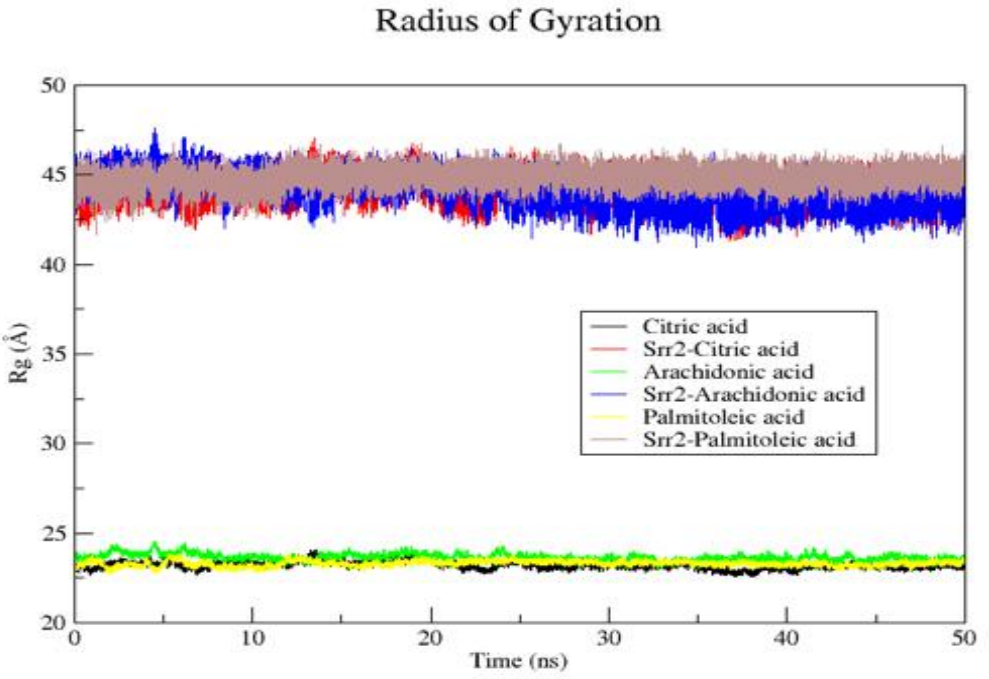


FIGURE 4.17: Simulation trajectories based different structure analyses. D. Rg.

Chapter 5

Conclusion and Future Recommendation

The aim of this research was to identify the compounds using computational methods for the treatment of neonatal infectious disease i.e neonatal meningitis that could be used as a drug in near future. For the current study eight ligands were choosen after data mining analyses on literature databases. The protein used for virtual screening was serine rich repeat protein Srr2. CB Dock 2 an automated version of Auto Dock vina was used for the docking studies. Protein ligand interactions of these ligands were analyzed using ligplot plus version v.1.4.5. After the detailed analysis of their binding score, physiochemical properties and ADMET properties three best scoring compounds citric acid, arachidonic acid and palmitoloic acid were recognized as hit compounds. Physiochemical properties and pharmacokinetics properties determined the final density of compound as drug or non drug. Arachidonic acid, palmitoleic acid and citric acid are identified as lead compound. From the above mentioned physiochemical properties and ADMET properties it is concluded that arachidonic acid shows best binding with serine rich repeat protein and its activity is also better as comopared to cefotaximide. All the software and tools used in the current research research study are reliable and authentic.

Arachidonic acid, citric acid and palmitoleic acid are the effective ligands that target the Srr2 and prevent GBS-Induced newborn meningitis. Their capacity to interfere with the development of biofilms, bacterial adhesion and virulence regulation highlights their potential as therapeutic agents in the treatment of GBS infection.

Postbiotics offers safety, immunomodulatory benefits and the capacity to maintain the microbial diversity making them a prospective therapy option for newborn meningitis. Postbiotics are superior over standard antibiotics in many ways in treating illness due to their benefical results from the immunomodulatory qualities, safety record and capacity to maintain microbial diversity. In contrast antibiotics have potential to cause toxicity, damage to development and disturbance of the gut flora.

Postbiotics came from the natural resources are usually regarded as safe for use in neonates. Moreover Postbiotics have positive effects on the gut-brain axis, regulating immunological responses and reducing inflammation. Postbiotics can also be used in combination with the traditional antibiotics and can improve treatment outcomes and also lower the antibiotic resistance.However it is clear that by using the Postbiotics we can compete with neonatal infections.

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