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In Silico Study of Propolis as a Potential Healing Agent for Gastric Ulcer

by

M Farhan Illahi

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Faculty of Health and Life Sciences
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I would like to dedicate this thesis to my Family, Friends and Teachers

CERTIFICATE OF APPROVAL

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Abstract

Propolis or bee glue is a resinous mixture produced by honey bees. Propolis is used for its medicinal properties in almost all the diseases. With increasing drug resistance problem, the interest to explore the natural products with medicinal properties is increasing. The purpose of this research work is to find out the compounds from the propolis that can be used for the cure of gastric ulcer. These compounds of propolis were find-out from the literature that reported their presence in the treatment of ulcer. Proteins whose PDB IDs are 5X7B (CagA), 5KSB (Hla-DQBI), 6VXI (ABCG2) and 6ODY (VacA) were selected from studying the pathway of ulcer in humans for this research work. 3D structures of these proteins were prepared for molecular docking after finding out their specific drug pockets. Molecular docking was performed for this purpose and after that selected compounds of propolis for the four proteins, were tested against the pharmacokinetics and toxicological properties. Selected Propolis compounds that pass Lipinkis and vebers rule for the oral bioavailable drugs are for gastric ulcer are Myricetin, -amyrins Acetate, Quercetin, Methyl pinoresinol, Syringic acid, Pinostrobin chalcone, Glangin, Naringenin and Artepillin C. These 9 compounds can be further validated on animal models to provide new ulcer treatment.

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Abbreviations

AAR	Additional Allowed Regions
AI	Aliphatic Index
B-amyrins	Beta Amyrins
CAPE	Caffeic Acid Phenethyl Ester
CCL4	Carbon Tetrachloride
DR	Disallowed Regions
EC	Extinction Coefficient
EEP	Ethanolic Extraxt of Propolis
GAR	Generously Allowed Regions
II	Instability Index
LDL	Low Density Lipo-protein
MFR	Most Favoured Region
NFAT	Nuclear Factor of Activated Cells
PDB	Protein Data Bank
ROS	Reactive Oxygen Species
RNS	Reactive Nitogen Species
T-pI	Theoretical pI
-R	Overall Negative Charged Amino Acids
+R	Overall Positive Charged Amino Acids

Chapter 1

Introduction

The name of Propolis originated from the word that was used by Aristotle in Greek and this word has two parts that are Pro means before and polis means city. Collectively this name means before the city or the Defender of the City [1]. Propolis of bee is glue that is produced by the bee. This glue is resinous, gummy and a balsamic material that bees collect from the flowers. Bees use this glue for the protection of their hive from the growth of microbes such as fungi and bacteria and apart from it, this is used as a construction material of the hive [2]. Bee propolis composition depends on the botanical region from which it is obtained. Bee propolis helps in the mainataince of the homeostasis, reduction of the vibration, keep check on the air flow, helps in the prevention of the putrefaction and also from the squatter [3]. The chemical composition of the propolis is responsible for its biological activity and the chemical composition depends on the plant from which the bees collect the resin for the production of honey and the propolis. There are many chemical types of the propolis according to the source plant have been registered. There is a core role played by the chemical diversity of the propolis in the propolis studies. The characteristics that are shown by the most of the Bee propolis includes opaque shiny and irregular shape, solid form at the room temperature and when the temperature rises from the room temperature it becomes sticky. The color of propolis varies from dark green, brown and black. It is sweet in taste but it can be bitter sometimes. All these characteristics vary

from hive to hive, season to season, botanical region, species of bee that produced specific propolis, and the geographical conditions that are present at the specific location from which the propolis is obtained and the location of resin collection by the honey Bees [4].

It is still used as a medicine for the treatment of wounds and burns by the people in the Balkan States. They also used it for the sore throat and stomach ulcer [5]. The propolis that is being extracted from the ethanol is being known for its anti-inflammatory effects for hundreds of years [6]. It is also been used as an immune-modulatory agent for centuries [7]. From the 12th century, bee propolis is being used as a medicine for the diseases of mouth, infections of throat and for the dental caries [8]. It is official in the United States to use the bee propolis as a Pharmacopeia and in Canada it is considered as a natural health product [9, 10]. In recent times it is gaining popularity due to its pharmacological and phytochemistry property. As the composition of the Bee propolis depends on many factors but some main components that are almost present in each type of propolis includes Chalcone, polyphenolics, aromatic acid, triterpenes and their esters [11].

Owing to resistance to antibiotics by pathogens, current research has been focused towards the usage of old medicine/natural products for handling and control of diseases. Resistance has caused increasing nosocomial infections in pathogen. Propolis is one of natural products that have been veried on pathogens and other in organisms causing community-acquired infections. Besides the well-known pathogens, confrontation has also been seemed in opportunistic microorganisms [12].Propolis is moderately non-poisonous and shows an extensive variety of antimicrobial activities against a variety of microorganisms, parasites, and infection [13].

Other organic and pharmacological properties have additionally been investigated for propolis [14]. The therapeutic and antimicrobial properties of propolis have been generally revealed and have a long history [15]. In various forms of topical, propolis is used as a natural remedy in various health food stores. It is also

utilized in beauty products or as a prevalent alternative drug for self-medication of different syndromes [16]. Recent uses of propolis incorporate details are cold disorder (upper respiratory tract infection, inuenza and common cold) and in addition to dermatological properties used in wound heal up, treatment of burns, genitals, acne, neurodermatitis and herpes simplex [17]. Due to its antimicrobial, antitumor, anti-inflammatory, antioxidant and immunomodulatory activities, it is being used in complementary medicines.

It is likewise utilized in toothpaste and mouth freshener and to treat gum disease and stomach. It is broadly utilized in beauty care products and in human being nourishments and drinks. It is easily accessible in market as a creams, container, throat capsules, mouthwash arrangements and powder, furthermore in several filtered items through which the wax were extracted. Due to its antioxidant, antiviral and antimicrobial characteristics, its broadly utilized in human being, and animals medication, pharmaceutical and beauty care product [18].

Peptic Ulcer disease (PUD) includes gastric ulcers (GU) and duodenal ulcer (DU) that is defined as the loss of the continuity in the part of the gastrointestinal tract wall that penetrates the muscular mucosa with least diameter of 0.5 cm [19]. It is worldwide disease with prevalence of 10 percent of adult population. In the pathogenesis of the PUD, *Helicobacter pylori* (*H.pylori*) infection plays a key role that is present in almost 90-100 percent in GU and in DU patients it is 60-90 percent. Geographic location and socioeconomic status plays an important role in this [20].

In the pathogenesis of *H.pylori* related diseases, multiple virulence factors such as cagA, vacA and dupA, are involved [21]. CagA gene can be associated with the peptic and gastric ulcer as it acts as a marker for a genomic pathogenicity island [22]. Human HLADQB1 genes might play important roles in *H. pylori* infection in Indonesian people [23].

