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# THERAPEUTIC POTENTIALS OF BLACK TEA MEDICAMENTS

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF **BACHELOR OF TECHNOLOGY**

IN

**BIOTECHNOLOGY**


*Submitted by*

**Satabdee Mohapatra**

**110BT0456**

*Under the guidance of*

**Prof. Sirsendu Sekhar Ray**

Dept. of Biotechnology and Medical Engineering
National Institute of Technology, Rourkela

2014

National Institute of Technology, Rourkela

# CERTIFICATE

This is to certify that the thesis entitled "**THERAPEUTIC POTENTIALS OF BLACK TEA MEDICAMENTS**" submitted by Ms SATABDEE MOHAPATRA in partial fulfilment of the requirements for the degree of Bachelor of Technology in BIOTECHNOLOGY embodies the bonafide work done by her in the final semester of her degree under the supervision of the undersigned. The thesis or any part of it has not been submitted earlier to any other University / Institute for the award of any Degree or Diploma.

**Dr. Sirsendu Sekhar Ray**

Assistant Professor

Department of Biotechnology and medical engineering

National Institute of Technology, Rourkela

# ACKNOWLEDGEMENT

If words are reflected as symbols of appreciation and token of acknowledgement, then words play the role of thanks to exhibit the deeply embedded feeling of gratitude. I am greatly indebted to, who either through guidance, discussion or providing facilities for the thesis work, have served as a beacon light or crowned my efforts with success. I would really like to take this opportunity to thank my project guide, **Prof. Sirsendu sekhar Ray**, Department of Biotechnology and Medical Engineering, NIT Rourkela, for motivating and guiding me throughout the time. I am sincerely thankful to **Prof. Krishna Pramanik**, Dept. of Biotechnology and Medical Engineering, NIT, Rourkela, for providing the necessary facilities for this work. I gratefully extend my sincere thanks to all faculties and to all teaching and non-teaching staff members of Department of Biotechnology & Medical Engineering, National Institute of Technology Rourkela, Orissa.

I also owe a debt of gratitude to Ms. Somya Asthana, Mr. Susant basuri, Mr. Mohit Gangwar, Mr. Tarun Agarwal, Mr. Rik Dhar and Ms Priyanka Goyal for all the help and support I got from them. This final year project has brought the best in me and I have given everything to be able to live up to the expectations of all. I would like to express my heartily thanks to my friends Aniket Mazumdar, Prajna Kabiraj, labmates and others in the department for their help and support.

Finally, I would like to express my heartfelt thanks to my parents for their blessings, support and constant encouragement and very special thanks to God for showering the blessings on me.

Satabdee Mohapatra

Biotechnology Engineering.

# ABSTRACT

Tea; a product of Camellia Sinensis plant is the most broadly used beverage in the world. Tea phytochemicals have been studied for the prevention of various chronic diseases, such as cancer, obesity, diabetes, jaundice, hypertension and inflammation related disorders. Whereas many studies have revealed the potential efficiency of tea for the prevention of these physiological disorders, the underlying mechanisms remain unclear. In this work, we evaluated the evidence and discussed the significance of proposed mechanisms for the prevention of the diseases such as Alzheimer's, obesity, diabetes mellitus type II, hypertension and asthma by tea. Molecular docking method was used to explore the ability of tea phytochemicals to inhibit the key enzymes. The three dimensional structure of target enzymes were either retrieved from protein data bank or modelled using swiss model. Autodock4.2 software was used for molecular docking that applies Lamarckian Genetic Algorithm. The ligand structures were retrieved from PubChem and KNApSAcK-3D database. PreADMET web server was used for Toxicity and ADME predictions. Based on this analysis, it has been found that theaflavin, rutin and 8-c-ascorbyl epigallocatechin could be the potential lead molecules as they act as inhibitor for most of the target enzymes and has a good drug score and also qualifies the toxicity and ADME test. Further the tea extract is loaded in liposomal drug delivery system and pectin-HEMA hydrogel system to analyse their drug delivery potential and it was found that liposomal system is best suitable for delivery to brain and hydrogel system better serve as colon specific delivery system. We concluded that these phytochemicals or their derivatives can be used for further in-vitro and in-vivo studies to produce valuable lead drug candidates.

**Keywords:** Phytochemicals, Molecular Docking, ADME & TOX, liposomal encapsulation, hydrogel

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# CHAPTER 1: INTRODUCTION

## 1.1- Introduction

With all these technological inventions and advancement in science, we are capable of finding cure for the prevailing diseases and increasing our life longevity. But with each advancing day new diseases are coming into existence and we face new challenges every day to invent medicine and to protect ourselves. Due to changing life style we are facing many problems, diseases related physiological disorders that is due to environmental changes such as obesity, diabetes, jaundice, asthma and other diseases due to mutation. Still the challenge doesn't end here. The medicines that are invented or come to market are not free from side effects. Each have some unwanted consequences, some are mild while others have deadly permanent consequences. To avoid all these consequences and to cure the diseases we need to search for some natural products.

The medicinal use of natural compounds that are isolated or derived from different natural sources like plants, animals or micro-organisms; recorded in human history possibly from thousands of years. However, it was not until the nineteenth century that scientists isolated active components from various medicinal plants. Among all the various medicinal plants and herbs tea is the most ancient herb used by Chinese and it is the most extensively used beverage in the modern world. Thus now a days many researchers are making attempt to isolate the active compounds from tea for various specific diseases and making marketed drug products out of it.

## 1.2- Tea and its phytochemicals :

Tea is one of the most broadly consumed drinks worldwide due to its various reasons such as its easy availability, good taste and last but not least several health benefit for human consumption [1]. Two main categories of tea that dominates as beverage are black and green teas. Among them green tea is the non-fermented form of tea that is used mostly in China and Japan, while black tea is more prevalent in Europe, North America and India [2]. Another type named oolong tea is a transitional variant between the green type and black tea that is rich in catechins and oligomerized catechins [3]. The

commercially available tea variety is acquired from leaf of *Camellia Sinensis* plant. Two principal variety species of the plant are used one being the small-leaved Chinese variety plant (*Camellia sinensis* subspecies *sinensis*), grown extensively used in China and Japan, used for most other types of teas, and the other type is the large-leaved Assamese plant (*Camellia sinensis* subspecies *assamica*), predominates in South and South-East Asia, which was generally used as black tea, though in previous years some white and green have been produced [4]. Black tea is more oxidized than other types such as green, white and oolong teas. There are two ways of processing black teas. The Crush, Tear, and Curl method or orthodox method. In the CTC method fanings leaves or dust grades are produced. The method of orthodox processing is done either by hand or by machine. For production of high quality tea hand processing method is used.

The usefulness of tea are credited to the presence of phytochemicals in it. Phytochemicals are the chemical compounds which naturally occur in plants. Some of the phytochemicals are accountable for colour and other organoleptic properties. The term phytochemical is usually used to refer to natural chemicals that may have significant biological properties, e.g.; antioxidants, and recognised as non-essential nutrients. The typical tea phytochemicals are epigallocatechin gallate, flavonoids, tannins, caffeine, polyphenols, boheic acid, theophylline, theobromine, anthocyanins, gallic acid. The extract from green tea has strong antioxidant due to the presence of (+)catechin, (-)-epicatechin (EC), (-)-epigallocatechin, (-)-epicatechin-3-gallate and EGCG. Moreover, black tea leave production involves enzymatic oxidation of the polyphenols in tea leaf to various new products such as theaflavins and thearubigens. The chief theaflavins constituents in black tea are theaflavin, theaflavin monogallateA, theaflavin monogallateB and theaflavin digallate. Oolong tea is a partially oxidized type and hence retains a substantial amount of these catechins. These phytochemicals adds several advantages to tea one of them is the high antioxidant activities that is due to the presence of polyphenols which enable the tea to search free radicals. Because the tannins present in tea known to form bond with proteins, it has uses as anti-cancer, diuretic and anti-diarrhoeal effect. The tannins also have diuretic and anti-cancer activity. Tea appears to inhibit the cholesterol absorption hence helps in reducing cholesterol level. The stimulation effect by of caffeiene is the best know effect of tea. Caffeine works by affecting adenosine receptors and blocking the

phosphor-di esterase enzyme. Tea is also known to reduce cardiovascular disease and induce body weight loss.

Hence using tea phytochemicals as ligand we can inhibit many enzymes that play vital role various disease pathways and thus can cure the disease.

## 1.3- Enzymes:

Till date tea is known to have activity on various enzymes that works in disease progression pathway. Some of those enzymes are acetyl cholinesterase, alpha amylase, alpha glucosidase, beta glucuronidase, pancreatic lipase and angiotensin converting enzyme.

### A. Acetyl cholinesterase

AChE is a serine hydrolase enzyme that catalyses hydrolysis of the neurotransmitter acetylcholine (ACh), thus resulting in termination of ACh action at the cholinergic synapse. AChE has been the main focus of research aimed at the discovery of its effective inhibitors for their use in treatment of Alzheimer's disease (AD). AD is the most common cause of dementia prevailing in our ageing society. It is estimated that AD account for 50 and 60% of dementia cases in old age and characterised by progressive, neurodegenerative disorder. The other symptoms linked to AD involve primary memory loss, decline in cognitive dysfunction and the late stages of the disease are often associated with language deficits, agitation, mood disturbances, depression and psychosis. Many drugs had been developed in recent years against AD but a total cure for the disease is not yet available and the drugs are often associated with long term side effects.

### B. Alpha Amylase

The α–amylase; a family of endoamylases is one of the chief products secreted by salivary gland and pancreas. The enzyme plays a vital role in digestion of polysaccharides such as starch and glycogen by cleaving α-D-(1-4) glycosidic bonds and often found in plants, microorganisms and higher organisms. The enzyme α-amylase action result in oligosaccharides with varying length with an α-configuration and α-limit dextrins as end products, which contains a mixture of maltotriose, maltose and branched oligosaccharides of 6–8 glucose units that constitues both α-1,4 and α-

1,6 linkages. There are many other amylo-lytic enzymes which take part in the starch breakdown process, but α-amylase contribution is a prerequisite for the initiation of this process.

**C. Alpha Glucosidase**

It is a carbohydrate-hydrolase which releases alpha-glucose instead of beta-glucose. Alpha –glucose molecule is released by the enzyme, by hydrolysing the terminal non-reducing 1-4 linked alpha- glucose residues. The substrate selectivity of the alpha-glucosidase enzyme is because of sub site attractions of the active site of enzyme. There are two mechanisms proposed for enzyme's action that include a nucleophilic displacement and an oxocarbenium ion intermediate pathway.

Alpha-glucosidase inhibition play a vital role in treating type II diabetes and its inhibitors are marketed as oral anti-diabetic drugs that work by preventing the digestion of carbohydrates. Carbohydrates are converted into monosaccharide compositions that can be easily absorbed by the intestine. Thus, the inhibitors of alpha-glucosidase helps in reduction of the impact of carbohydrates in blood sugar thus work against type II diabetes.

**D. Beta Glucuronidase**

Beta-glucuronidase; a family-2-glycosyl hydrolase enzyme that chop the residue of β-D-glucuronic acid from the non-reducing termini of glycosaminoglycan. In Homo sapiens, the lysosomal β-glucuronidase degrades sulphated glycosaminoglycans. The experimentally determined X-ray structure of a human β-glucuronidase shows that it is a tetramer, with each monomer having 653 AA residues and 80 kDa size. It was discovered that each monomer of the human. Several studies reported that the heightened activity of the enzyme lead to variety of pathological conditions, including urinary tract infection, renal diseases, transplantation rejection, epilepsy, neoplasm of bladder, testes, larynx, and breast. Among other therapeutic importance, one of the major advantage of this enzyme is its role in enterohepatic circulation and thus in jaundice. Jaundice; a disease characterized by yellowish discoloration of the skin, sclera, and mucous membranes due to elevated bilirubin as result of abnormal bilirubin metabolism and/or excretion.

A probable mechanism for controlling the bilirubin mechanism is β-Glucuronidase inhibitor which can interrupt circulation of the bilirubin in enterohepatic region and hence facilitates faecal excretion and lower serum bilirubin concentration. By inhibiting human milk β-Glucuronidase the intestinal absorption of the bilirubin is shown to decrease in a rat model with bile duct and duodenal catheters.

**E. Angiotensin Converting Enzyme**

ACE is a zinc-dependent mono-carboxypeptidase which plays a crucial role in balancing vasoconstrictor and proliferative actions of angiotensin II with the vasodilatory and ant proliferative effects of angiotensin. For the treatment of hypertension ACE is a important target because of the enzymes involvement in a number of blood pressure-related systems, for example' the kinin nitric oxide system and renin-angiotensin system. In the RAS mechanism, angiotensin I is chopped into the potent vasoconstrictor angiotensin II by ACE, and the hypotensive peptide, bradykinin and kallidin are inactivated by ACE in KNOS. Therefore, unnecessary action of ACE results in increased vasoconstriction and hypertension. Altered expression of this enzyme is related to cardiac, vascular, and renal dysfunctions. In addition, blocking the synthesis of angiotensin II by ACE inhibitors or its actions by angiotensin II receptor blockers has shown to increase cardiac ACE expression. The overexpression of ACE by gene transfer technology protects the heart from hypertension-induced cardiac remodelling. Inhibition ACE is the potential mechanism for antihypertensive treatment.

**F. Lipase**

Lipases are the enzymes that help in triacylglycerol, phospholipids and fat digestion. The human lipases are two types that includes the pre-duodenal and the extra-duodenal lipases. Pancreatic lipase (PL), is the major lipo-lytic enzyme produced and secreted by human pancreas, and it has a crucial role in the effective triglycerides digestion. PL eliminates fatty acids from the 'a0' and 'a' location of dietary triglycerides, producing mono-glycerides, saturated and polyunsaturated long chain fatty acids as the lipo-lytic yields. Lipase is accountable for 50–70% of dietary fats hydrolysis.

Inhibition of pancreatic lipase is one among the most broadly studied for determining potential efficiency of natural products as anti-obesity means. Orlistat, one among the two clinically accepted drugs for treatment of obesity, has mechanism of acting through PL inhibition. Being a best-selling drugs worldwide, still it has various unpleasant digestive side effects like oily stools, spotting among others. The success of orlistat has prompted research for the identification of newer inhibitors of lipase that lack these disagreeable side effects.

**G. Lipoxygenase**

Lipoxygenase enzyme are found in different forms such as 5-lipoxygenase (5-LOX), 15-lipoxygenase (15-LOX) etc. 5-LOX initiates the process of synthesis of the leukotriene (LTs) from arachidonic acid. LTs are known to cause constriction, increase vascular permeability and mucus secretion in the lung, and hence are potent vasoconstrictors of coronary arteries, and also have the potential to induce inflammatory reactions. Because of these properties, LTs are viewed as powerful mediators which may play crucial roles in inflammatory disorders, asthma, vascular diseases and cancer. The cellular catalysis reaction and expression of 5-LOX is regulated tightly. Hence, 5-LOX is found basically in mature leukocytes or dendritic cells. The cells which are unable to produce and express 5-LOX, their transcription system is blocked by DNA methylation.

The (5-LOX) from Human has been considered since long as a probable therapeutic target for inflammatory diseases. While asthma being the principle disease target various other diseases have also been hypothesised in the research literatures as possible targets for 5-LOX inhibition, few named; allergic rhinitis, idiopathic pulmonary fibrosis, atherosclerosis, chronic obstructive pulmonary disease, atopic dermatitis ischemia reperfusion injury and acne vulgaris. 5-LOX is also an implication in seborrhoea dermatitis; a skin disease.

Quite a number of inhibitors for 5-LOX have been found. The 5-LOX inhibitors are generally categorised into, reductive, iron ligands and competitive/mixed inhibitors. Till now only one chemical compound is being approved as one among the drug

against inflammation, i.e.; Zileuton. Zileuton is amongst a potent and selective 5-LOX inhibitor but its mode of action is quite unusual for a therapeutic agent.

## 1.4- Computational Drug Discovery:

Computational drug discovery offers noble strategy for fast and frugal drug discovery and development technique. This technique diminishes efforts, time and cost of drug discovery and development. The recent improvements in bioinformatics and remarkable increase in the accessibility of both biological macromolecule and small molecule information has brought us significantly nearer to computational drug discovery.