Environmental factors such as smoking, alcohol consumption, high salt in diet, steroidal and non-steroidal anti-inflammatory drugs, h.pylori infection are not the only cause of the peptic ulcer but genetic predisposition also play an important

role [24, 25]. Proteins are large molecules that are composed of one or more long chains of amino acids. They are very important for the body functions especially as structural components such as body tissues, hair or muscle etc. A small change in any protein can lead to very drastic results. One of the most possible and important genetic factor can be the changes in the ABCG2 gene that can be involved in the development of PUD. ABCG2 gene encodes ABCG2 protein with 655 amino acids that a half transporter belongs to the ABC transporters superfamily. ABCG2 is localized in the apical membrane cells of the digestive tract that confirm its protective role that limits the accumulation of harmful xenobiotics in cells/organs. If it loses its function that will result into the development of gastric ulcer [26–28]. Another gene that plays important role in the *H.pylori* infection is human HLA-DQBI gene and its association has been shown with peptic and gastric ulcers in some populations [29].

Molecular docking is a process that is used for identifying a relationship between a compound and a protein with respect to some disease to find out potential drug targets against that protein for a specific disease. It is a sort of virtual screening method that can help to find out some potential targets from a large list of candidate compounds. It works on the binding affinity of protein and compound that is also known as ligands [30].

Due to the antioxidants and antimicrobial activities of the propolis, interest in it has been increased. In this research, molecular docking will be performed to find out the compounds from the propolis that can play important role in the treatment of ulcer. One protein will be docked against all the components found in the propolis [31].

1.1 Problem Statement

Propolis being an apicultural product has multiple biological properties. In the treatment of some illness and various pathological conditions, propolis, shows a promising role. By keeping in view the growing interest in the natural product as

medicines, a need arise to explore the propoliss role in the healing of ulcer, and to develop new and effective strategies, that will help to improve the currently available gastrointestinal therapies in the clinical practice.

1.2 Aims and Objectives

We endeavoured to undertake an in silico assessment of propolis to determine their effect on the gastric ulcer due to its medicinal properties such as including anti-inflammatory, immune-stimulant, anti-oxidant, anti-tumour, a neuro-protective and anti-microbial activity that had been proved by numerous scientific studies.

- To investigate the interactions of propolis against specific protein targets playing role in gastric ulcer through molecular docking.
- To find out the credibility of the selected compounds for oral bioavailable drugs by predicting the pharmacokinetics and toxicological properties.

1.3 Proposed Solution

- To find out the physiochemical and toxicological properties of propolis compounds for the credibility of selected compounds as oral bioavailable drugs.
- To explore propolis compounds that can be used as potential candidates for the treatment of gastric ulcer

Chapter 2

Literature Review

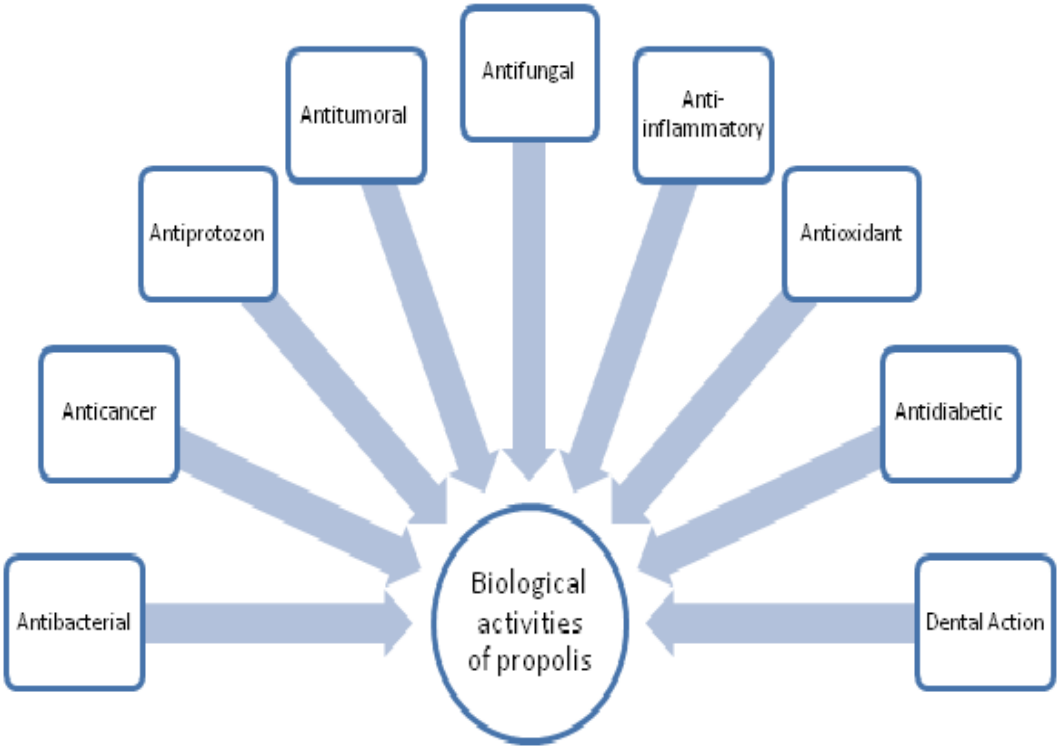

FIGURE 2.1: Biological activities of propolis [32]

2.1 History of Propolis

Propolis is from the time of the presence of the honey and there are many evidences that suggest its use by the Persians, Romans and ancient Egyptians [32]. Ancient

Egyptians use the propolis in many ailments and do the ornaments [33]. They learn this from the bees that use propolis as an embalming substance as the bees cover their hives with the propolis and they transport the dead bees from the hive with the propolis and wax [34]. Bees protect their hive from the infections that can be caused by the decomposition of the carcass. According to the ancient Jews, propolis is being used as the medicine and they use the word tzori that is Hebrew word [35]. It is used due to its therapeutic properties that are mentioned in the Old testament. There is a biblical balm of Gilead that is almost indistinguishable from the propolis and it is described as a gift in the Bible that is presented by the Queen of Sheba to the King Solomon. It was grown around the dead for almost 1500 years in Judae and it becomes popular due to its medical properties and aroma. The resins that are involved in the propolis productions are from many trees that includes *P. nigra*, *P. balsamifera* and *P. gileaddensis* [36].