Various in silico approaches will assist in identifying various drug targets via bioinformatics tools. These tools even be familiarized to distinguish the target enzymes and assist in analysing their structures for probable binding sites. The computational method help in production of various candidate molecules, in the confirmation of their resemblance to other drug characteristics, these molecules and the target protein docking, ordering them consistently with their binding energies affinity, and finally further to enhance binding characteristics optimizing the molecules. Various web server and software are present that can be used for finding active site for example CASTp, Q site finder, pocket finder.

For identification of a consistent and effective drug for target enzyme; protein-ligand auto docking are used. The main aim of protein-ligand docking is to calculate the binding energy of the protein-ligand reaction complex at given atomic coordinates. Recent growths in energy functions and search algorithms made computational docking approaches a valuable tool to discover and study the protein-inhibitor interaction

The protein catalytic sites and active residues are the key factors for the flexible docking. Autodock4.2 software is an automated docking tool that help in predicting how the ligand molecules are binding to receptor of known 3D structure during docking and the software also allow to model the binding parameters of ligand with various number of different conformational clusters and finding possible minimum binding energy.

The exploitation of available complementary experimental and informatics techniques intensifies the vision of success in several stages of the drug discovery procedure, starting from the identification of the novel targets and disclosure of their functions to the

discovery and development of principal compounds with preferred properties. Leading roles of computation in drug discovery are; Virtual screening, in silico ADME/T prediction & de novo design and advanced strategies for finding protein-ligand binding.

## 1.5- Drug delivery Techniques:

### A. Liposomal Encapsulation

The spontaneous formation of bilayered phospholipid membrane closed structures with internal aqueous environment when phospholipids are dispersed into water is known as liposome. They are small spherical shaped vesicles which can be produced from nontoxic surfactants, cholesterols, glycolipids, membrane proteins, and long chain fatty acids.

The lipid based vesicles, i.e.; liposomes are microscopic (either unilamellar or multilamellar) vesicles which are formed due to phospholipids self-assembly in an aqueous media that results in closed bilayered structures.

Liposomes are excellent agents for targeted drug delivery system as they can target a drug or any nanomaterial to the particular site of action in body thus helping in enhancing the efficiency. These vesicles can act as a reservoir from which the entrapped chemical/material is slowly released over time. Hence, to maintain therapeutic drug levels in blood stream a sustained release process can be exploited. Another advantage is that liposome surfaces can be modified by attaching polyethylene glycol (PEG) units to the lipid bilayer thus resulting in the increase of circulation time of liposomes in the bloodstream vividly. There is commercialization of liposomal formulations of anti-tumour drugs and antifungal agents nowadays. Molecules of various weight starting from low molecular weight compound such as glucose to high molecular weight macromolecules such as proteins and peptides have also being incorporated in liposomes. Aqueous compartments of the liposome contains water soluble contents while lipid soluble compounds/drugs and amphiphilic drugs/chemicals integrate themselves in the phospholipid bilayers of the liposome. Different routes of administration of liposome encapsulated drugs are intravenous injection, oral delivery, local application and these can be used for treating various diseases.

**B. Pectin-HEMA Hydrogel**

Pectin consists of a complex set of polysaccharides, rich in galacturonic acid, found in the primary cell walls and is also one of the major component of the middle lamella, where it helps to bind cells together. The pectin is mainly used as a gelling agent, thickening agent and a stabilizer in food. It is also used to design medicines in the cases of high cholesterol, high triglycerides, and to prevent colon and prostate cancer. Pectin prevents poisoning caused by lead, strontium, and other heavy metals and is also administered in patients suffering from diabetes and gastroesophageal reflux disease.

Hydroxyethylmethacrylate or HEMA is the monomer used in making the polymer poly hydroxyethylmethacrylate. This polymer is hydrophobic in nature; however, when subjected to water it swells due to the presence of hydrophilic pendant group. It absorbs from 10 to 600% water relative to the dry weight due to the hydrophilic nature. This swelling property of polymer, allowed it to be first material used in manufacture flexible contact lenses.

Hydrogel is a hydrophilic network of polymer chains which sometimes can be found as a colloidal gel with water as dispersion medium. Hydrogels are highly absorbent natural or synthetic polymers that can contain more than 99.9% water. The degree of flexibility of hydrogels is very similar to natural tissue, because of their significant water content. Hydrogels are commonly used as scaffolds in tissue engineering. Hydrogel-coated wells known as “smart or intelligent gels” are environment sensitive and have been used for cell culture. These smart hydrogels have the capability to sense the changes in temperature, pH, or the concentration of metabolite and acts by releasing incorporated loads as result of such a change and a sustained-release drug delivery systems is achieved.

## 1.6- Objectives:

The primary objectives of current project work are:

1.6.1 To identify the different phytochemicals present in tea.

1.6.2 To analyse the phyto chemicals (from PubChem and knapsack Id) of selected molecule for their potential to be drugs.

1.6.3 To analyse and dock all chosen molecules for the identification of novel drug candidates.

1.6.4 To study molecular, ADME, toxicity properties of novel drug candidates.

1.6.5 To design different drug delivery techniques and its characteristic analysis.

## 1.7- Plan of Work:

| **Plan of work** | **7th Semester** | | **8th Semester** | |
|---|---|---|---|---|
| | **Mid sem** | **End sem** | **Mid sem** | **End sem** |
| **Literature Review** | | | | |
| **Identification of tea phytochemicals** | | | | |
| **Molecular docking and property analysis** | | | | |
| **Drug Delivery techniques** | | | | |
| **Manuscript writing** | | | | |

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Diseases and the enzymatic pathways:

### 2.1.1 Alzheimer's diseases:

A devastating neurodegenerative disorder with progressive loss in memory [5]. The enzyme responsible for Ach synthesis is significantly reduced resulting in decrease in levels of ACh in the brain. The site in AChE enzyme that is responsible for noncatalytic actions, is found out to be in the proximity of the peripheral anionic site (PAS) of the enzyme [6, 7]. Peripheral anionic site is a well-known site located at the entry site of the deep catalytic gap of the enzyme AChE, and it plays a vital role in binding inhibitors and substrates.

Recently various efforts have concentrated on enhancing the transmission in cholinergic synapse by blocking the action of AChE which degrades ACh at the synaptic junction. AChE inhibitors (ChEIs) are the new targets for treatment of AD. Various ChEIs are available with varying degrees of efficacy, which slows down the dementia associated with AD [8-10]. The United States Food and Drug Administration (U.S. FDA) have been approved four drugs for treating the Alzheimer's disease that act by inhibiting the AChE; those are Aricept, Exelon, Reminyl and Tacrine [11]. These four drugs represent diverse classes of AChE inhibitors and have different pharmacologic properties beyond inhibition of AChE. AChE inhibitors has various side effects which are usually attributable to marginal cholinergic effects that includes vomiting, nausea and diarrhoea. To decrease the side effects natural compounds should be considered for AD drug. Various research works have shown poly phenols such as cucumin, epigallocatechin-3-gallate, reserveratrol and theaflavin, can be considered as alternative candidates for Alzheimer's treatment.

### 2.1.2 Diabetes Mellitus Type II:

Diabetes mellitus type II is a common disease that affects people of all ages. Type 2 diabetes happens in genetically susceptible persons and is preferred

by weight excess [12-14]. Decreasing the absorption of dietary energy molecules such as fats and carbohydrates can ameliorate these metabolism related disorders. This can be achieved by inhibiting the significant enzymes that are responsible for metabolism of carbohydrates which includes alpha-amylase and alpha glucosidase.

Alpha amylase also known as α -1,4-glucan-4-glucanohydrolases is one of the chief products secreted from the human pancreas. The chief action of α-amylase is the catalysis of early hydrolysis reaction of starch into smaller oligosaccharides by cleaving the α-D-(1-4) glycosidic bonds. Neither terminal glucose residues nor α-1,6-linkages can be cleaved by α-amylase [15-18]. The catalysis reaction of α-Amylase goes through a double displacement mechanism that involves the formation of covalent intermediate of β-glycosyl enzyme and its hydrolysis by the use of active site residue carboxylic acids [19, 20]. The residues, in particular, Asp197, Glu233, and Asp300 were defined to serve the purpose of catalytic residues [21, 22]. Acarbose, a marketed drug extensively used for treatment of type II diabetes mellitus, is a pseudo-tetra saccharide. The major side effects that are common to inhibitors of α-amylase; bloating, abdominal discomfort, diarrhoea and flatulence occur in about 20% of patients.

The breakdown of carbohydrates in the small intestine can be prevented by using alpha-glucosidase inhibitors; and hence decreases the postprandial increase in level of blood glucose in humans [23-25]. For this attribute alpha glucosidase inhibitors can be used as potential medication targets for treatment of diabetes mellitus. Acarbose, voglibose and miglitol are the two marketed drugs that a help keep blood sugar levels within a target range by slowing the digestion of starches [26-30]. Alpha-glucosidase inhibitors are competitive inhibitors of the enzymes. Acarbose also blocks pancreatic alpha-amylase in addition to inhibiting membrane-bound alpha-glucosidase. Pancreatic alpha-amylase hydrolyses complex starch structure to small oligosaccharides in the lumen of the small intestine. By inhibiting the enzymes rate of digestion of carbohydrate can be reduced. Less amount of

glucose is absorbed because the carbohydrates are not broken down into small molecules such as glucose. The short term effect of these drugs on diabetic patients is to decrease current blood glucose levels while the long term effect is a small reduction in haemoglobin A1C level.

### 2.1.3 Obesity:

Obesity is a multifaceted disease with symptoms of an excessive weight increase in comparison to height because of increased deposition of fat like adipose tissue that accredited to a higher calorie intake than expenditure of energy. Pancreatic lipase has a crucial role in the effective digestion of triglycerides and is accountable for the hydrolysis of 50–70% of total dietary fats [31, 32]. This enzyme has been widely used for the determination of the potential efficacy of natural products as anti-obesity agents [33]. Orlistat, the saturated derivative of lipstatin is the only authorized anti-obesity drug that has been shown to act through inhibition of pancreatic lipase (PL), which is a key enzyme for the digestion of dietary triglycerides [34].

### 2.1.4 Jaundice:

Jaundice is a disease that characterized by yellowish discoloration of the skin, sclera, and mucous membranes due to elevated bilirubin as result of abnormal bilirubin metabolism and/or excretion. There are two type of bilirubin present in our body; the unconjugated or indirect bilirubin which is insoluble in water or conjugated to glucuronic acid/direct bilirubin which makes it soluble in water. Degradation of heme results in production of bilirubin that undergoes conjugation with glucuronic acid and excreted as bilirubin glucuronide via bile into the intestine. Intestinal β-Glucuronidase can chop the glucuronide bond to yield unconjugated bilirubin that can be absorbed by the intestine back into the blood circulation [35, 36]. Breast milk also contains some amount of beta-Glucuronidase that contributes in neonatal jaundice, because of its potential to de-conjugate bilirubin conjugates in intestine, yields bilirubin which is better absorbed by intestine [37].

A probable mechanism for treating jaundice is β-Glucuronidase inhibitor which can interrupt the enterohepatic circulation of bilirubin by acting on its active site residue and thus facilitating excretion in faeces and serum bilirubin concentration is lowered [38]. The human β-glucuronidase active site residue has been found based upon the experimentally determined active site in E. coli β-galactosidase and corresponds to the amino acid residues Glu451, Glu540 and Tyr504. Some of the presently marketed formulas based on this mechanism is enzymatically hydrolyzed casein [39]. Casein hydrolysate formula inhibits β-Glucuronidase, and infants consuming such formula have less jaundice than infants receiving routine formula [40, 41]. The major β-Glucuronidase inhibitor in casein hydrolysate is L-aspartic acid [42]. Thus β-Glucuronidase inhibitor are potential target for treatment of hyperglycaemia.

### 2.1.5 Hypertension:

Hypertension or otherwise called high blood pressure, also sometimes known as arterial hypertension, is a prolonged medical disorder which is characterized by elevation in blood pressure in the arteries. Of several causes of hypertension one is the Angiotensin converting enzyme that plays a vital role in vaso-constriction and dilation and thus affects the blood pressure. ACE is a key target for the treatment of hypertension because of its involvement in a number of blood pressure-related systems, for example' the renin-angiotensin system (RAS) and the kinin nitric oxide system (KNOS). In the RAS, the ACE cleaves angiotensin I into the potent vasoconstrictor angiotensin II, and in the KNOS, hypotensive peptide, bradykinin and kallidin are inactivated by ACE [43]. Therefore, unnecessary action of ACE results in increased vasoconstriction and hypertension. The mechanism of action of ACE shown below. Angiotensin-converting enzyme (ACE) inhibitors can be considered as potential agents for the treatment of heart failure, hypertension and other cardiovascular and renal diseases. ACE inhibitors have been proven more effective than other hypertensive substances in reducing proteinuria and retarding the progression of renal damage in patients with various types of nephropathy. Some of the synthetic

ACE inhibitory marketed drugs are Captopril, Lisinopril and Enalapril that act as anti-hypertensive [44]. However, pharmacological ACE inhibitors use results in a range of side-effects that includes cough, skin rashes, angioedema, reduced renal function and foetal abnormalities [45].

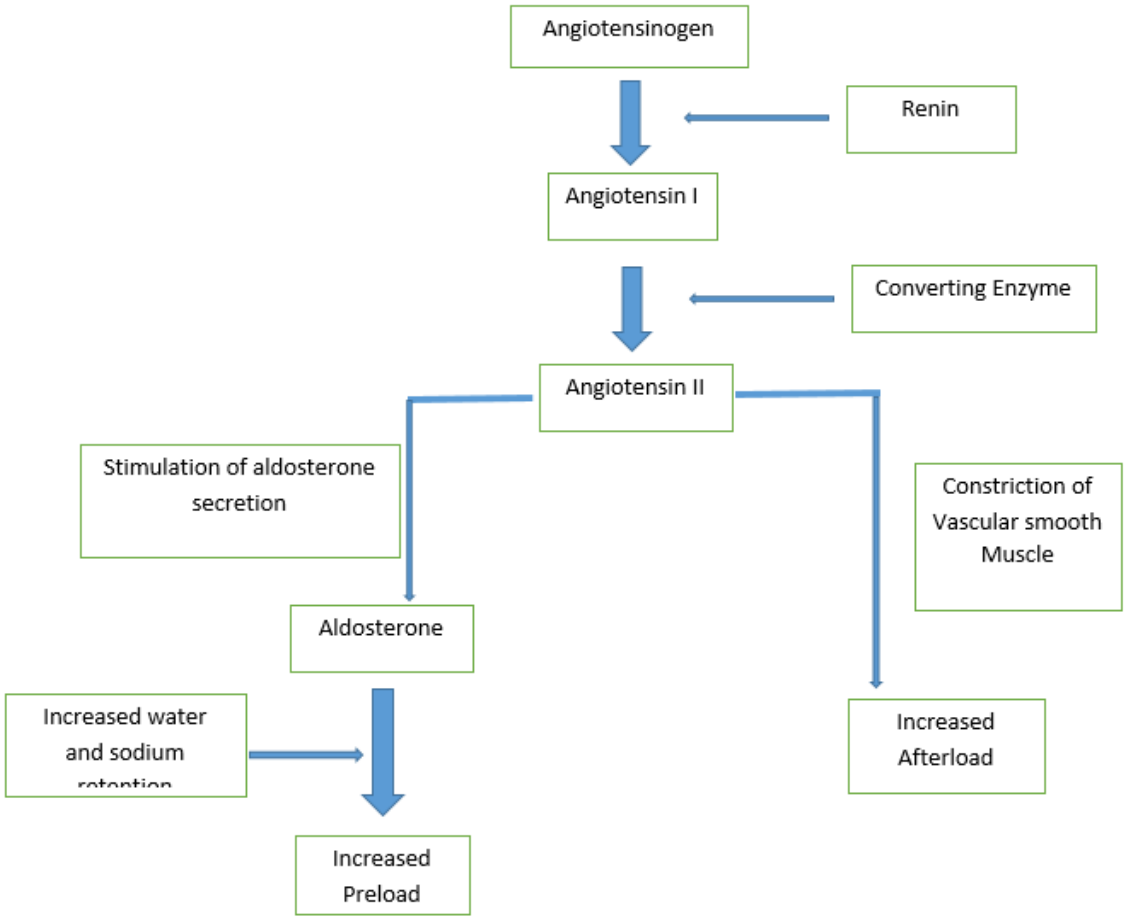



Figure 1: Pathway of renin angiotensin system.

### 2.1.6 Asthma:

Asthma disease is characterized by episodic hyper secretion of mucus, bronchoconstriction and inflammation of the air passage. Several research studies suggest that compounds derived from the activities of arachiodonate 5-lipoxygenase on arachiodonic acid have a role in the physiological pathway of asthma [46, 47]. The products of 5-LOX pathway include 5-HETE,

sulfidopeptide leukotrienes, have been detected in body fluids after induced Broncho-constriction [48]. Hence inhibition of these 5-LOX products may act as potential drug target for asthma treatment. Many inhibitors for 5-LOX have been described [49-51], that can be categorised into three types, iron ligands, reductive and competitive inhibitors [52], nevertheless, only one compound has been permitted as a drug, zileuton [53, 54]. Zileuton is a potent and selective 5-LOX inhibitor but its mode of action is unusual for a therapeutic [52, 55].

## 2.2Drug Delivery Techniques:

Method of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals is provided by various drug delivery systems. The technological inventions are necessary for administering drug at precise rate and also slow and targeted delivery are other attractive methods that have been pursued vigorously [56-58].

LIPOSOMAL DRUG DELIVERY SYSTEM

The concentric bilayered lipid structures consist of amphipathic phospholipids. There are various classification of liposomes based on the number of bilayer such as MLV (multilamellar), SUV (small unilamellar), or LUV (large unilamellar). The liposomal size range varies from 0.025 μm to10 μm in diameter. Methodology of preparation and composition of ingredients regulates size and morphology of the resulting liposomes. Liposomal encapsulation are used for delivery of various compounds such as drugs, vaccines, and genes for a diversity of disorders [59]. Enhanced efficiency and reduced toxicity are provided by drug delivery systems. Long circulating macromolecular carriers such as liposomes are known to exploit the ‘enhanced permeability and retention’ effect for preferential extravasation from cancer vessels [60]. Stable formulation, improved pharmacokinetics, and a degree of ‘passive’ or ‘physiological’ targeting characteristics are provided by liposomal drug delivery systems [61]. Another category of liposome known as immune-liposomes appear to be non-immunogenic and are capable of long circulation even with repeated administration [62]. There are several studies showing liposomal encapsulated dopamine can be efficiently delivered into the brain and its degradation in circulation can be prevented [63]. The mechanism of action is that

liposomes get attached to the membranes of cell and fuse with them, then releasing the encapsulated drug into the cell. The other mechanism suggest that in phagocytic cells, liposomes are taken up, the phospholipid walls are acted upon by organelles called lysosomes, and the medication is released. Still the Liposomal delivery systems largely under experimentation; the precise mechanisms of liposomal action are under study, as the ways of targeted delivery to specific diseased tissue [64].

PECTIN-HEMA HYDROGEL SYSTEM

The use of biodegradable polymers for the development of sustained and site-specific drug delivery system has increased dramatically over the past few decades. Synthetic polymers can be tailored to develop a wide range of properties thereby offering greater advantages than the natural ones. However the use of synthetic polymers in pharmaceutical formulations are restricted since their processing techniques are based on certain properties. Therefore, this has led to the demand for safer drug carrier systems. Hydrogels are the hydrophilic polymer networks that are swollen in water, but are not dissolved in it. These are three-dimensional cross-linked polymeric structures that are able to swell considerably in an aqueous environment and are proved to have an extraordinary capacity (>20%) for absorbing water within the polymeric network structure. It was observed by Ranjha et al that the swelling characteristics, pH sensitivity, and the biodegradability of these cross-linked hydrogels could be modified to desired extent by varying the monomeric compositions and degree of cross-linking and also by using other suitable polymer component [65]. Pectin is a heterogeneous, hydrophilic naturally occurring polysaccharides that are the major constituents of citrus by products and is widely used as a gelling and stabilizing agent in food, pharmaceuticals and cosmetics. Poly-HEMA was chosen to modify pectin as it is a well-known biomaterial and is the first one to be successfully employed in the biological use. The synthetic hydrogels prepared using these poly-HEMA is known to possess a high mechanical strength, a relatively high water content in swollen state, and resistance to various chemicals [66]. In addition to its non-toxicity, non-antigenic properties and good biocompatibility, molecularly engineered hydrogels based on pHEMA have shown to be potential carriers in drug delivery for water soluble anticancer drugs, including 5-

fluorouracil, topical mitomycin-C, and cytarabine, and also in dental, ophthalmic, and neural tissue engineering applications.

# CHAPTER 3: MATERIALS AND METHODS

## MATERIALS AND METHODS:

## 3.1. Requisites:

### 3.1.1 Bioinformatics softwares and tools used

1. NCBI
2. Protein Data Bank (PDB)
3. CASTp
4. PubChem
5. ArgusLab
6. PreADMET
7. PRODRG2
8. AutoDock4.2
9. LigPlot+
10. UCSF Chimera

### 3.1.2 Chemicals and Reagents used

1. Lecithin- HIMEDIA
2. Cholesterol- HIMEDIA
3. Chloroform-Rankem
4. Methanol- Lobal chemie
5. PBS
    a. NaCl- HIMEDIA
    b. KCl- Qualigen
    c. Na2HPO4- Qualigen
    d. K2HPO4-

## 3.2. Insilico Study

### 3.2.1. Protein structure retrieval:

The three dimensional structures of target enzymes were retrieved from PDB (Protein Data Bank; http://www.rcsb.org/pdb/). The enzymes selected for targeting the diseases have been compiled in Table 1. Miscellaneous ligands and other hetero-atoms like water, ions were removed from the protein models for active site predictions and further docking studies using Argus Lab Software.

**Table 1**: Potential Drug targets to target various diseases.

| **Enzyme** | **Disease** | **Organism** | **PDB Id** |
|---|---|---|---|
| Acetyl cholinesterase | Alzheimer's | Homosapiens | 4M0F |
| Alpha-amylase | Diabetes mellitus Type II | Homosapiens | 1HNY |
| Alpha-glucosidase | Diabetes mellitus Type II | Homosapiens | 2QMJ |
| Beta- Glucuronidase | Jaundice | Homosapiens | 1BHG |
| ACE | Hypertension | Homosapiens | 1O86 |
| Lipase | Obesity | Homosapiens | 1LPB |
| Lipoxygenase | Asthma | Homosapiens | 3V92 |

### 3.2.2 Modeling:

To build 3D model of protein structure, Homology modeling was used that takes experimentally found structures of linked family members as templates. SWISS-MODEL workspace; that is a Web-based integrated modeling expert system. For a given protein, an archive of experimental protein structures is examined to identify appropriate templates. On the basis of a sequence alignment between the template structure and the target protein, a three-dimensional model for the target protein is created. Model quality evaluation tools are used to estimate the reliability of the resulting models from SWISS-MODEL.

Lipoxygenase enzyme doesn't have good quality PDB structure as those contain multiple mutations. Hence its structure is modelled using SWISS-MODEL. First a new modelling project was created. Then target sequence is inserted in FASTA format and the model was built by clicking on 'BUILD MODEL'. Homology modeling is currently the most accurate computational method to generate reliable structural models and is routinely used in many biological applications.

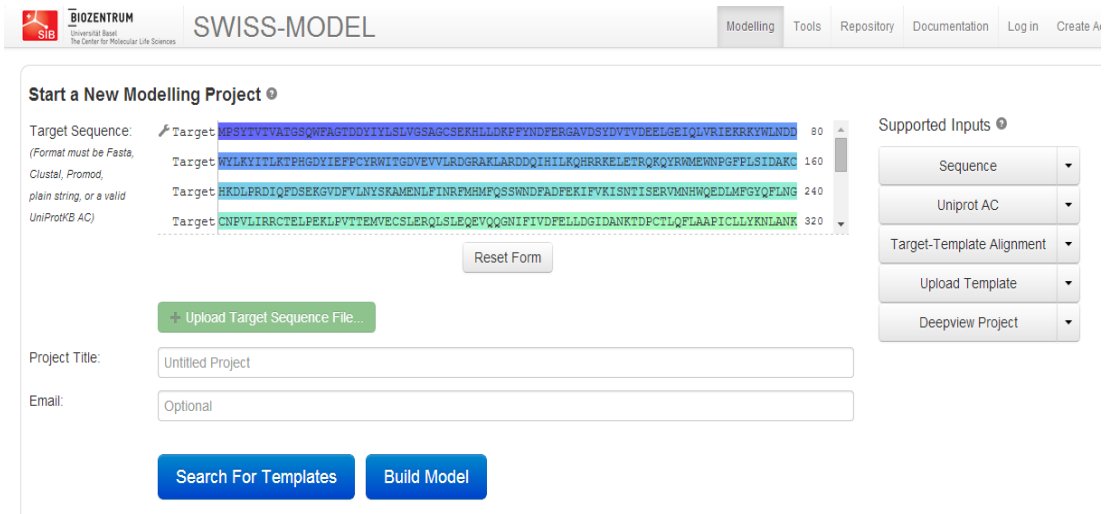



Figure 2: Screenshot of protein model building in SWISS-MODEL.

### 3.2.3 Protein Active Site Predictions:

The active sites of targeted enzymes were predicted by using CASTp Calculations (Computed Atlas of Surface Topography of proteins). Amongst the predicted site, the site having the catalytic amino acids was chosen for the docking of the ligand. The catalytic amino acids of the each protein were analysed from UniProt (http://www.uniprot.org/).

### 3.2.4 Ligand selection:

The three dimensional structure of phytochemicals of black tea were screened from KNApSAcK-3D database (http://knapsack3d.sakura.ne.jp/), PubChem database (http://pubchem.ncbi.nlm.nih.gov/) and ChemSpider database (http://www.chemspider.com/). The PDB structure of the ligand was deduced using PRODRG Server (davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg). Ligand optimization

and energy minimization was further done using Argus lab software. Apart from this, the three dimensional structures of standard inhibitors for all the aforesaid proteins were also deduced from PubChem database (http://pubchem.ncbi.nlm.nih.gov/) and were used as reference inhibitory molecules.

### 3.2.5 ADME properties and toxicity testing of ligands using PreADMET server

An important block in the drug discovery process, particular in the later stages of drug discovery, is the analysis of the ADME and toxicity properties of the lead drug candidates. To avoid the failure of more than 50% of drug nominees because of ADME/Toxicity deficits most pharmaceutical corporations has employed a collection of in vitro ADME/Toxicity screening software that aims at discarding compounds within the discovery section which are seem to fail any down the line test. One of the web based application is PreADMET quickly predicts the drug-likeness and ADME/Toxicity knowledge of drug candidates. The web address http://preadmet.bmdrc.org/ was accessed to open PreADMET server. Chemical structure of ligand was drawn and submitted in the displayed screen. The result was saved carefully.

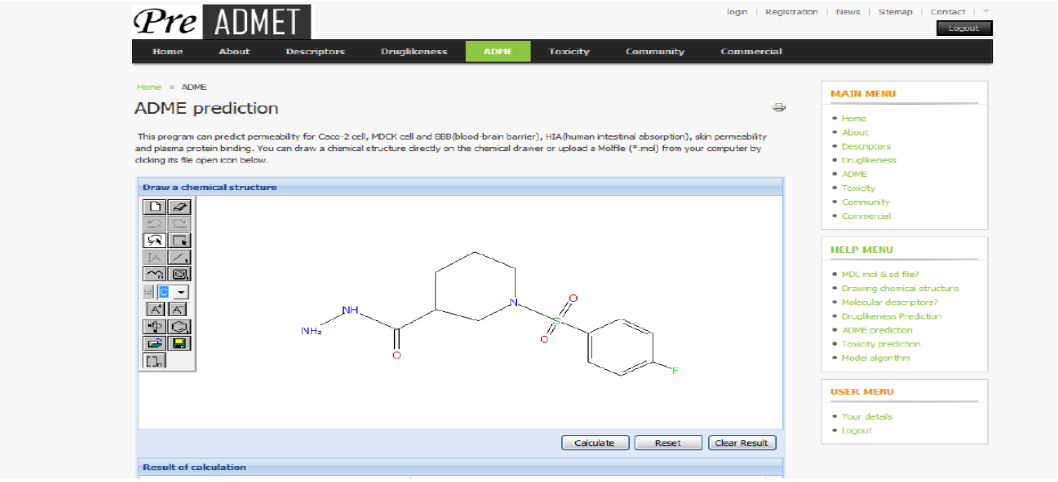



FIGURE 3: Screen shot of molecular property analysis in Pre ADMET server.

### 3.2.6 Molecular Properties and Drug likeness:

The phytochemicals of tea were further examined for its drug likeness and molecular properties using Osiris web server. Molsoft; A Cheminformatics system which

provides varied range of software tools that supports manipulation of molecules and their processing, SD file conversion, molecule standardization, tautomer generation, molecule fragmentation, modeling of molecule and drug design, high quality molecule representation, database tools supporting sub-structure and search for similarity, also hold fragment-based virtual screening, bioactivity prediction and data visualization. The web address http://www.organic-chemistry.org/prog/peo/ was accessed to open molecular property viewer, i.e., Osiris. Then molecule structure was drawn and property were studied.

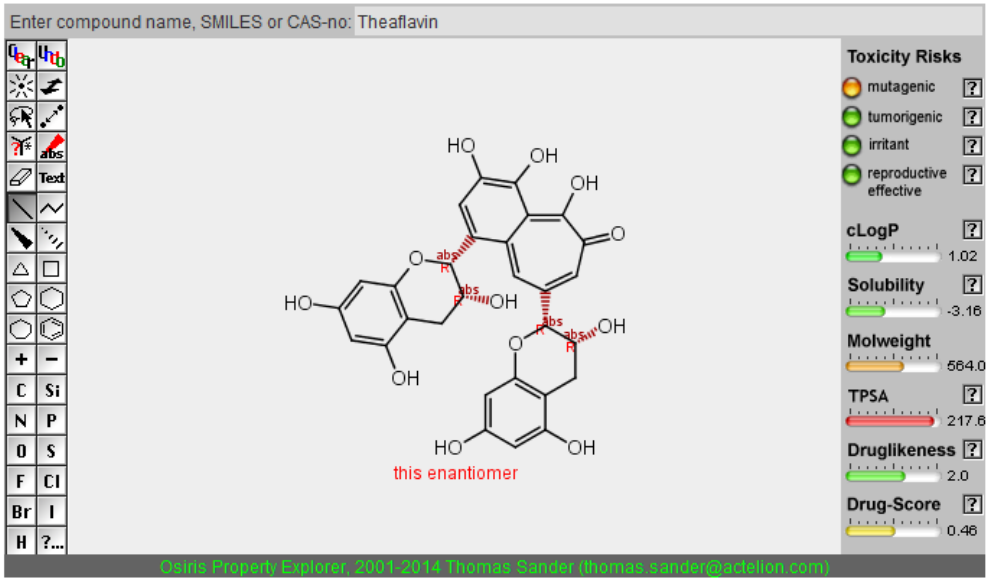



FIGURE 4: Screen shot of Osiris molecular analysis web server showing molecular properties and drug score of theaflavin.

### 3.2.7 Molecular Docking:

The computational docking of black tea phytochemicals and standard inhibitors were performed into the active site of corresponding protein models using AutoDock4.2 software (version 1.5.6). Lamarckian genetic algorithm is used as search function in the AutoDock 4.2 software. Lamarckian genetic algorithm is a fusion of genetic algorithm and local search algorithm which uses a parameterized scoring function of free energy to evaluate binding energy with regular docking procedure on the basis a 150 arbitrarily placed individuals population size; a maximum number of 2.5*107 energy evaluations, a mutation rate of 0.02, a crossover rate of 0.80 and an elitism

value of 1 [67]. For each ligand molecule 15 autonomous docking runs were carried out and the results that obtained were grouped according to rmsd criteria of 1.0 Å. Autogrid calculates the grid maps that represents the proteins and grid size was set to 60*60*60 points with grid spacing of 0.375 Å. Polar hydrogen's were added to protein macromolecular models during docking using the hydrogen's module and then, Kollman united atom partial charges were assigned. The coordinate of the docked protein along with the ligand was visualized using UCSF chimera and LigPlot+ [68].