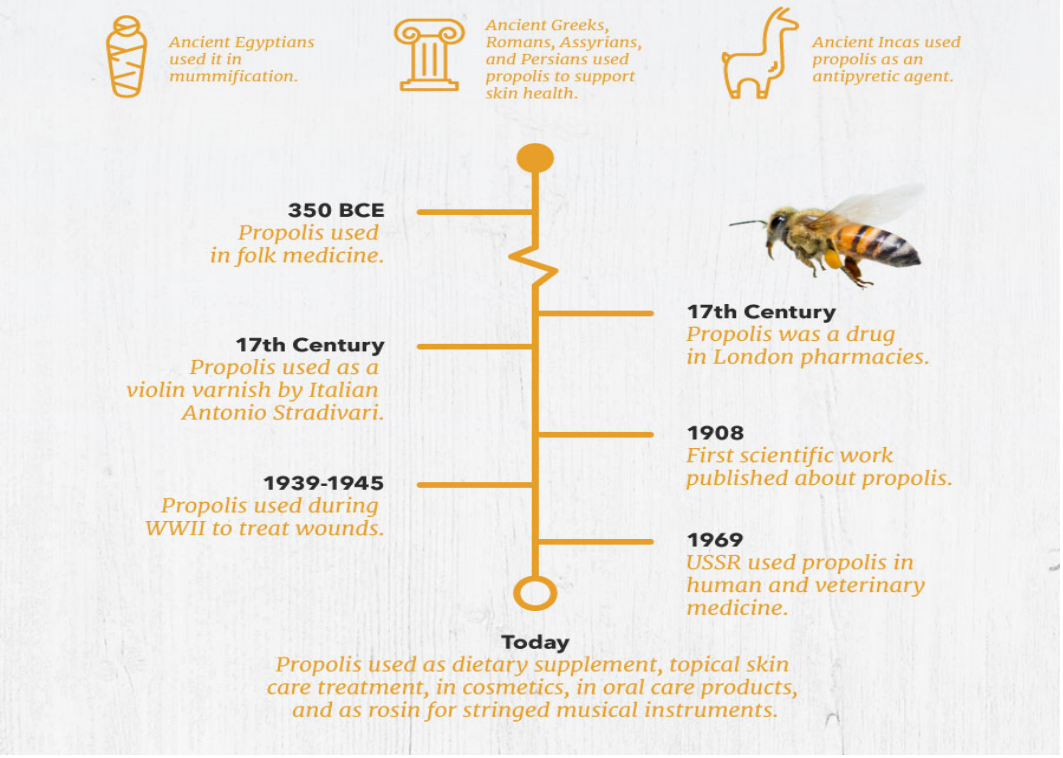

FIGURE 2.2: History of propolis [33]

One of the special components that are found in the propolis is the balm of Gilead that is used in the Holy Temple in Jerusalem, two times a days, there are multiple

Hebrew names of the balm of Gilead that includes nataf, kataf, tzori and afarsemon and they can be traced in multiple sages that includes shimon Ben-Gamliel, Rambam, modern biblical botanist Yehuda Feliks and Saadia Gaon [37].

In past, propolis is used in conventional drug. Solely rare documents about use of propolis are available. Some sources as of the twelfth-century dene pharmaceutical measures comprising bee glue which were used for handling of oral and pharyngeal infections as well as dental caries. In the Georgian original medical piece of writing dated toward c. 1486 Karabadini (Book of Medical Treatment), the writer proposes that propolis is worthy against dental deterioration [38]. Advantageously, the consciousness of therapeutic properties of propolis made in conventional society medication and, in addition, propolis was still widely utilized in home grown prescription on the regions of Eastern Europe. Altogether, propolis has been frequently called Russian penicillin [39].

2.1.1 Propolis in Early Modern Times

The interest of European people developed into the propolis after the Renaisaance theory of the ad fonts and it also brought the interest of the people in the teaching and medicine practices. In the famous herbal book named as the The History of Plant (1597), the use of the resin or the clammy substance is used as a healing ointment and it is obtained from the black poplar tree buds and it is very good against all the inflammation, brusises and squats [40]. During the seventeenth century, the propolis was included in the pharmacopoeiasin in England and it is used as a healing ointment [41]. A botanist and physician Nicholas Culpeper and he presented his work in the book known as The poplar tree and he states that the the ointment called Populneon, which is made of this Poplar, is singularly good for all heat and inflammations in any part of the body, and lesser the heat of wounds. CAPE also acts as anti-cancer agent. Propolis is shown to be involved in the inhibition of the peroxidation of the Low density Lipo-Protein (LDL) and nitration of proteins during the in vitro studies. It is used to dry up the milk of womens breasts, when they have weaned their children [42].

2.2 Properties of Propolis

2.2.1 Melting Point

At 25 C to 45 C propolis a soft, sticky and a pliable substance. It becomes hard and brittle in particularly frozen conditions. The brittle behavior of propolis remains event at higher temperatures and it becomes stickier and gummier above the 45 C. At 60C to 70 C, propolis becomes liquid but the melting point of some samples can be as high as 100 C [43].

2.2.2 Solubility of Propolis

Due to the complex structure of the propolis is cannot be used directly and commercially it is extracted with the suitable solvent.

TABLE 2.1: Different solvent used for extraction of Propolis [44]

Water	Methanol	Ethanol	Chloroform	Acetone
Anthocyanins Starches Tannins Polypeptides Saponins Terpenoids Lectins	Anthocyanins Terpenoids Saponins Tannins Xanthoxyline Totarol Quassinoids Lactones Flavones Phenones Polyphenols Polypeptides Lectins	Tannins, Polyphenol Polyacetylenes Terpenoids Sterols Alkaloids	Terpenoids Flavonoids	Flavonols

Some of the most common solvents that are used for the extractions of propolis include water, ethanol, methanol, chloroform, ether, acetone and dichloromethane as shown in Table 2.1.

The chemical composition of the propolis depends on the geographical regions and method used for extraction. Due to this reason the solvents must be chosen carefully [45].

2.2.3 Chemical Components of Propolis

Propolis is dark in color mostly brown or the dark green and has a very pleasing flavor of the popular buds, wax, honey, and the vanilla, in the meanwhile it can also be of bitter taste. Propolis gives an aromatic smell when it is scalded and this is due to the presence of theresins in the propolis [46]. The aroma and the chemical composition differ with the geographical regions. At low temperature, when propolis is cold, it is hard and brittle while becomes sticky when heated and warmed. There are investigations done on the chemical composition and properties of the propolis [47]. Alphaamylase [48] and some other polyphenolic compounds, flavones, esters, phenolic acid and fatty acids are present in propolis [49–52].

In Propolis, there are twelve different flavonoids are present that includes acacetin, pinocembrin, rutin, chrysin, naringenin, catechin, luteolin, galangin, apigenin, kaempferol, quercetin and myricetin. Two phenolic acids are also present in addation with the flavonoids and these are cinnamic acid and caffeic acid. From three different propolis extracts, the levels of the chemical compounds were checked and these extracts were ethanolic, aqueous-ethanolic and aqueous-glycolic extract. There is a great percentage of the caffeic acid, quercetin, chyrsin and galangin was present in the propolis that was extracted by the aqueous-ethanolic extract. While in the ethanolic preparation there is a great amount of the caffeic acid, chrysin and resveratrol is present.