## 3.3 Drug Delivery Technique:

### 3.3.1. Preparation of crude tea extract

Dried tea leaves were collected, powdered and then 40g of the powder is added in 2ooml of water. Then it is boiled for 15min, filtered and the filtrate is dried in rotary vacuum evaporator at 30-35 rpm and $100^0$C. 3.5gm of powdered extract obtained after complete drying in Rotary Vacuum Evaporator. The powder is stored in deep refrigeration for further experimentation.

### 3.3.2. Liposome Encapsulation

#### A. Preparation of Encapsulated Liposome

Liposomal Encapsulated tea was prepared by thin film hydration Method. In this method the lipid must first be dissolved and mixed in an organic solvent to assure a clear and homogeneous mixture of lipids. A solution is made of 6 ml chloroform and 3 ml methanol to which 0.72g of lecithin and 0.06 of cholesterol is added. The solution is vortexed for 30-40 minutes till the lecithin and cholesterol is fully dissolved. This solution is added to round bottom flask of the rotatory vacuum evaporator. The temperature of the water bath attached to the rotatory vacuum evaporator is raised to 66 degree Celsius. The solution is rotated at 25 rpm till the all solvent gets evaporated and a thin lipid film is visible. 36 ml of PBS is prepared and 0.36gm of powdered tea extract is added to it, then the solution is added to round bottom flask and the flask was vigorously vortexed till the entire film is dissolved in the aqueous medium.

Once the lipids are thoroughly mixed in the organic solvent to assure a clear and homogeneous mixture of lipids, then the solvent is removed to produce a lipid film. Then lipid film obtained is carefully dried to remove remaining organic solvent by placing the flask on a vacuum pump. Then medium of aqueous solution is added to the container of dry lipid and agitated. The hydrated lipid suspension was then downsized by a diverse techniques, such as extrusion or sonication.

**B. Characterisation of Liposome**

**a. Downsizing of prepared liposomes:**

Disruption of LMV suspension using sonication typically produces SUVs. It can be done using bath or probe sonicators. Sonication of the prepared suspension is achieved by placing the liposomal suspension in a bath sonicators and then sonicating for 5 to 10 min above $T_c$ of lipid. After sometime the liposomal suspension start to clarify to produce a slightly dim transparent solution. The haze produced in the suspension is because of scattering of light induced by remaining large liposome particles lingering in the suspension of lipid. To yield a clear transparent solution of small unilamellar vesicle these large residual particles are removed by centrifugation. Mean size of liposome and their distribution is inclined towards their concentration in solution, composition, time and power of sonication, temperature, sonicators tuning and volume.

**b. Phase contrast microscopy:**

It is an optical microscopy method which is based on the principle of conversion of phase shifts in the incident light that is passing through a clear, transparent specimen to the intensity changes in produced image. Basic principle of the method is to make changes in phase observable in the phase contrast microscope by separating the background light illumination from the sample scattered lights that make up the forefront details and can be manipulated inversely.

**c. Zeta potential and Particle size measurement:**

A Zetasizer was used to analyse particle size by Dynamic Light Scattering and to measure Zeta Potential. Dynamic Light Scattering is a technique used to measure size of a molecule. The diffusion of the particles in the solution moving in Brownian motion is measured by DLS which is converted to size distribution and

size applying Stokes-Einstein equation. Smaller particle cause the intensity to fluctuate more rapidly than larger particle. The interference of scattered light from the particle causes a fluctuation in the intensity of light scattered at a particular observation angle which reflects particular size. DLS always gives hydrodynamic diameter; it is the value that denotes to how a molecule diffuses in a solution so it is mentioned as hydrodynamic diameter.

**d. Drug kinetics study:**

Drug release from the encapsulated liposomes was calculated using dialysis method.

**Preparation of dialysis bag:** The glycerol included as humectant in the dialysis bag was removed by washing the dialysis tubing in running water for 3-4 hours. Removal of sulphur compounds can be done by treating the tubing with 0.3% (w/v) sodium sulphide solution at $80^0$C for around 1min. After that it is cleaned by the help of hot water wash ($60^0$C) for approximately 2min. Then it is acidified with a 0.2% (v/v) water solution for removing the acid.

**Preparation standard curve:** A standard curve for different tea concentration is prepared by analysing the concentration by OD method in UV-VIS spectrophotometer at 380nm.

Liposomal concentration is dispersed in 1ml PBS solution and placed in a tube with one end covered with dialysis membrane. The tube was hung above a glass beaker containing 100ml of PBS inside it, for which the portion of the dialysis membrane with the formulation dipped into the PBS. The beaker was placed in magnetic stirrer, then stirring was maintained at 100rpm at $37^0$C with samples were taken in every one hour over a course of 10 hours and assayed for drug content at 380nm spectrophotometrically.

### 3.3.3. Pectin-HEMA Hydrogel

**a. Preparation of solution:**

Pectin solution of 1% concentrations was prepared by dissolving pectin in distilled water kept at $50^0$C. The temperature was gradually increased and set at $70^0$C and then finally at $90^0$C. The solution was constantly stirred with the help of

a magnetic stirrer and a homogenous solution was prepared. The solutions prepared were further kept for degassing. Also, APS and TEMED solutions of 40% strength was prepared.

**b. Preparation of Hydrogels:**

The hydrogels were prepared in 15 mL bottles that were purchased from Rankem. Hydrogels were prepared in these bottles to maintain the size and the uniformity. The preparation of the hydrogels were done by adding 1000 µL of the pectin solution of different concentration in 450 µL of the pure HEMA solution. Then after proper mixing of these two solutions, APS and TEMED both 25 µL were added respectively. A control was also prepared which did not contain any pectin. The total reaction mixture of 1.5 mL, was kept constant for all the different concentrations of pectin used. The prepared hydrogel solutions were then kept in a hot air oven at $37^0$C for the gels to form.

**c. Characterization of the hydrogels**

**<u>Swelling study at different pH</u>**

The initial weight of the wet hydrogels were taken. These hydrogels were then kept in a Vacuum drying oven (Labtech) for around 48h to get fully dried. Dried hydrogels were then weighed ($W_d$) and immersed in (10) mL of pH 7.4 and pH 9 phosphate buffer solutions and thus the swelling experiments were carried out. The hydrogels were taken out at different time intervals and blotted carefully to remove the excess surface water using laboratory tissue. These wet swollen samples were then weighed ($W_t$) at the above mentioned time intervals. All the swelling experiments at different pH were performed in triplicates. The swelling ration were calculated using the equation:

$$\textbf{Swelling ratio (\%)} = \frac{W_t - W_d}{W_d} \ \%$$

where,

$W_t$= Weight of wet hydrogels**,** $W_d$= Weight of dry hydrogels

**e. Drug Release study:**

Tea loaded pectin-HEMA hydrogel were prepared. The loaded hydrogel dispersed in 1ml PBS solution and placed in a tube with one end covered by dialysis membrane. The tube was hung above a glass beaker containing 100ml of PBS inside it, due to which the part of the dialysis membrane with the preparation immersed into the PBS. The beaker was placed in magnetic stirrer and the stirring was maintained at 100 rpm at 37°C with samples were collected every one hour over a period of 10 hours and assayed spectrophotometrically for drug release at 380nm.

# CHAPTER 4: RESULT AND DISCUSSION

## 4.1. Insilico drug discovery:

### 4.1.1 Modelling of Lipoxygenase

The modelling of Lipoxygenase enzyme was done in Swiss model web server. Ten models were obtained which are subjected to further validation. The validation data is given below. Comparing the validation data of different properties model 8 is selected for further experimentation. Model 8 has 0% disallowed structural score, comparatively high Ramachandran plot score, highest percentage of allowed structural model and comparatively high verified structure.

Table2: Verification of Lipoxygenase protein model.

| Model no. | Ramachandran Plot score (%) | Allow (%) | Gener (%) | Disallowed (%) | Verified (%) | Errat (%) |
|---|---|---|---|---|---|---|
| 1 | 92.9 | 6.6 | 0.3 | 0.2 | 95.56 | 82.432 |
| 2 | 93.2 | 6.1 | 0.5 | 0.2 | 98.22 | 82.583 |
| 3 | 93.4 | 6.1 | 0.2 | 0.3 | 97.93 | 85.435 |
| 4 | 93.2 | 6.4 | 0.2 | 0.2 | 98.96 | 82.432 |
| 5 | 93.1 | 6.3 | 0.5 | 0.2 | 95.7 | 84.685 |
| 6 | 92.9 | 6.4 | 0.3 | 0.3 | 98.52 | 82.282 |
| 7 | 93.2 | 6 | 0.3 | 0.5 | 96.89 | 83.634 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8 | 93.2 | 6.4 | 0.3 | 0 | 96.89 | 87.234 |
| 9 | 93.2 | 6.3 | 0.3 | 0.2 | 96.3 | 84.084 |
| 10 | 93.2 | 6.3 | 0.2 | 0.3 | 98.96 | 83.033 |

### 4.1.2 Active site prediction:

Active sites of the target PDF protein of all organisms were predicted by CASTp, active site prediction tool. CASTp computation is based on the pocket algorithm of the alfa shape theory. The active site of protein with structural pockets and cavities were calculated for all test enzymes comparing the CASTp result and UniProt data. The result is shown below:

Table3: Active site of the target enzymes.

| Name of enzyme | Acetyl cholinesterase | Alpha-Amylase | Alpha-Glucosidase | Beta-Glucuronidase | ACE | Lipase | Lipoxygenase |
|---|---|---|---|---|---|---|---|
| PDB Id | 4M0F | 1HNY | 2QMJ | 1BHG | 1O86 | 1LPB | - |
| Active site amino acid | SER-203, HIS-447, GLU-334 | GLU233, ASP300 | ASP529, GLU532 | GLU451 | GLU391 | SER152 | LEU608 |

### 4.1.3 Docking study:

As part of our ongoing effort towards the design and development of effective drug against most prevailing chronic diseases for subsequent use in medical applications, we have studied the inhibition efficiency of the selected black tea phytochemicals for the possible drug targets. The docking of protein with ligand molecules was done step by step according to the standard procedure. It was done by automated docking tool AutoDock4.2. In order to find the novel drug against each dieses under consideration first we docked those enzymes with reference molecule. The reference molecule that selected were marketed drugs which act in

selected inhibitory pathway. For most of the ligands, docking explicitly generated the crystallographic binding orientation within the protein cavity. For each ligand, 10 independent docking run were initiated with randomized populations and docking results for individual run were clustered if their final docked positions were within a tolerance of 0.5 $A^0$.

The docking result of enzymes of selected diseases with respective reference molecule are listed below:

Table 4: Docking study of target enzymes with the reference molecules.

| **ENZYME** | **Name of Inhibitors** | **B.E.(kcal/mol)** | **IC (µm)** |
|---|---|---|---|
| **Acetyl cholinesterase (4M0F)** | Donepezil | -9.88 | 0.057 |
| | Galantamine | -9.24 | 0.17 |
| | Rivastigmine | -7.72 | 2.2 |
| | Tacrine | -6.99 | 7.53 |
| | Huperzine | -8.66 | 0.45 |
| **Beta-Glucuronidase (1BHG)** | Saccharolactone | -5.42 | 583.2 |
| | Silymarin | -8.78 | 0.368 |
| **Alpha- amylase** | Acarbose | -5.04 | 202.9 |
| **Lipase (1LPB)** | Orlistat | -5.9 | 47.38 |
| | Esculetin | -6.7 | 12.35 |
| **Alpha-glucosidase (2QMJ)** | Miglitol | -6.82 | 9.98 |
| | Voglibose | -9.26 | 0.163 |

| | | | |
|---|---|---|---|
| **ACE (1O86)** | Benazepril | -10.15 | 0.036 |
| | Captopril | -6.08 | 35.01 |
| | Enalapril | -7.94 | 1.51 |
| | Lisinopril | -10.52 | 0.019 |
| | Temocapril | -10.54 | 0.019 |
| | Zofenopril | -7.95 | 1.5 |
| **Lipoxygenase (MODEL 8)** | Azelastine | -4.4 | 594.5 |
| | Carbamazine | -5.54 | 87.64 |
| | nerdihydro guaiaretic acid | -5.54 | 86.63 |
| | Zileuton | -6.68 | 12.78 |

The docking of enzymes with the selected candidate ligands were done at the respective active site of the enzyme. All the catalytic residues are present in active site pocket of the enzyme and thus helpful in stable interactive bond formation with ligand molecules. Docking result were interpreted on the basis of binding energy and inhibition constant. This high binding energy suggest stable interaction thus a very high inhibition efficiency and low inhibition constant. Inhibition constant (Ki) value obtained from the docking result agrees with it. From all docking results we sort out top two docking result for each enzyme-ligand complex and represent in tabulated form below. We have sorted this result according to decreasing order of binding energy.

Table 5: Best docked ligands and their UCSF chimera interaction analysis.

| **ENZYME** | **Name of test Inhibitors** | **Binding Energy** | **IC (µM)** | **H-Bonds** | **Interacting Amino acid** |
|---|---|---|---|---|---|

| | | (kcal/mol) | | | |
|---|---|---|---|---|---|
| **Acetyl cholinesterase (4M0F)** | theaflavin (114777) | -11.77 | 0.0068 | 8 | TYR124 (3), GLY126, GLU202, ASN87, MET85, GLY448 |
| | Vitamin E (2116) | -10.77 | 0.0128 | 0 | - |
| | DONEPEZIL* | -9.88 | 0.0568 | 0 | - |
| **Alpha-Amylase (1HNY)** | 8-c-ascorbyl(-)-epigallocatechin (3001587) | -8.69 | 0.4289 | 6 | HIS201, GLU233, ASP197, ARG195, Intramolecular(2) |
| | Rutin (5280805) | -8.58 | 0.5177 | 6 | GLU233, HIS305, HIS201, LYS200, TRP59(2) |
| | ACARBOSE* | -5.04 | 202.91 | 8 | |
| **Alpha-Glucosidase (2QMJ)** | orientin (5281675) | -8.92 | 0.2899 | 5 | GLN603, ASP327(4) |
| | 8-c-ascorbyl(-)-epigallocatechin (3001587) | -7.57 | 2.8 | 7 | ASP203, ASP327(3), TYR605, GLY604, Intramolecular (1) |
| | VOGLIBOSE* | -9.26 | 0.1634 | 3 | |
| **Beta-Glucuronidase (1BHG)** | Theaflavin (114777) | -8.83 | 0.3358 | 3 | GLU540, ASN450, SER503 |
| | Strictinin (73330) | -8.81 | 0.3491 | 7 | GLU540(2), ASN450, ASN484, GLU451, TYR508, LYS606 |
| | Silymarin* | -8.78 | 0.368 | 3 | GLU540, TYR505, PHE206 |
| **ACE (1O86)** | Theaflavin (114777) | -10.94 | 0.0095 | 9 | SER355(2), TYR360, PHE359, ASN66, ASP358, ALA63, Intramolecular (2) |

| | | | | | |
|---|---|---|---|---|---|
| | Rutin (5280805) | -9.74 | 0.0723 | 8 | ASN70, ALA356, TRP357, GLU411, GLU384, ARG522, TYR523, Intramolecular (1) |
| | RAMIPRIL* | -11.07 | 0.0077 | 1 | |
| **Lipase (1LPB)** | 8-c-ascorbyl epigallocatechin 3-o-gallate (c00008969) | -10.14 | 0.0371 | 5 | SER237, HIS236, GLY161, ASP164(2) |
| | Schaftoside (442658) | -9.78 | 0.0677 | 6 | ASP164, SER137, ILE163(2), Intramolecular (2) |
| | ORLISTAT* | -5.9 | 47.38 | 1 | |
| **Lipoxygenase** | Hexadecane (5366244) | -6.75 | 11.27 | 0 | - |
| | ZILEUTON* | -6.68 | 12.78 | 0 | - |

***Standard inhibitors***

**4.1.4 Interaction study:**

As mentioned above, protein-ligand interaction analysis is necessary for better understanding of the underlying molecular mechanisms of this wide-ranging inhibitory activities of the candidate ligands against the target enzymes. To describe the enzyme-ligand complex stability the molecular interaction in terms of Hydrogen bond & hydrophobic interaction were studied using UCSF chimera and Ligplot. The result for only the best posture of each nominated potential lead molecules were presented below. A Ligplot figure gives a schematic representation of the hydrogen bonds and non-bonded interactions between ligand and the residues of the protein with which it interacts. Higher stability comes from higher binding affinity which in turn depends on, if the ability of ligands to form hydrophobic interactions with the binding site hydrophobic amino acids is higher. Figure below shows the interaction between the best selected ligands and the targeted enzyme. It can be seen that selected ligands have large number of H-bond and

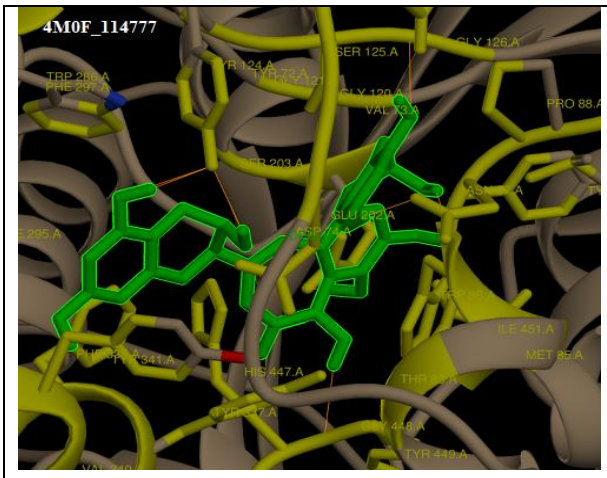
hydrophobic interactions, where no. of H-bond is less there hydrophobic interaction number is higher and vice versa.

Table 6: Interaction study of best docked ligand-target enzymes using LigPlot

| **PROTEIN** | **Active AA residue** | **LIGAND** | **H-bond** | **Hydrophobic interaction** |
|---|---|---|---|---|
| **Acetyl cholinesterase (4M0F)** | SER203 | Theaflavin (114777) | TYR133, GLN71, HIS497, ASN87, TYR124, GLY126 | GLY120, LEU130, GLU202, ILE451, TRP86, GLY448, GLY121, PRO88, TYR337, TYR341, TRP286, PHE297, SER125, PHE338, ASP74 |
| | | Vitamin E (2116) | NA | GLU202, SER203, HIS447, PHE338, TYR337, TRP286, TYR341, GLY122, PHE297, ASP74, TYR124, SER125, GLN71, TYR72, ASN87, TYR133, TRP86, GLY120, GLY121 |
| **Alpha Amylase (1HNY)** | ASP197 | 8-c-ascorbyl(-)-epigallocatechin (3001587) | HIS305, ASP195, GLU233, ASP356 | LYS200, HIS201, LEU162, TYR151, TRP59,TRP58,ASP300, HIS299, TYR62, ILE235 |
| | | Rutin (5280805) | TRP59, GLU233, HIS201, LYS200, GLU233, HIS305, ASP356 | TYR62, LEU162, GLU63, TRP58, ASP19, ALA198, TYR151, GLY306, ILE235 |
| **Alpha-Glucosidase (2QMJ)** | ASP443 | 8-c-ascorbyl(-)-epigallocatechin (3001587) | ASP327, GLN603, ASP203, MET144 | TYR605, TRP406, TRP539, ASP542, ARG526, ASP443, TRP441, ILE364, HIS600, TYR299, PHE575 |
| | | Orientin (5281675) | GLN603, ASP327 | ILE364, TRP441, ASP542, ASP443, TRP406, PHE450, MET444, ASP203, ALA576, TYR299, TYR605, GLY602, PHE575 |
| **Beta Glucuronidase (1BHG)** | GLU451 | Strictinin (73330) | HIS385, LYS606, GLU540, ASP207, ASN484, **GLU451**, ASN486 | TRP587, TYR508, SER485, HIS455 |

| | | | | |
|---|---|---|---|---|
| | | Theaflavin (114777) | LYS606, TYR584, ASN450, HIS385 | SER503, TYR508, HIS509, TYR205, GLU451, PHE206, GLU540, ASP207, TRP587 |
| **ACE (1O86)** | GLU384 | Theaflavin (114777) | ASN66, TYR360, ASP358, SER355, HIS353, **GLU384**, TYR523 | TYR63, TRP357, ALA356, PHE391, ARG533, VAL518, PHE513, ALA354, GLU411 |
| | | Rutin (5280805) | ASN70, ALA356, **GLU384**, HIS387, HIS383,GLU411,ZN701 | PHE513, VAL357, GLU143, HIS353, SER355, TRP357, ALA354, VAL380, PHE391, TYR520, GLU2000, TYR523, HIS543 |
| **Lipase (1LPB)** | SER237 | 8-c-ascorbyl epigallocatechin 3-o-gallate (C00008969) | ASP164, ARG341, **SER237**, HIS236 | LEU349, ILE163, PHE162, HIS348, TYR199, PRO265, PHE300, LEU298 |
| | | Schaftoside (442658) | ARG341, PHE162, **SER237** | LEU349, ALA345, ALA344, ILE163, PHE300, TYR199, ILE294, HIS348, ALA263, GLY161, ASP164 |
| **LIPOXYGENASE** | LEU608 | Hexadecane (5366244) | NA | NA |

The pictorial representation of best docked molecule is in figure below. The right side figure shows the LigPlot+ analysis in which green dotted line shows the hydrogen bonding between the drug candidate and amino acid residues of protein . The purple coloured molecule is the drug candidate. In the UCSF Chimera analysis the orange and blue coloured line shows hydrogen bonding and the middle fluorescent molecule is the drug candidate and the side coloured ones are the amino acid of the protein within area less than 5 Å. The analysis of docked molecule were done and result shown in above table.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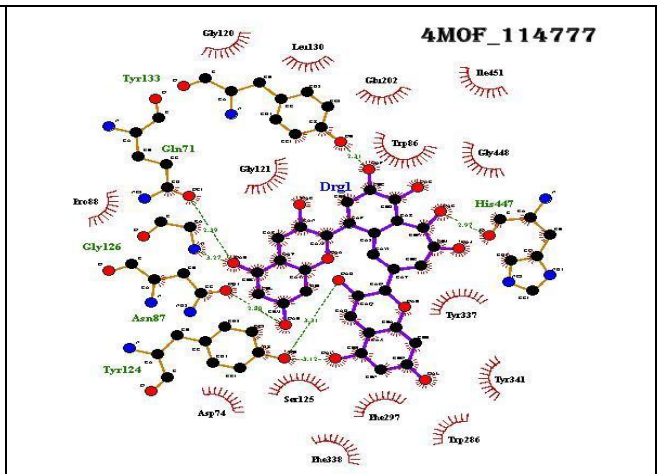
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Ile451
Gln71
Trp86
Gly448
Gly121
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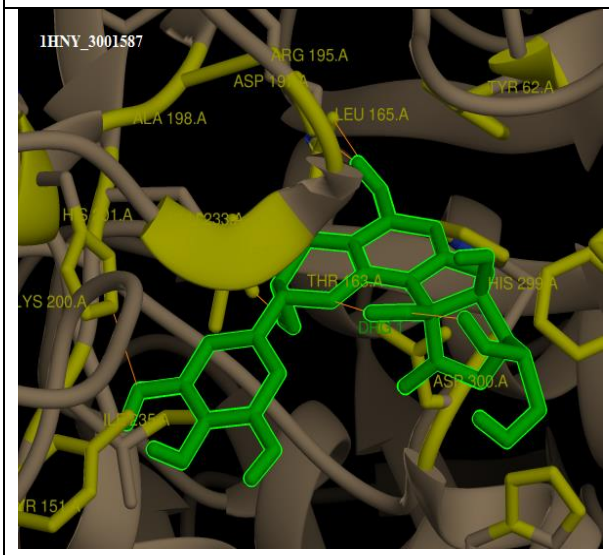
Ser125
Tyr341
Asp74
Phe297
Trp286
Ile338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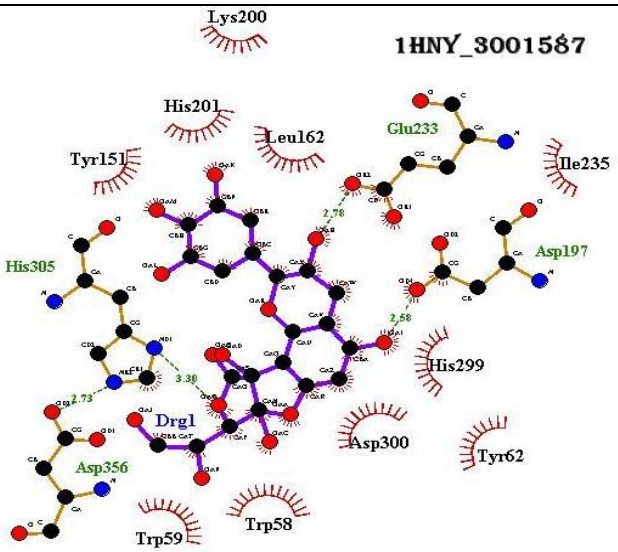
LEU 165.A
ALA 198.A
TYR 62.A
THR 163.A
HIS 299.A
LYS 200.A
ASP 300.A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Lys200
His201
Leu162
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His305
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His299
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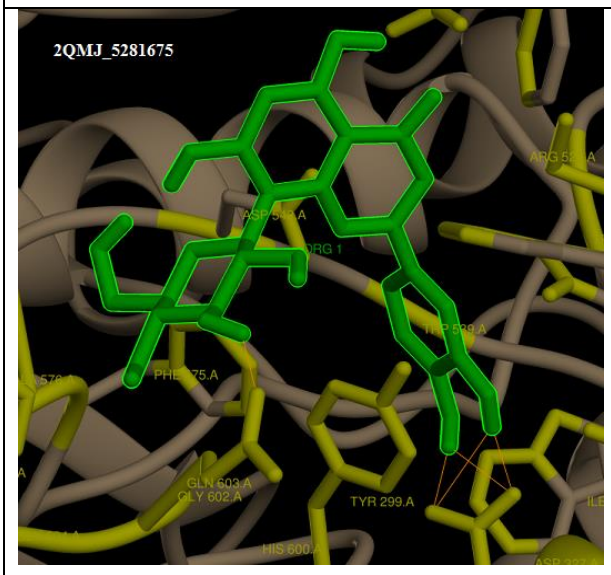
Asp356
Trp59
Trp58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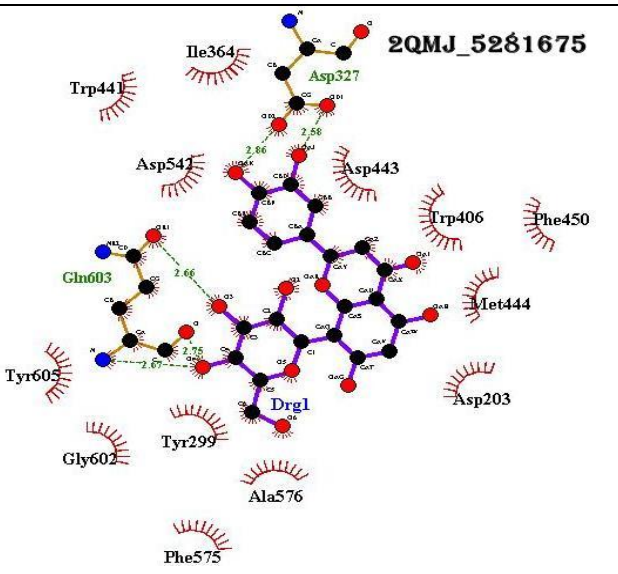
GLN 603.A
GLY 602.A
TYR 299.A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Asp327
Trp441
Asp541
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Trp406
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Gln603
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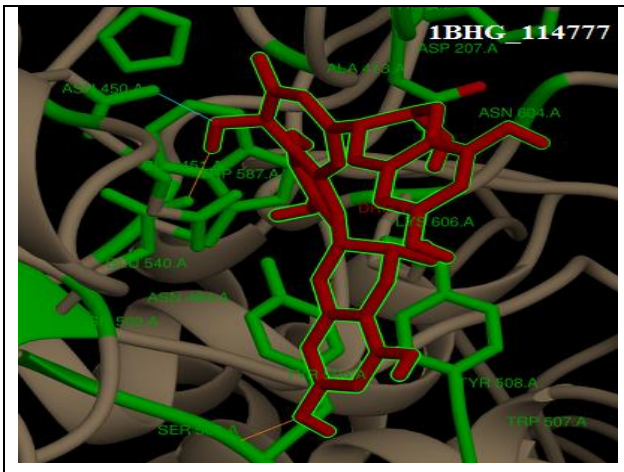
Tyr299
Gly602
Ala576
Phe575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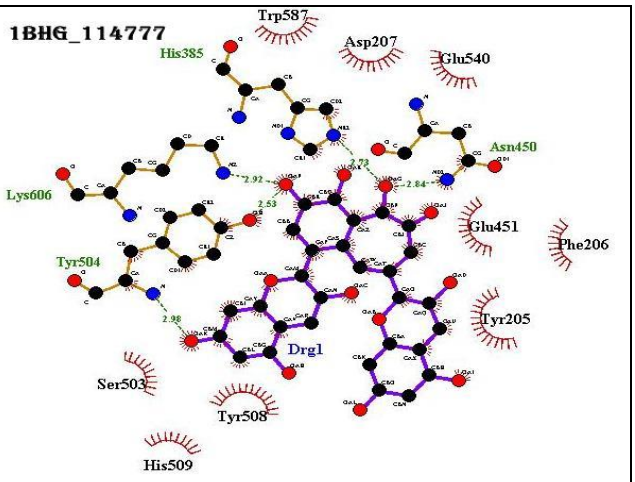
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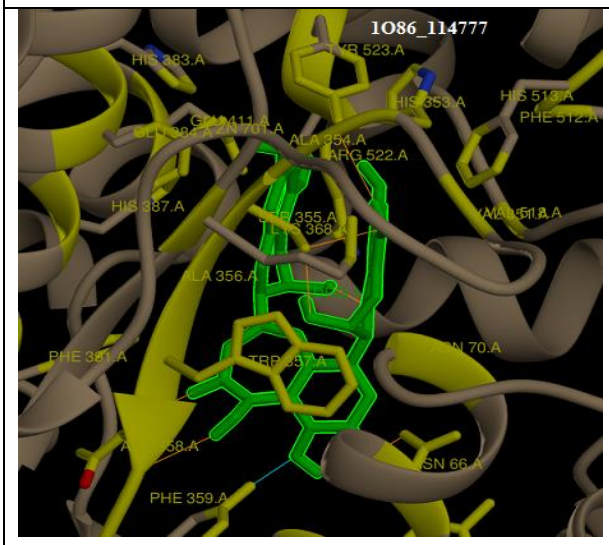
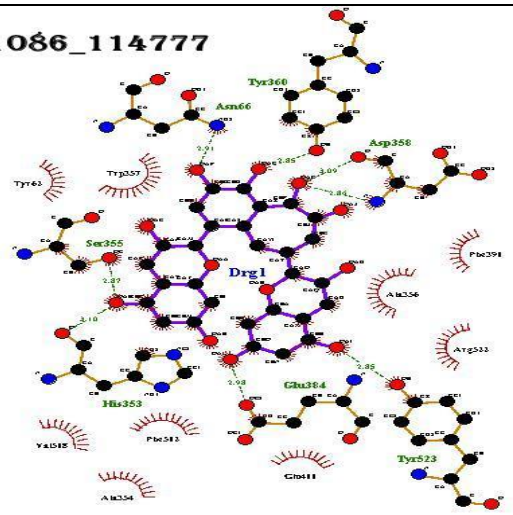
Asp207
His385
Glu540
Asn450
Lys606
Glu451
Phe206
Tyr504
Tyr205
Drg1
Ser503
Tyr508
His509