Almost 11% of the caffeic acid and other flavonoids were present in very low amount and unidentified compounds constitute the 85% of the total composition

in the aqueous-glycolic extract. According to the investigators, for a qualitative and quantitative analysis, a method known as the Capillary Zone Electrophoresis (CZE) is present. Through this method extracted propolis contains 72.7% phenolic acid esters, 1.1% phenolic acids, 6.5% dihydrochalcones, 2.4% aliphatic acids, 1.9% flavanones, 1.7% chalcones, 0.7% tetrahydrofuran derivatives and 4.6% flavones are present [53, 54]. Some of the biologically active components present in the propolis are the 72% (+) titerpenoids and 8% ditetpenoids. Another method known as High-Speed Countercurrent Chromatograph (HSCCC) that uses pre-fractionation and successive steps of purification. As a result, many bioactive components are isolated and characterized from a very complex fraction of proplis. The components isolated are the sandaracopimaric acid, (+)-ferruginol, (12E)- and (12Z)-communic acid, -acetoxy-19(29)-taraxasten-20a-ol, cycloartenol, (+)-totarol, five triterpene acetates, free fatty acids, two labdane fatty acid esters, 15-o-oleoyl and 15-o-palmitoyl-isocupressic acid [55, 56].

2.3 Health Benefits of Propolis

Propolis plays a very important role in dealing with multiple diseases. Some of the health benefits of the propolis are summed up in Table 2.2. All the major properties of the propolis are described below one by one.

TABLE 2.2: Health applications of propolis

Health benets	Propolis activity	Type of studies	References
Reproductive care	Anti-oxidant	Animals	[57]
	Hormone balance	Animals	[58]
	Anti-oxidative agent	Animals	[57]
	Reduce premenstrual Syndrome	Humans	[59]
	Post-menopausal treatment	Humans	[60]

Neurodegenerative and aging diseases	Longevity promoting	Animals	[61]
	Alzheimers diseases	Animals	[62]
	Mental illness	Humans	[63]
Wound healing	Fibroblast migration	Animals	[64]
	Collagen production	Human	[65]
	Vasodilatation	Human	[66]
GI Disorder	Antiparasitic	Human	[67]
	Antiulceration	Human	[68]
Gynecological care	Antifungal	Human	[69]
	Antifungal and antibiofilm	Human	[70]
Oral health	Antibacterial	Laboratory	[71]
	Daily mouthwash	Human	[72]
	Toothpaste disinfection	Laboratory	[73]
	Toothpaste against gingivitis	Human	[74]
	Oral therapeutic drug	Human	[75]
Oncology treatment	Anti-breast cancer	Human	[76]
	Antimelanoma cancer	Animals	[77]
	Anti-lung cancer	Human	[78]
Dermatology care	Acne Vulgaris	Human	[79]
	Collagen metabolism	Animals	[76]
	Diabetic foot ulcer	Human	[77]

2.3.1 Anticancer Effect of Propolis

From the main chemical components of the propolis, two components have an antiproliferative property and these compounds are the Caffeic Acid phenethyl Ester

(CAPE) and chrysin. This property is due to the suppression of the complexes of the cyclins and the arrest of cell cycle in the cancer cells by the effects of the CAPE or chrysin [80]. The in vivo and in vitro studies show that there is an inhibitory effect of the CAPE and chyrsin on the progression of the tumor cells and it can also be used as a chemotherapeutic or chemopreventive anti-cancer drug. For the assessment of the chemoprevention, the best candidate is the squamous cell carcinoma (SSC) because the lesions are amenable for the oral delivery of chemopreventive agents [81]. When the propolis is injected or given through dietary administration, it has the ability to inhibit the occurrence and progression of the oral lesion malignancy. During the treatment the effects of propolis can be visually monitored and modulation of the inhibition of the genes can be performed as molecular targets that are used for the validation of the chemopreventive approaches. Another therapeutic effect of propolis also can be the induction of the apoptosis [81, 82]. But this mechanism is seemed to be dependent on the type of the propolis that is being extracted and the presence of the compounds in that particular type of the propolis. According to the recent studies the flavonoids and the astazanthin and the flavonoids that both are present in the propolis protect the cells from the beta-Amyloid that is involved in the induction of the apoptotic death of the cells [83–86]. There are many other biological activities of the propolis that also includes immunottimulant activity. Against many enevironmnetal mutagens that includes 1-nitropyrene, 4-Nitro-O-Phenylenediamine, 2-amino-3-methylmidazao, benzo[a]pyrene and quinoline it shows antimutagenic effect [87].

The chemo defensive movement in cell culture and animal models might be going to the result in ability to preclude DNA making in tumor cells, the potential toward provoke apoptosis of tumor cells, and their property to start macrophages controlling the ability of B, T and NK cells. Additionally, giving expectation that, they will have similar defensive action pastime in human being due to consequences advice that flavonoids from propolis count on a shielding activity against the lethality of the chemotherapeutic or radiation in mice [88]. Propolis mixed with adjuvant most cancers prevention agent remedy may additionally improve the adequacy of chemotherapy with the aid of improving the symptom on leukocytes, liver, and

kidneys and consequently empowering dosage acceleration [89].Though the caffeic acid, an antimetastatic activity,phenethyl esters (CAPE) from poplar propolis and Artepillin C from Baccharis propolis have been recognized as the greatest eective antitumor agent in various polyphenols [90, 91]. In human lymphocytes, anticarcinogenic capability of propolis in vitro was discovered. Plasma checks had been acquired from 10 sound males, non-smoking volunteers, which had been incubated and offered to increasing concentrating of propolis (0.01, 0.05, 0.1, 0.2, 0.5, 0.7, and 1.0 mL) [92]. This suggest that micronucleus concentration had been 1.4770.38 - 4.0270. 64 Mitotic record costs have been somewhere in the range between 19.4572.22 - 0.2870.33. The contrasts between the manipulate and uncovered cells were statically important (pp; 0: 05) [93]. In peripheral human being lymphocytes in vitro are acquaintance to various concentrations of propolis cannot produce a cancer-causing inuence. Though, it showed that propolis might have a cancer-causing impudence in high concentrations by increasing micronucleus rates [94].

2.3.2 Antioxidant Properties of Propolis

This property of propolis is related with some of the biological properties that it shows such as chemoprevention. The powerful antioxidants are the flavonoids that are present in the propolis and they also protect the cell membrane from the lipid peroxidation because they are capable of the scavenging free radicals [95].

The Reactive Oxygen Species (ROS) and the Reactive Nitrogen Species (RNS) are involved in the cellular ageing and cellular death with some other factors in some conditions. Some of the types of deaths caused by RNS and ROS include cardiovascular diseases, cancers, arthritis, diabetes, Alzheimers disease and the Parkinsons disease [96, 97]. The cellular levels of the H_2O_2 and the NO can be reduced by the propolis due to its anti-inflammatory properties [98]. As the inhibitors of the oxidative stress, a wide range of propolis compounds has been described. These compounds include CAPE that is involved in the blockage of the production of the ROS in several systems [99]. CAPE also acts as anti-cancer

agent. Propolis is shown to be involved in the inhibition of the peroxidation of the Low density Lipo-Protein (LDL) and nitration of proteins during the in vitro studies. The antioxidant activity in animals [100] and humans [101] can be increased by the propolis during the in vivo studies and it leads towards the decreased peroxidation of the lipids [102, 103]. Hydrogen peroxide (H_2O_2) that induces the DNA damage in the cultured fibroblasts is also inhibited by the Turkish propolis [104].

A remarkable medical property of the ethanolic extract of propolis (EEP) is described by the Krol *et al* 2004 that shows protection against the gamma radiations [105]. For this study, the experiment was done on the mice and the anti-oxidativative effects of propolis was find out that can be involved in the radical scavenging ability of propolis. According to their experimentthat the luminal H_2O_2 chemiluminescence can be inhibited by the increased amounts of the EEP. This demonstration shows the anti-oxidative capacity of the propolis because of the high contents of the flavonoids in the in vitro study. Another study was done to for the purpose of investigation the antioxidant activity of the propolis that was deprived of the CAPE. Two propolis that were with and without CAPE, and the active components of the propolis shows the free radical scavenging effect, that is dependent on the dose. The results show the inhibition of the xanthine oxidase activity due to the antilipoperoxidative capability. As compared to the propolis extract that was without the CAPE, propolis extract containing CAPE shows more active behavior. According to the experimental studies the CAPE plays a very important role in the antioxidant activity of bee propolis [106]. Apart from CAPE, the antioxidant behavior of another component the propolis known as the tectochrysin is investigated. It also shows role in the decrease of the activities of the serum transaminase that shows elevation due the hepatic damage that was induced by the CCl4 intoxication in the rats. It also increases the antioxidant activity of the enzymes that in return decreases the production of the Malonaldehyde (MDA) [107]. CAPE also acts as anti-cancer agent. Propolis is shown to be involved in the inhibition of the peroxidation of the Low density Lipo-Protein (LDL) and nitration of proteins during the in vitro studies.

2.3.3 Antibacterial and Antiviral Activities of Propolis

As there are multiple types of flavonoids are present in propolis that has an antibacterial and anti-inflammatory properties [108] that can be used as powerful natural antibiotics [109]. The flavonoids play a very important role in the cure of respiratory disorders that can be common cold or the influenza viruses [110]. A variety of the potent polyphenols is present in the propolis that has the capability of enhancing the antistaph activity of some pharmaceutical drugs majorly antibiotics such as streptomycin [111]. Velikova *et al.*2001 [112, 113] and Marcucci *et al.*2010 [114] report the antibacterial activity and chemical composition of the propolis. For the effective prevention of contamination of *E. coli* and *S. aureus*, natural antibiotics such as propolis can be used [115]. Against a wide range of the gram-positive rods propolis shows its antibacterial activity but in the case of gram-negative bacteria it is only limited to the bacilli [116, 117]. Ugur and Arslan perform some tests for the verification of the antimicrobial activity of propolis and according to them this activity depends on the sample of propolis, propolis dosage, and solvents that are being used for the extraction of propolis [118]. The growth of the *B. cereus* and *S.aureusis* inhibited by the 125-500 ug/ml propolis [119]. The polyphenols content plays a very important role in the antimicrobial activity of the propolis [120]. The growth of bacteria can be inhibited by the propolis as it prevents the division of cells that as a result form pseudo-multicellular bacterium. Apart from this, propolis is also involved in the inhibition of the synthesis of the protein that causes the partial bacteriolysis and in return the cytoplasmic membrane is being disorganized [121]. Against the Herpes simplex virus type 1 the activity of the 3-methyl-but-2-enyl caffeate was investigated in vitro. 3-methyl-but-2-enyl caffeate that is a very minor compound of the propolis is very effective in the reduction of the virus titer and the synthesis of the viral DNA effectively [122]. Another compound that is also isolated from the propolis known as the isopentlyferulated shows its role as an inhibitory agent in the in vitro studies against the activity of the infectious influenza viruses [123]. The mortality is decreased and the survival length of the infected mice with the influenza virus A/PR8/34 (HONI) is done by using the aqueous extract of propolis [124]. Some of the compounds that includes:

melliferone, moroni acid, three known triterpenoids, betulonic acid, four known aromatic compounds and anwuweizonic acid were extracted from the Brazilian propolis and are tested against the activity of the HIV in H9 lymphocytes [125].

By agar diffusion method, the antimicrobial activity of the propolis that is composed from Gujarat by agar diusion method beside *Asparagus nigar*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, and *Candida albicans*. Ethanolic extracts of trial (conc. 200 mg/mL) presented lowest action of gram-negative bacteria (*P. aeruginosa* and *E. coli*) but great antibacterial action, gram-positive is *Bacillus subtilis*. Though, *A. Niger* did not shows any action the yeast (*C. albicans*) presented the reasonable zone of inhibition. But, 40% was least the methanolic extracts [126, 127].

2.3.4 Anti-fungal Activity of Propolis

Through the sensitivity tests that were conducted on the 80 strains on the Candida yeasts, 20 strains of *Candida tropicalis*, 20 strians of the *Candida albicans*, 15 strains of the *Candida guilliermondiiand* 2 strains of the *Candida krusei*, the antifungal activity of the propolis was studied [128]. The order of the sensitivity tests for the antifungal activity was in this order: *C. albicans* is greater than *C.tropicalis* is greater than *C. krusei* is greater than *C. guilliermondii*. Kovalik investigated 12 patients that were suffering from the chronic sinusitis that is caused by the *Candida albicans* [129]. 8 out of 12 cases were of those in which the fungus shows sensitivity towards the propolis but it shows resistant in 2 cases and weak sensitivity is shown by fungus towards the propolis. The treatment of the alcohol-oil-emulsion of propolis is given to the patients. The emulsion 2-4 ml was introduced after the irrigation with isotonic serine into the sinuses every day or after one day. The conditions of the patients get improved after 1-2 propolis treatments. After treatement 5-8 patients the clinical recovery occurred in 9 patients and other three patients show improvements. All the patients were recovered after the 10-17 days. The growth of the *Candida albicans*, *A. ochraceus*, *Penicillium viridication*, *Aspergillus flavus* and *p.notatum* is inhibited by the pure extracts of propolis with the

concentrations of 15-30 mg/ml. The 0.25-2.0 mg/ml concentration of the propolis is enough for the repressed growth of the *A.sulphureufor* 10 days [130]. 38 strains of fungi and 60 strains of yeast [131] and *Aspergillus parasiticus* strain NRRL 2998 [132] is prohibited by the ethanolic extract of propolis (EEP). Another 2 extracts of propolis names as the ethanolic and dimethyl-sulphoxide were active against the Trypanosomacruzi [133] lethal towards the *Trichomonas vaginalis* [134].