1O86_114777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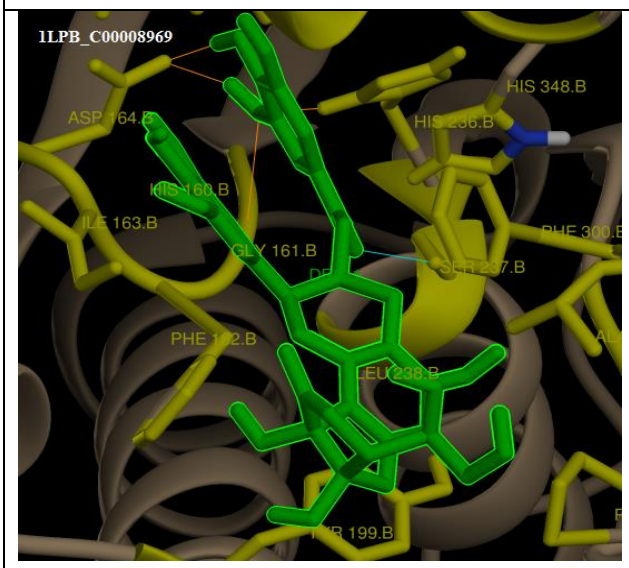
Asp358
Tyr357
Ser355
Drg1
Phe391
Ala356
Arg522
Glu384
His353
Phe543
Tyr523
Gln411
Ala354


1LPB_C00008969

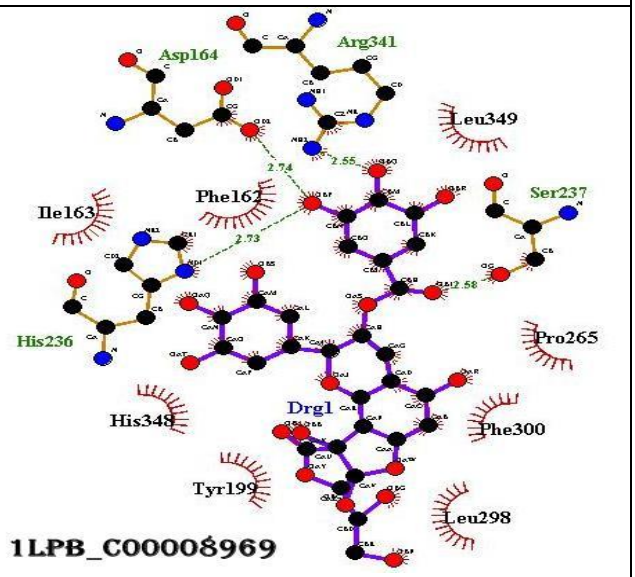

Asp164
Arg341
Leu349
Phe162
Ile163
Ser237
His236
Pro265
Drg1
His348
Phe300
Tyr199
Leu298
1LPB_C00008969

FIGURE 5: UCSF and LigPlot+ interaction analysis. The left side figure show the UCSF chimera post-docking analysis, the middle green colored molecule represent ligand and yellow colored ones are the neighboring amino acids in 5 Å region that are interacting with ligand. The right side figures exhibits the Ligplot+ result. In the LigPlot result; dotted line represents the H-bond of the ligand with the amino acid of the protein represented in green. Spikes on the arcs show the hydrophobic interaction with the amino acids presented in black

The results of table show hydrogen bonding between drug candidate and amino acid residues of target protein. Furthermore, from the UniProt we identified the active amino acid residue for each protein that is bonded with metal residue. The active metal binding amino acid residue is crucial for the function of protein. This residue directly participates in catalysis and if we hinder this residue to make bond with metal ion, the biological functioning of protein is totally busted. This will add plus point to docking result. The position of active metal binding residue in the protein sequence is also shown in the table.

**4.1.5 Molecular, ADME properties calculation and toxicity testing result:**

The molecular properties calculation of 2 best docked molecules was done by molsoft online server, the ADME properties calculation and the toxicity testing was done by PreADMET server. The Molsoft calculates the molecular weight, number of hydrogen bond donor, number of hydrogen bond acceptor, octanol-water partition coefficient (LogP), and solubility (LogS). The PreADMET calculates the absorption, distribution, metabolism, excretion and toxicity properties of drug candidates.

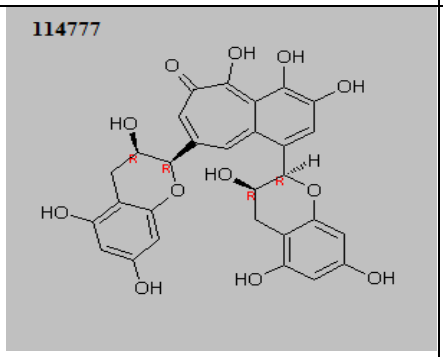
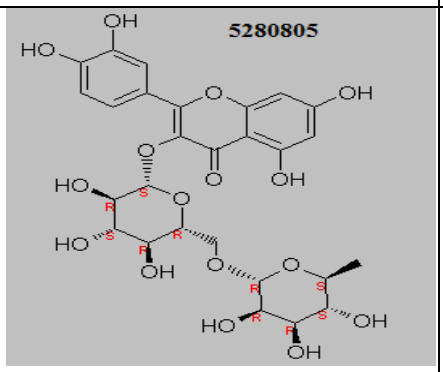
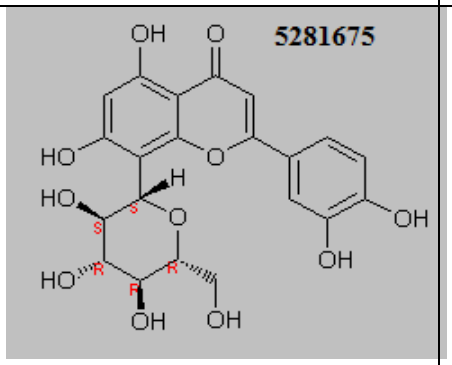
Table 7: ADME and toxicity analysis of best docked ligands.

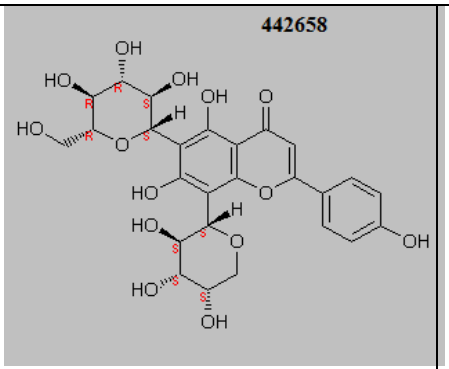
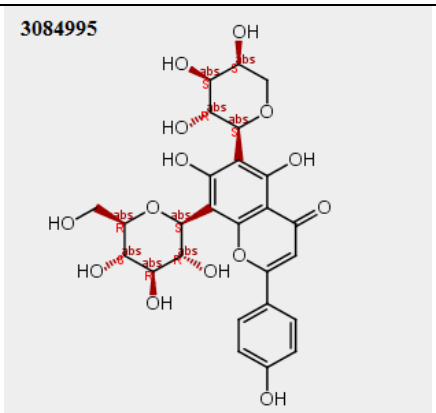
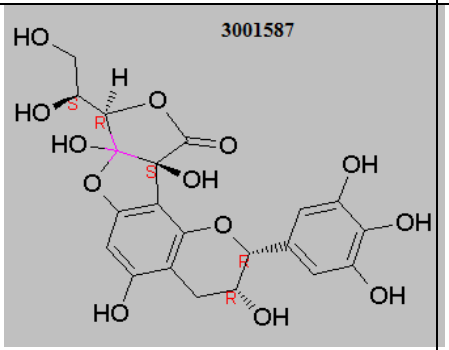
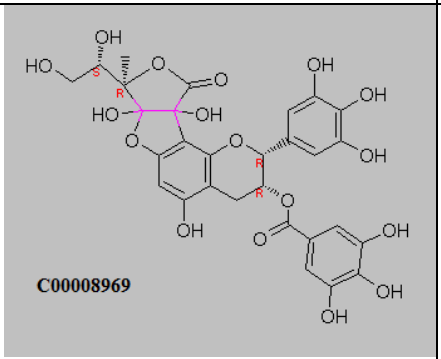
| Molecule Id | Absorption | | | | Distribution | | Carcinogen i- city test | |
|---|---|---|---|---|---|---|---|---|
| | HIA (%) | Caco-2 cell (nm/se | MDCK cell (nm/sec) | Skin ( $\log K_p . C_m / sec$ | PPB (%) | BBB (%) | Mou se | Rat |

| | | c) | | ) | | | | |
|---|---|---|---|---|---|---|---|---|
| Theaflavin (114777) | 17.867 | 14.818 | 0.04596 | -4.52829 | 100 | 0.036 | -ve | -ve |
| Vitamin E (2116) | 97.832 | 29.119 | 38.9051 | -0.51599 | 100 | 19.90 | -ve | -ve |
| 8-c-ascorbyl(-)-epigallocatechin (3001587) | 4.7156 | 5.8293 | 0.70946 | -4.62943 | 39.6 | 0.028 | -ve | -ve |
| Rutin (5280805) | 2.8612 | 7.9126 | 0.32694 | -4.6667 | 43.9 | 0.029 | -ve | -ve |
| Schaftoside (442658) | 4.2104 | 6.708 | 0.522638 | -4.79521 | 39.6 | 0.028 | -ve | -ve |
| Orientin (5281675) | 14.99 | 2.995 | 0.6968 | -4.68557 | 63.1 | 0.034 | -ve | -ve |
| Strictinin (73330) | 0.822 | 12.854 | 0.09971 | -4.32686 | 100 | 0.03 | -ve | -ve |
| 8-c-ascorbyl epigallocatechin 3-o-gallate (C00008969) | 1.573 | 11.716 | 0.04342 | -3.94587 | 100 | 0.03 | -ve | -ve |
| Hexadecane (5366244) | 100 | 37.788 | 66.1719 | -0.52629 | 100 | 18.63 | -ve | -ve |

The above tables reveal the properties of molecule as a potent drug candidate. All molecules have well moderate Caco-2 cell permeability, low MDCK cell permeability, human intestinal absorption, weakly bound to plasma protein and inactive BBB penetration. All of these molecule show negative result in carcinogenicity test.

Table8: molecular property and drug likeness study of best docked ligands.

| Ligand | Structure | M.W. | Clog P | Solubility (Log S) | Drug-likeness | Drug-score |
|---|---|---|---|---|---|---|
| Theaflavin (114777) |   | 564 | -1.02 | -3.16 | 2 | 0.46 |
| Rutin (5280805) |   | 610 | -1.26 | -2.4 | 3.31 | 0.57 |
| Orientin (5281675) |   | 448 | -0.42 | -1.97 | -0.71 | 0.32 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Schaftoside (442658) |  | 564 | -1.9 | -1.81 | -5.83 | 0.12 |
| Isoschaftoside (3084995) |  | 564 | -1.89 | -1.81 | -5.83 | 0.16 |
| 8-c-ascorbyl(-)-epigallocatechin (3001587) |  | 480 | -1.54 | -1.25 | 1.82 | 0.72 |
| 8-c-ascorbyl epigallocatechin 3-o-gallate (C00008969) |  | 646 | -0.66 | -2.05 | -1.39 | 0.5 |

Molecular properties of the best ligands screened from docking analysis are shown in Table2. The hydrophilic property of a ligand is determined by cLogP. Low hydrophilicity results in high logP value which signifies poor permeation or absorption. For compounds to have a rational probability of being well absorbed their logP value must not be greater than 5.0 (http://www.organic-chemistry.org/prog/peo/). In our result all the best candidate ligands exhibit value less than 5.0 and thus can be concluded to have good permeability. The calculation of LogS was done to evaluate the aqueous solubility of the ligand molecule which is a necessary condition for the knowledge its absorption and distribution. As per the given parameters LogS value should be greater than -4.0 and all the selected candidate molecules have values accordingly thus qualifies the test. A positive value in the druglikeness test shows that the molecule contains predominantly fragments that are commonly present in commercially available drugs. But a negative score does not necessarily mean that the molecule cannot be a potential drug. At the end a drug score is calculated for each molecule which is a result obtained from combine outcome of cLogP, solubility, druglikeness, molecular weight and toxicity risks that may be used to judge the molecule's overall probability to qualify as a drug.

From the docking analysis it is observed that Theaflavin (114777), Strictinin (73330), Rutin (5280805), Orientin (5281675), Schaftoside (442658), Isoschaftoside (3084995), 8-c-ascorbyl(-)-epigallocatechin (3001587), and 8-c-ascorbyl epigallocatechin 3-o-gallate (C00008969) could be the probable lead molecule for the inhibition of the target enzymes. Comparing the molecular properties tabulated above it can be observed that 8-c-ascorbyl(-)-epigallocatechin, and 8-c-ascorbyl epigallocatechin 3-o-gallate have very good drug score. On the other hand theaflavin has a considerable overall drug score and have good inhibition efficiency for most of the target enzymes.

### 4.2. Drug delivery Techniques:

#### 4.2.1 Liposomal Encapsulation:

**Particle size Analysis**

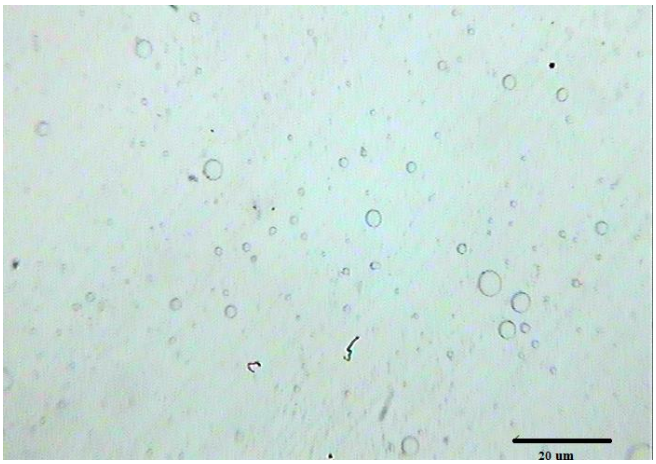



FIGURE 6: Liposome; viewed under Phase contrast microscope.

The above figure shows the liposomes as seen under phase contrast microscopy. The droplets depicts the bilayered liposomal structure.

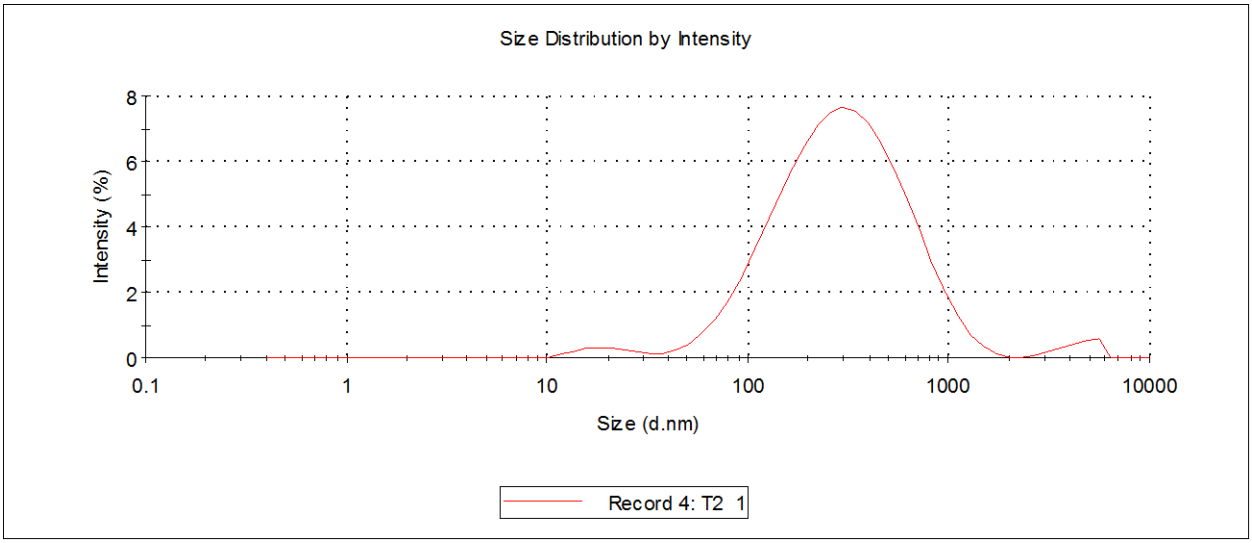



FIGURE 7a: Average size characterisation of the liposome (particle size distribution by intensity)