2.3.5 Anti-Inflammatory Activity of Propolis

Two compounds that were derived from the propolis of the honeybee hives named as caffeic acid and phenethyl ester shows anti-inflammatory properties. As in the onset of the many inflammatory diseases, T-cells play a key role so Mrquez *et al.* 2012 [135] examines the immunosuppressive activity in the T-cells. The results show that the phenolic compound played a very key role in the inhibition of the T-cell receptor-mediated T-cell activation. These studies and experiments show that the CAPE inhibits the gene transcription of the interleukin IL-2 and its synthesis that stimulates the T-cells. The DNA binding and transcriptional activities of the nuclear factor (NF)-B, activator protein 1(AP-1) and nuclear factor of activated cells (NFAT) is examined for inhibitory mechanism of the CAPE at the transcriptional level. According to their results, the NF-B dependent transcriptional activity is inhibited by the CAPE but it does not affect its cytoplasmic degradation [136].

Irritation is the composite biological reaction of vascular tissues to destructive stimuli, such as free radicals, pathogens, damaged cells and irritants. The key response of the host is an anti-inammatory action [137]. The action of propolis has been looked into by Almeida and Menezes. NADPH-oxidase ornithine decarboxylase, myeloperoxidase movement, tirosine-proteinkinase, and hyaluronidase from guinea pig pole cells have inhibitory properties of propolis. Through the existence of flavonoids dynamicand cinnamic acid of these anti-inammatory action can be described [138]. The former comprises of naringenin,quercetin, andacacetin; the latercontainscaeic acid (CA) and caeic acid phenyl ester (CAPE) [139]. Previous

incorporates, naringenin,quercetin, and a cacetin the last includes caeic corrosive (CA) and caeic corrosive phenyl ester (CAPE) [137].Galangin and CAPE, being average famous propolis components, showed anti-inammatory action and essentially restrained carrageenan oedema, carrageenan pleurisy, and adjuvant joint pain aggravations in rats. The lipoxygenase pathways of arachidonic corrosive digestion amid aggravation in vivo are mainly restricted the dietary propolis. The Caeic corrosive, quercetin, and naringenin were a less intense modulator of arachidonic corrosive digestion than CAPE [138, 139].

2.3.6 Anti-Ulcer Activity of Propolis

During the in vitro studies it was investigated by the Boyanova *et al.*Propolis has an inhibitory effect on the growth of the Helicobacter pylori [140]. Against the 38 clinical isolates of *H.pylori* the activity of 30 % Ethanolic Extract of Propolis (EEP) is being evaluated by using the agar-well diffusion method. Against the 73.1% of the *H.pylori* isolates the growth is inhibited by the dried propolis dics. Ethanol was used as a controlling mechanism in this study. The effect of propolis was also being tested on the 18 *Campylobacter*. The zone of inhibition was 15mm for the *H.pylori* isolates and 11.6mm is for the *Campylobacter spp.* According to them, there are antibacterial activities possessed by the Bulgarian propolis and it can inhibit the growth of the *Campylobacter jejuni* and *Campylobacter coli* [141]. According to the Tossoun et al.for the management of the chronic skin ulcers can be treated [142].

Another study was done by M.Kucharzewski *et.al.* 2013 [143] to find out the effect of propolis ointment towards the healing of the chronic venous leg ulcers. For this study, 56 patients were considered and divided into two groups. 28 patients included into the group I with ulceration area of 6.9 to 9.78 cm and their treatment includes the application of propolis ointment and compression of short stretch bandage. 29 patients added into the group II with ulceration area of 7.2 to 9.4 cm and their treatment was done with the Unnaboot leg compression. Group II was not given the topical propolis treatment. The efficacy of both treatments

was compared in patients with resistive venous leg ulcers. After 6 weeks of the treatment all the patients from group I healed very quickly but the group II patients healed after 16 weeks of the treatment. From this it can be concluded that the combined treatment constituting both propolis ointment and bandage compression stocking is much effective in healing venous leg ulcer as compared to the Unnas boot compression only [143].

2.3.7 Hepatoprotective Effect of Propolis

Defensive capability of a propolis changed into assessed alongside mercury-incited oxidative pressure then most cancers prevention agent enzymatic adjustment in liver of mice. By using the increasing lipid peroxidation and oxidized glutathione level and introduction to a mercuric chloride incited oxidative fear alongside corresponding abatement in glutathione and extraordinary most cancers prevention agent proteins. Mercury inebriation strayed the movement of marker liver compound in blood. Conjoint remedy of propolis repressed lipid peroxidation and oxidized glutathione level even though improved stage of glutathione. Action of cancer prevention marketers catalysts that is catalase, superoxide dismutase, glutathione S-transferase, and glucose 6-phosphate dehydrogenase, became moreover reestablished correspondingly closer after propolis organization to control. Arrival of serum transaminases, lactate dehydrogenase, soluble phosphatase, and Y-glutamyltranspeptidase become basically reestablished closer to control after propolis remedy. Results propose that propolis as the cancer prevention agent in opposition to mercury-actuated poisonous rst-class and gives proof that it has remedial ability as hepatoprotective specialist [144].

For the protection of the liver of rats from the injury of carbon tetrachloride, the aqueous propolis Extract (APE) shows its properties. It was done by decreasing the leakage of the cytosolic enzyme Lactate Dehydrogenase (LDH) that decrease the lipid peroxide generation and helps in the maintenance of the cellular contact by reduced glutathionine [145]. The acetemainophen induces the protective effects of the propolis on the hepatotoxicity. The mechanisms of the hepatoprotective

effects of the propolis were also investigated. The cytottoxity of AA is significantly decreased by the pretreatment with the PP (1, 10, 100, 200 and 400 u/ml, 24 h) in the rat hepatocyte culture. The method was dose-dependent. The mortality and the incidence of the severe hepatice necrosis induced by AA were decreased by the pre-treatment with PP (10 and 25 mg/kg, P.O., 7 days). After the 7 days treatment of the PP the hepatic enzyme activities of the cytochrome P450 monooxygenases (P450s), Phenolsulpho transferase, UDP-glucuronyl transferase and gluththione S-transferases were measures in both mice and rats. The activity of the P4502E1 is decreased in the rats with the PP (50 and 100 mg/kg, P.O.) but the activities of GST and PST increased significantly. While in the mice that were treated with the (10 and 25 mg/kg, P.O.), the activities of the P4501A2, 2B1, 3A4 and 2E1were inhibited but it enhances the activity of the PST. According to these results, it has been shown that on the hepatic injury the PP has a protective effect and it inhibit the phase I and phase II enzymes [146].

2.3.8 Anti-Diabetic Effect of Propolis

In rats, the antihypertensive effect of propolis is also shown [147]. The rats that are diabetic, the levels of the fasting blood glucose (FBG) were decreased after the administration of the propolis extracts and it also decreases the malonaldehyde (MDA), Total Cholestrol (TC), nitric oxide (NO), Low-Denisty Lipoprotein Cholestrol (LDLC), Triglyceride (TG), Very Low-Density Lipoprotein Cholestrol (VLDL-C) and the levels of the High Density Lipoprotein Cholestrol (HDL-C) Superoxide Dismutase (SOD) increases in the rats. This concludes that the propolis can be used to control the blood glucose level and helps in the modulation of the metabolism of the glucose and blood. This leads towards the decreased effects of the lipid peroxidation and helps in the scavenge the free radicals in the diabetic rats [148].

The impact of ethanolic listen of propolis against trial diabetes mellitus-related adjustments becomes inspected. Diabetes becomes incited tentatively in rats by using i.P. Infusion of streptozotocin (STZ) in measurements of 60 mg/kg between

for three innovative days. Blood urea nitrogen (BNU), creatinine, glucose, lipid prole, malondialdehyde (MDA), and urinary egg whites have been predicted. Superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), and MDA were predicted inside the renal tissue. The consequences indicated diminished frame weight and increased kidney weight in diabetic creatures [149]. Contrasted with the manage everyday rats, diabetic rats had higher blood glucose, BNU, create nine, add up to cholesterol, triglycerides, low-thickness lipoprotein-ldl cholesterol (LDL-C), MDA and urinary egg whites, and lower high-thickness lipoprotein-ldl cholesterol (HDL-C) tiers. In addition, renal tissue MDA becomes particularly expanded while SOD, GSH, and CAT were essentially diminished. Renal GSH, SOD, and CAT had been altogether increased whilst MDA turned into signicantly decreased [150]. These results may additionally suggest a strong cancer prevention agent impact of propolis which can enhance oxidative stress and delay the occasion of diabetic nephropathy in diabetes mellitus [151].

2.3.9 In-Silico Approaches

It is reported that the Okinawa propolis (OP) and its compounds have anti-cancer effects. It also have lifespan-extending effects on the Caenorhabditis elegans by the inactivation of the oncogenic kinase, p21-activated kinase 1 (PAK1). Nymphaeol-A (NA), nymphaeol-B (NB), nymphaeol-C (NC), isonymphaeol-B (INB), and 30-geranyl-naringenin (GN), are the five OP flavonoids that were evaluated for their anti-inflammatory, anti-diabetic, and anti-Alzheimers effects using in vitro techniques. Through inhibition of albumin denaturation (half maximal inhibitory concentration (IC50) values of 0.261.02 _M), nitrite accumulation (IC50 values of 2.47.0 _M), and cyclooxygenase-2 (COX-2) activity (IC50values of 11.7424.03 _M), they showed significant anti-inflammatory effects. They also strongly suppressed in vitro-glycosidase enzyme activity with IC50 values of 3.775.66 _M. However, only INB and NA inhibited acetyl cholinesterase significantly compared to the standard drug donepezil, with IC50 values of 7.23 and 7.77 _M, respectively.

Molecular docking results indicated that OP compounds have good binding affinity to the glucosidase and acetyl cholinesterase proteins, making non-bonded interactions with their active residues and surrounding allosteric residues. All the compounds follow Lipinskis rule of five and does not show toxicity by following all the toxicity parameters. Their high reactive nature with the kinetic stability is demonstrated through Density functional theory (DFT) based global reactivity descriptors. From this study, it can be concluded that the OP compounds might have benefits in the inflammation, type 2 diabetes mellitus and Alzheimers disease treatment [152].

Another study was done to find out the interaction between four components of propolis that includes Acacetin, Naringenin, Caffeic Acid Phenethyl Ester and Chrysin and the protective antigen. Molegro virtual docker (MVD) and Chimera 1.7 was used for molecular docking. The results of this study show that each ligand from these four ligands has an interaction with the protective antigen that can inhibit its interaction with the cell [153].

Another In vitro study was done to find out the potential use of propolis as a multi-target therapeutic product and another purpose of this study was also to find out the physiochemical properties, chemical composition, antioxidant, antibacterial, antioxidant, immunomodulatory and anti-cancer properties of propolis. HPLC was used to find out the main phenolic compounds of propolis. The results show that propolis shows inhibitory effects against all the tested gram-positive and gram-negative strains and also show high antioxidant activities. The propolis shows therapeutic properties in cytostatic, antibactetial and immunomodulatory effects [154].

2.3.10 Molecular Docking

When the human project got completed it resulted into the increased number of therapeutic targets in the drug discovery. Apart from drug discovery, multiple techniques came into being such as high-throughput protein purification, nuclear

magnetic resonance spectroscopy and crystallography. The development of these drugs contributes into the structural details of the proteins and protein-ligand complex that is also very important in the drug discovery [155, 156].