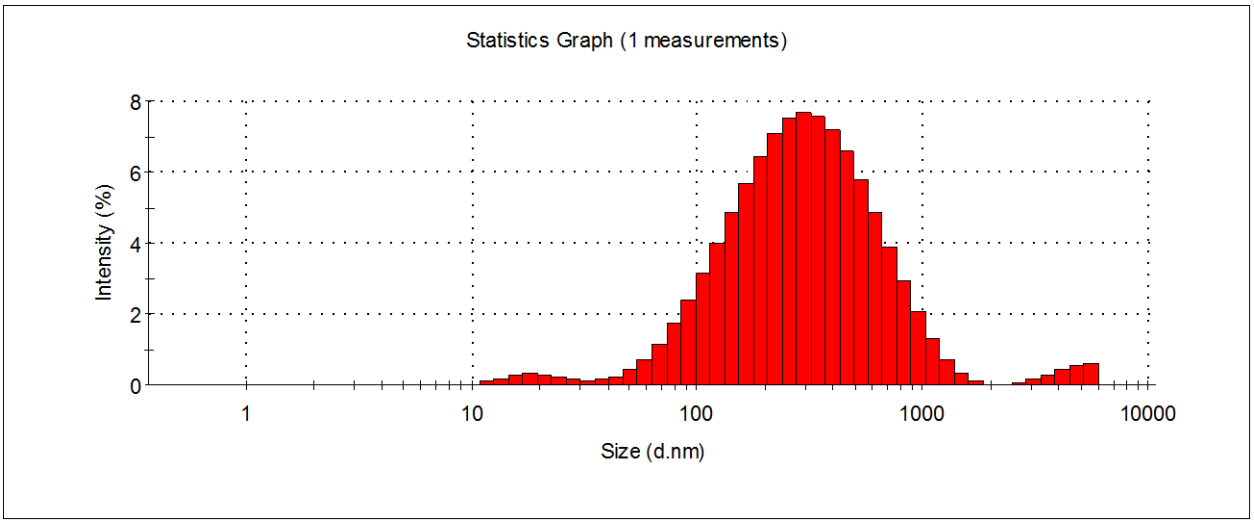



FIGURE 7b: Statistics graph of average size characterisation of the liposome (particle size distribution by intensity)

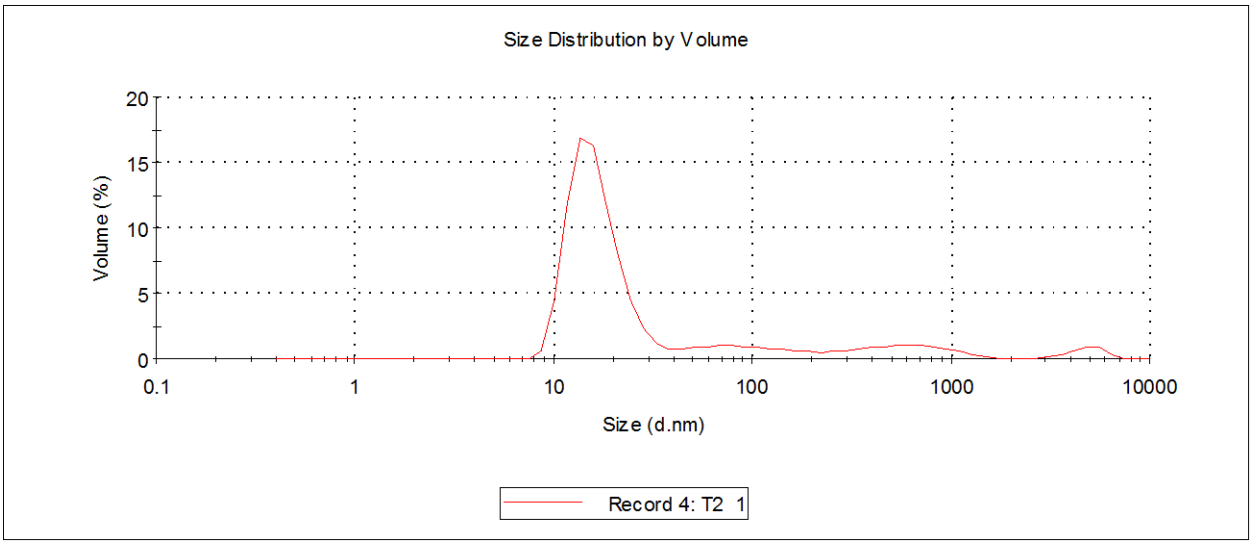



FIGURE 8a: Average size characterisation of the liposome (particle size distribution by volume)

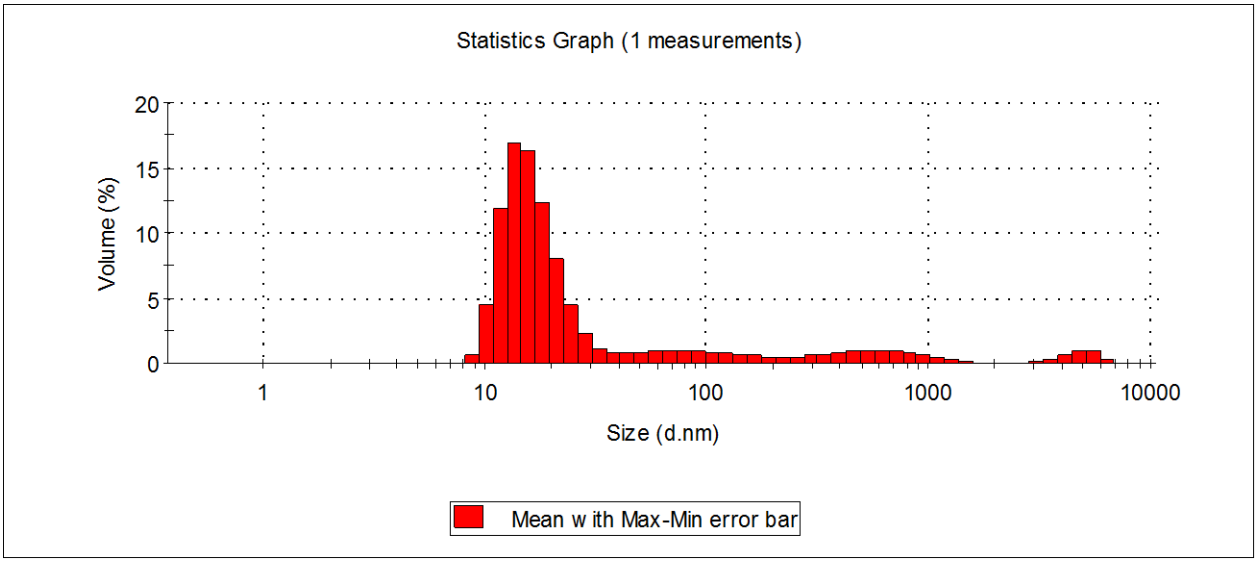



FIGURE 8b: Statistics graph (particle size distribution by volume).

The liposome size was found out by dynamic light scattering method. The result shows the size distribution in two different method, one by volume and another by intensity. Size distribution by intensity shows a broad peak with Z average of 224.5 nm. The z-average diameter values are the means of 3 repeat measurements made on the neat liposome samples. The size distribution by volume agrees with that of intensity. The size of the liposomes can be varied and downsized liposome can be obtained by increasing the sonication time.

## Zeta Potential

**System**

| | | | |
|---|---|---|---|
| **Temperature (°C):** | 25.0 | **Zeta Runs:** | 12 |
| **Count Rate (kcps):** | 199.8 | **Measurement Position (mm):** | 2.00 |
| **Cell Description:** | Clear disposable zeta cell | **Attenuator:** | 6 |

**Results**

| | | | Mean (mV) | Area (%) | Width (mV) |
|---|---|---|---|---|---|
| **Zeta Potential (mV):** | -18.5 | **Peak 1:** | 0.00 | 0.0 | 0.00 |
| **Zeta Deviation (mV):** | 0.00 | **Peak 2:** | 0.00 | 0.0 | 0.00 |
| **Conductivity (mS/cm):** | 13.0 | **Peak 3:** | 0.00 | 0.0 | 0.00 |
| **Result quality :** | Good | | | | |

FIGURE 9: The zeta potential data of prepared liposome.

Zeta potential result shows that the encapsulated liposome has the potential -18.5 mV. To be stable the zeta potential value need to between -30 mV to -40 mV. The prepared liposome is less stable and the stability can be increased by pH adjustment and salt addition. The importance of characterization of liposomal encapsulation is understanding their appropriateness for a diverse range of applications. Knowledge about zeta potential of liposome in solution help in predicting the stability and fate of the liposomes in vivo. Stability and aggregation of liposomes in solution due to association of the liposomes with other counter charged particles can be checked by measuring zeta potential of the subsequent complex.

**Drug kinetics**

Table9: Drug release study: data for standard graph

| Conc. | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 | 0.9 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|
| OD (380nm) | 0.105744 | 0.195411 | 0.3433 | 0.428338 | 0.506783 | 0.672445 | 0.768645 | 0.798333 | 0.886702 | 1.173153 |

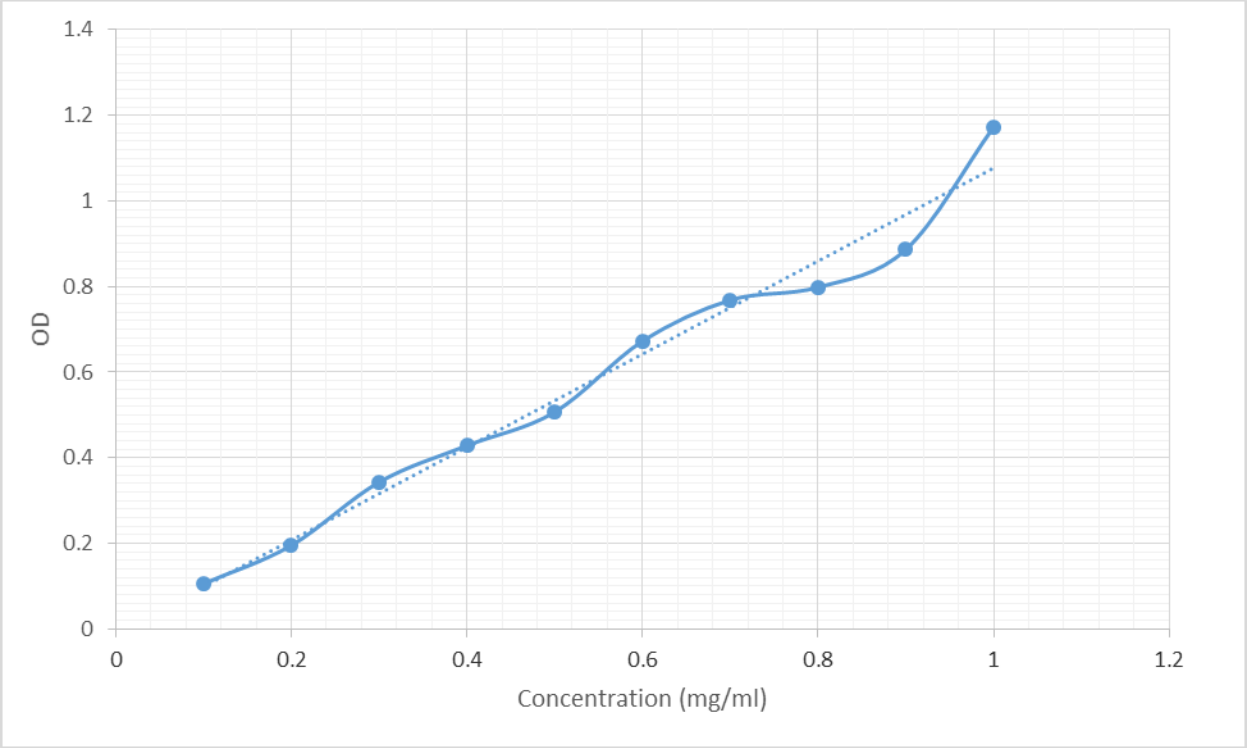



FIGURE 10: Standard graph depicting change in OD with increasing tea concentration.

Table 10: Drug release study in liposomal encapsulated tea.

| Time (hr) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Drug release | 0.2 | 0.32 | 0.4 | 0.55 | 0.64 | 0.76 | 0.84 | 0.9 | 1.07 | 1.09 |

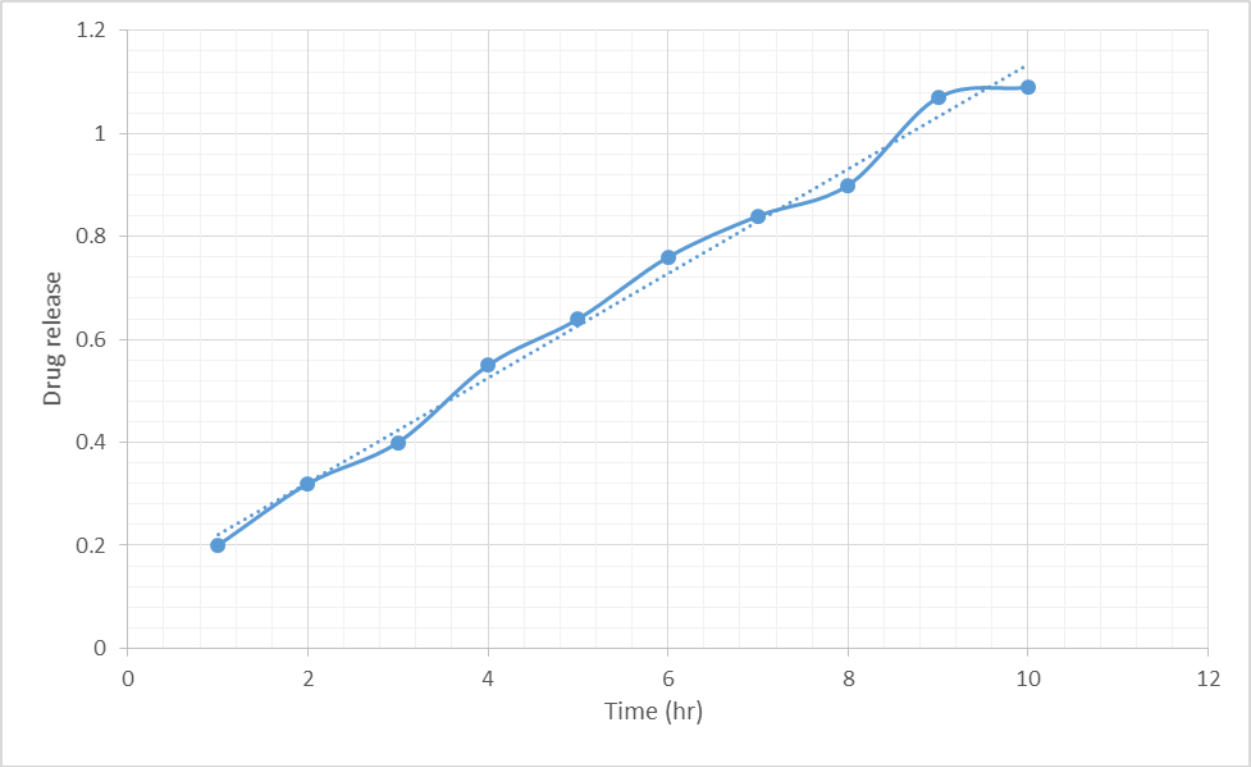



FIGURE 11: Test graph depicting drug release with time.

The drug kinetic study was done for 10 hours and it shows the drug release increases over time and after it achieves equilibrium and the curve achieves equilibrium. The highest amount released is approximately 0.95mg/ml. The drug release was found to be good as the inhibitory concentration needed for various diseases studied above by in silico analysis was in the range microgram. Hence this drug delivery system serves better release of drug for colon specific delivery.

### 4.2.2 Pectin-HEMA hydrogel:

**Swelling Test**

Table 11: Swelling test of pectin-HEMA hydrogel at pH 7

| Time | Swelling % | | | |
|---|---|---|---|---|
| Hrs (at pH 7, 37C) | P0 | Error | P1 | Error |

| | | | | |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 1 | 31.82931 | 5.8024 | 38.98804 | 4.07622 |
| 2 | 43.37992 | 6.18436 | 50.82303 | 1.9238 |
| 3 | 51.60138 | 6.26902 | 60.52184 | 3.68645 |
| 6 | 66.90237 | 10.20625 | 74.86602 | 11.94019 |
| 12 | 77.21329 | 16.39145 | 85.96142 | 18.79971 |
| 24 | 86.75034 | 24.88078 | 93.56734 | 21.23649 |
| 48 | 93.43828 | 27.45256 | 100.3104 | 20.18558 |

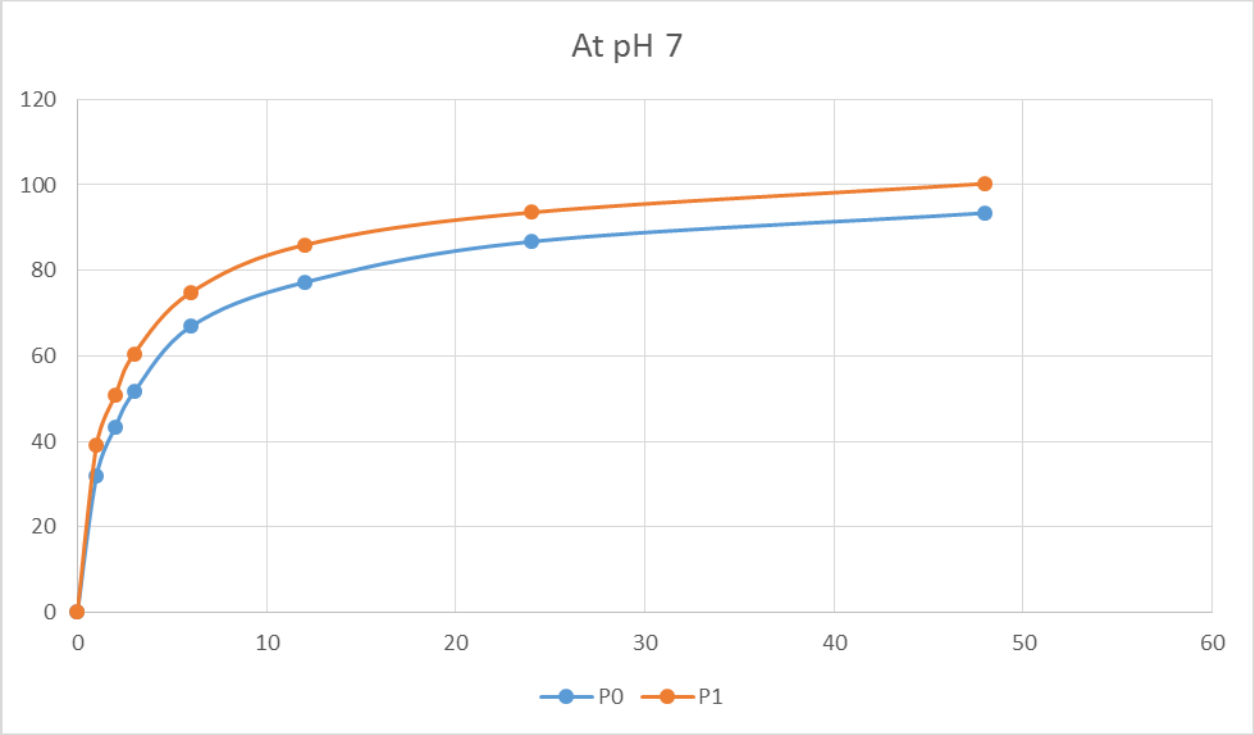



FIGURE 12: Swelling data distribution at pH 7.

Table 12: Swelling test of pectin-HEMA hydrogel at pH 9.

| Time | Swelling Percentage (%) |
|---|---|

| Hrs<br><br>(at pH9) | P0 | Error | P1 | Error |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 1 | 30.83345 | 1.26226 | 33.03208 | 0.82556 |
| 2 | 36.39013 | 1.34182 | 38.84771 | 0.99467 |
| 3 | 39.96303 | 1.01155 | 43.10153 | 1.74044 |
| 6 | 47.48652 | 0.50946 | 53.039 | 4.95752 |
| 12 | 50.67339 | 0.72345 | 57.99038 | 6.25878 |
| 24 | 52.67239 | 3.60201 | 61.80925 | 4.57658 |
| 48 | 57.96818 | 3.19823 | 69.22172 | 5.03515 |

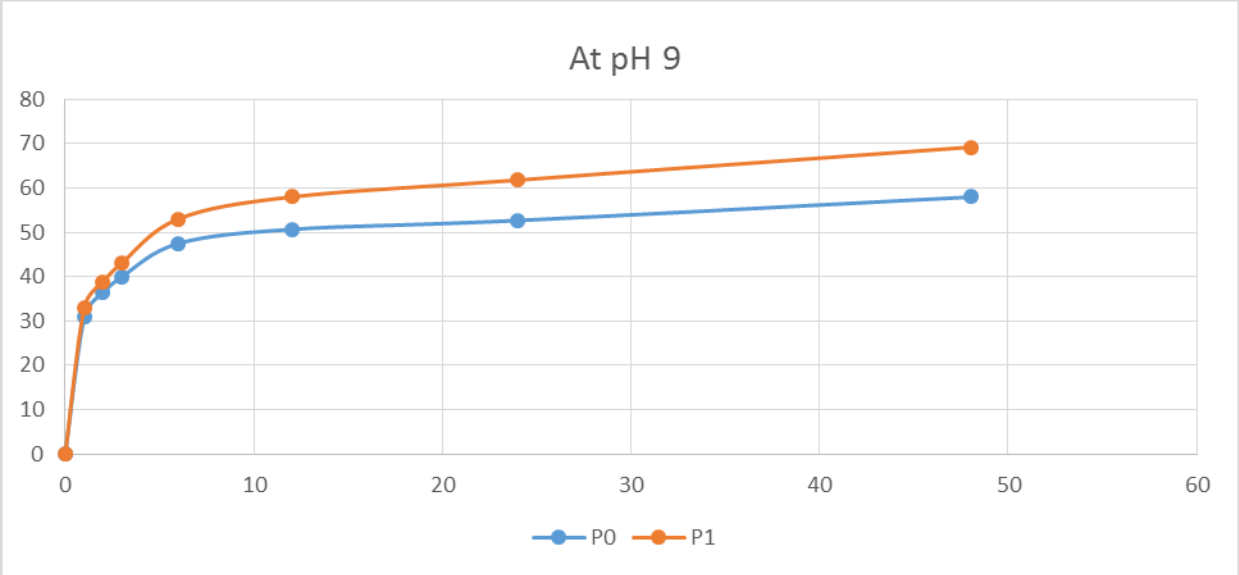



FIGURE 13: Swelling data distribution at pH 9.

Swelling studies were done in phosphate buffer solutions of varying pH 7and 9. Results showed that swelling increased with increasing time. P0 denotes the control sample while P1 is the sample with drug. P1 swelling increases more with time than the control. It can be seen that test

sample swells more than the control and with increasing pH swelling decreases. At pH 7 the maximum swelling is approximately up to 100gm while at pH 9 maximum goes up to 70 gm. Swelling decreased with increase in crosslinking ratio owing to tighter hydrogel structure.

**Drug Kinetics**

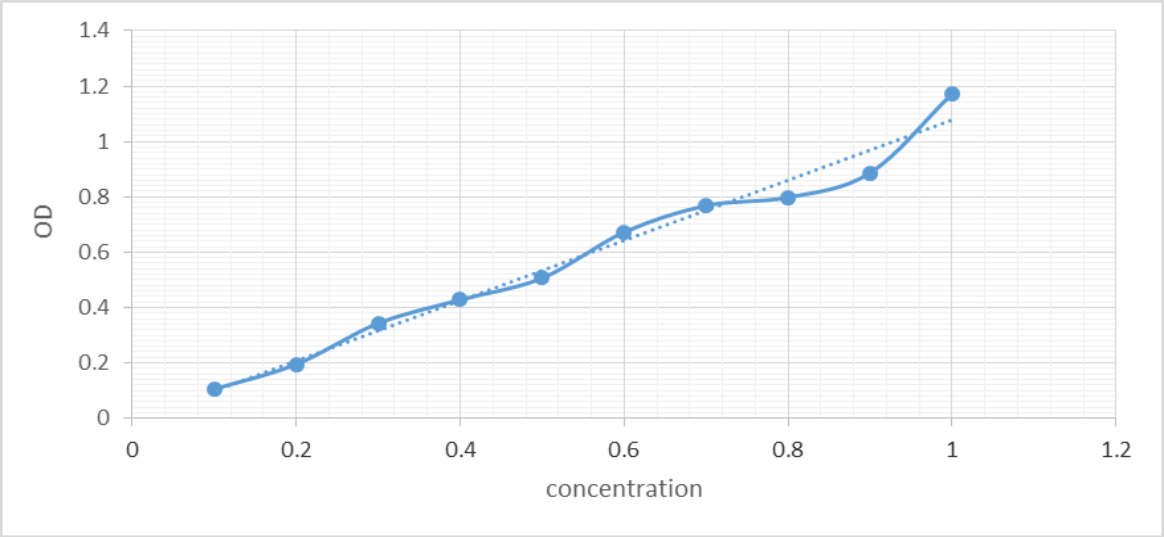



FIGURE 14: Standard graph of drug release.

The drug release study of pectin-HEMA hydrogel system was done for 10 hours and it shows the drug release increases over time and after it achieves equilibrium and the curve become parallel to x-axis. The highest amount released can be found out by comparing the test graph to standard which is approximately 1mg/ml. The drug release was found to be good as the inhibitory concentration needed for various diseases studied above by in silico analysis was in the range microgram. Hence this drug delivery system serves better release of drug.

Table 13: Drug release study of tea loaded pectin-HEMA hydrogel.

| Time | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Drug Release | 0.36 | 0.49 | 0.59 | 0.7 | 0.76 | 0.94 | 1.09 | 1.11 | 1.13 | 1.14 |

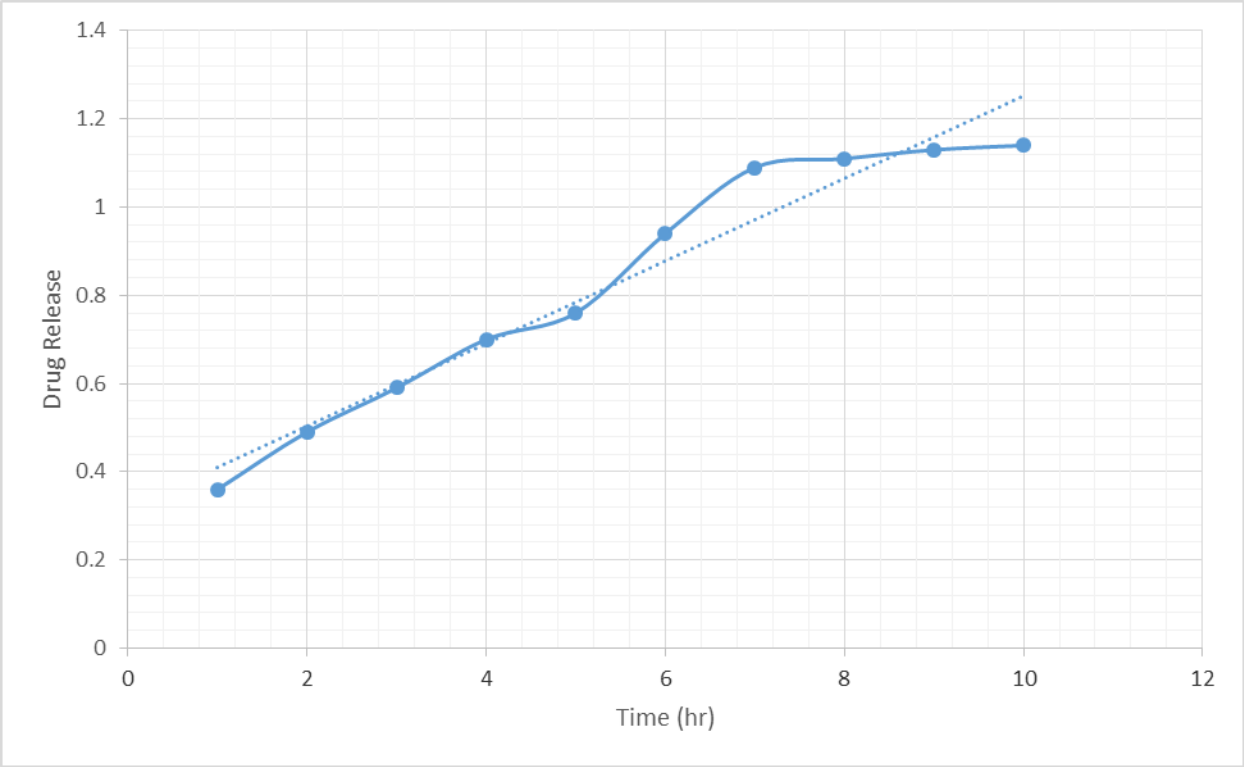



FIGURE 15: Test graph of drug release from pectin-HEMA hydrogel

# CHAPTER 5: CONCLUSION

## 5.1 Summary:

- In the current study tea phytochemicals are analysed for their therapeutic potential and specific phytochemicals for inhibition of target enzyme (acetyl cholinesterase, alpha amylase, alpha- glucosidase, beta glucuronidase, lipase, angiotensin converting enzyme and lipoxygenase) has been developed that can be used for further in-vivo analysis against respective diseases.
- Initially 149 phytochemicals from tea were tasted for their molecular property, toxicity and drug likeness and 19 molecules are found out to be non- toxic and non- carcinogenic.
- The docking analysis of these 19 phytochemicals against target enzymes show that theaflavin, rutin, schaftoside, strictinin, orientin, Vitamin E, 8-c-ascorbyl epigallocatechin 3-o-gallate and 8-c-ascorbyl epigallocatechin play a vital role in their inhibition.
- Among these best docked ligands for all the enzymes and the respective diseases, it was found that theaflavin and rutin are common inhibitory molecules for most of the enzymes that were under study.
- The two best docked ligands for each enzyme were further subjected to interaction analysis using LigPlot and UCSF chimera. The interaction study reveals that the protein-ligand complex were stable because of hydrogen bond and hydrophobic interaction with the active site residues.
- Two drug delivery systems were prepared and analysed for target specific delivery. Liposomal encapsulation system was characterised and particle size was found to be in nano range with ales stable zeta potential value which can be increased by specific salt addition. Drug release study was done by encapsulating tea extract in liposomes and the results shows that the system releases tea extract approximately up to 1mg/ml. liposomal encapsulation is best suitable for drug delivery to brain.
- Pectin-HEMA hydrogel system was prepared and characterized by swelling test and drug release study was done. Swelling test in two different pH reveals that the hydrogel swell more in neutral system and the test sample showed more swelling than control sample. This system is best suitable for colon specific drug delivery.

## 5.2 Conclusion:

Lower binding energy and inhibition constant are the key factors for the selection bet inhibitor. From the docking analysis it was found that theaflavin, rutin, schaftoside, strictinin, orientin, Vitamin E, 8-c-ascorbyl epigallocatechin 3-o-gallate and 8-c-ascorbyl epigallocatechin play a vital role in the inhibition of target enzyme. Out of the above mentioned molecules theaflavin rutin and 8-c-ascorbyl epigallocatechin show a good drug score and are common inhibitory molecule for most of the target enzymes. Hence all the outcomes suggest that these molecules can be act as potential drug targets and considered further in vivo analysis for production of drug. Two Drug delivery systems liposomal encapsulation system and pectin-HEMA hydrogel were prepared and analysed for proper characteristics. Liposomal encapsulation system was characterised and particle size was found to be in nano range with moderately stable zeta potential value which can be increased by specific salt addition. Drug release study was done by encapsulating tea extract in liposomes and the results shows that the system releases tea extract approximately up to 1mg/ml. liposomal encapsulation is best suitable for drug delivery to brain. Hydrogel system was prepared and characterized by swelling test and drug release study was done. Swelling test in two different pH reveals that the hydrogel swell more in neutral system and the test sample showed more swelling than control sample. This system is best suitable for colon specific drug delivery.

## 5.3 Future Outlooks:

The emerging new diseases with changing life style, the rising cost of development of drug and the lack of potential therapeutic representatives have been a chief concern now a days. A satisfactory potential substitute method is required as a solution of efficiency and wellbeing. In silico designing of drug molecules is one of the solution to the above problem which plays a vital role in different steps of development of a drug that is from pre-clinical procedure to late-clinical stage. This technological era has gifted a CADD that is, computer aided drug design as a novel work which starts with virtual screening and progress towards in-vitro and then in-vivo study. The various tools of bioinformatics helped in identifying potential drug molecules to target against different diseases. In this work the discussed drug molecules can be subjected to next step analysis to develop a new medicine.

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