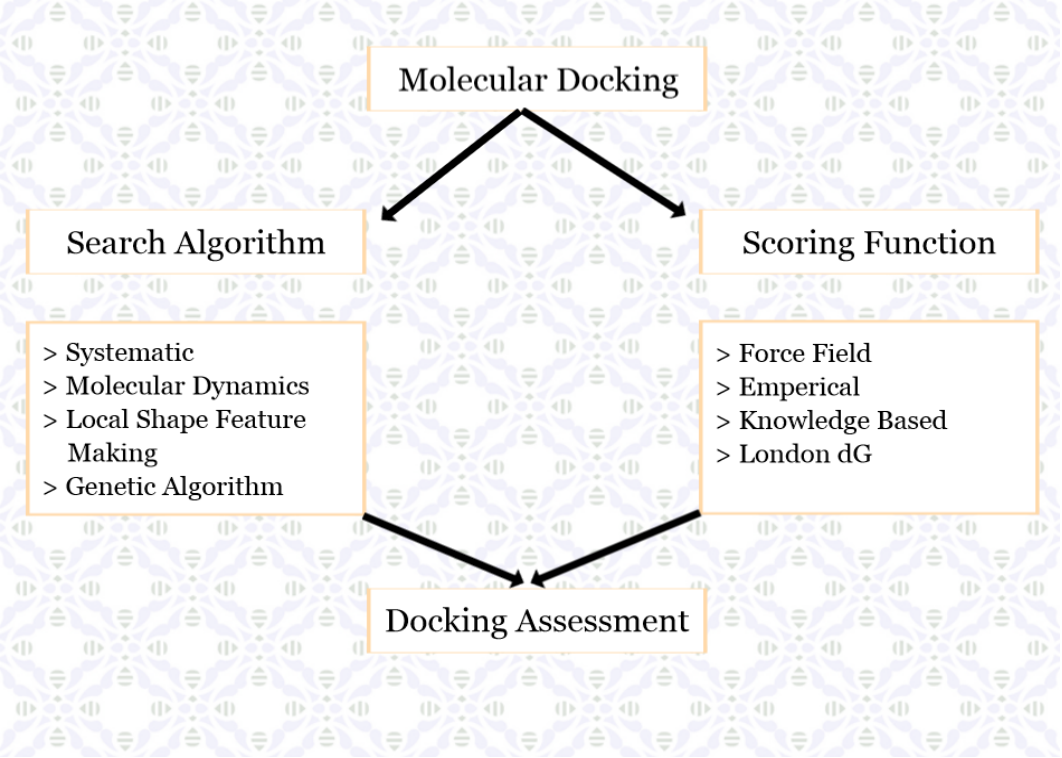

FIGURE 2.3: Functionality of molecular docking [157]

To model the interactions between a small molecule (ligand) and protein at an atomic level molecular docking can be used and it allow to characterize the behavior of the small molecules at the pocket or the binding site of the protein that is very important in drug discovery. It also helps to elucidate fundamental biochemical processes. Two basic steps involved in the process of docking that includes prediction of the ligand conformation, its position and orientation and to find out the binding affinity. These steps are involved in the sampling and scoring methods [154].

The purpose of the molecular docking is to predict the ligand-receptor complex by using computational methods [157]. The docking efficiency can be increased by knowing the binding site. This site can be recognized by comparing the protein with the other proteins of the same family that share similar function or with the

proteins that are co-crystallized with some other ligands [158]. When a protein has a high number of degrees of freedom than it will cause difficulties in molecular docking and also increases the computational cost of the calculations. The simplest docking can have only three translational and three rotational degrees of freedom of the protein and the ligands. It is considered as a rigid docking in which protein and ligands acts as two separate rigid bodies. There are different algorithms and scoring function for the assessment of molecular docking and different tools used different algorithms and scoring functions, Fig 2.3 shows different algorithm and scoring functions. Advancements in the algorithms now allow the ligands to fully explore the conformational degree of freedom in rigid-body receptor [159].

2.3.10.1 Available Docking Protein Software

There are many software that are available for molecular docking most of the software are desktop base and can be used in different languages such as python, C sharp etc. different molecular docking software along with their platform are shown in Table 2.3.

TABLE 2.3: Different tools for molecular docking [160]

Software	Description	Online / Desktop	License
AutoDock	Binding orientation and ligand target affinity is predicted by it.	Desktop based	Open source
Blaster	It combines ZINC databases with the Dock tofind the ligandor targeted protein.	Online and Desktop based	Free
Docking server	It integrates a number of computational chemistry software.	Online	Commercial

FlexX	It is an inncremental build based docking program.	Desktop based	Commercial
Gold	It base on Genetic algorithm, flexible ligand, partial flexibility for protein.	Desktop based	Commercial
Lead Finder	It is a program for molecular docking and biological activity.	Desktop based	Commercial
Molecular Operation Environment (MOE)	It has docking application withinMOE, choice of placement methods, and scoring functions.	Desktop Based	Commercial
VoteDock	Consensus docking method for prediction of protein-ligand interactions.	Desktop based	Academic

There are several software available for molecular docking. Most of them are desktop based software and used different languages like python, C sharp etc [160].

For this project we use MOE because it contains docking application within the tool and there are several other choices of placement method and scoring function

present in it. MOE can work on windows, macOS and Linux. Major application areas of MOE include structure based design, simulations and biological applications. It is very easy to use and has a friendly user interface [161].

2.3.10.2 Analysis and Visualization of Docked Compounds

TABLE 2.4: Visualization software [162]

Name	Description	Web or Desktop Based	License
Chimera	Visualization and analysis of molecular structures and related data, including density maps, trajectories, and sequence alignments .	Desktop	Free
PyMOL	A cross-platform molecular graphics tool, has been widely used for three-dimensional (3D) visualization of proteins, nucleic acids, small molecules	Desktop	Academic
LigPlot	Automatically generates schematic diagrams of protein-ligand interactions for a given PDB file.	Desktop	Academic

Post Dock	It processes a docking results database and displays an interactive pseudo-3D snapshot of multiple ligand docking poses such that their docking energies and docking poses are visually encoded for rapid assessment.	Desktop	Academic

Virtual screening of components has now become a standard technology in modern drug discovery. After the docked compounds the analysis and visualization process occur to measure and label the residue sites of protein and their attachment and visualize the docking result in 3D format. There are many softwares or tools available to analysis and visualize the docked compounds as mention in Table 2.4. Every tool shows the different representation of the docked compounds [162].

2.3.10.3 Pharmacokinetics

While dealing with the medicinal chemistry lessons, some of the major topics such as pharmaceutical, pharmacokinetic and pharmacodynamics phases are included. In pharmaceutical phase, drug administration route such as enternal or parenteral and its pharmaceutical form such as tablet, capsule or solution etc is included. There are four steps of pharmacokinetic phase that included absorption, distribution, metabolism and excretion (ADME). For the absorption of a drug, its key molecular properties include lipophilicity and solubility. In 1197, Lipinski and his coworkers published the Rule of Five (Ro5) for the pharmacokinetic parameters that base on the study of the properties of 2245 drugs from the World Drug Index (WDI) database that were approved for phase II clinical trials [163].

TABLE 2.5: Pharmacokinetic tools [164]

Name	Description	Desktop / Web	License
OSIRIS Property Explorer	It helps to draw chemical structures of drugs and to calculate their other drug relevant properties when the structure is valid	Desktop based	Academic
Molinspiration	It supports internet chemistry community by offering free on-line services for calculation of important molecular properties	Web based	Free

We use Molinspiration toolkit to predict pharmacokinetic properties of a drug. It is web based tool and everyone can use it freely. Molinspiration toolkit has user friendly interface. The input of Molinspiration toolkit is the compound smiley which can be gather from ChemSpider or PubChem websites.

Chapter 3

Methodology

3.1 Proposed Diagram

Fig 3.1 shows the detail outlines of our research methodology.

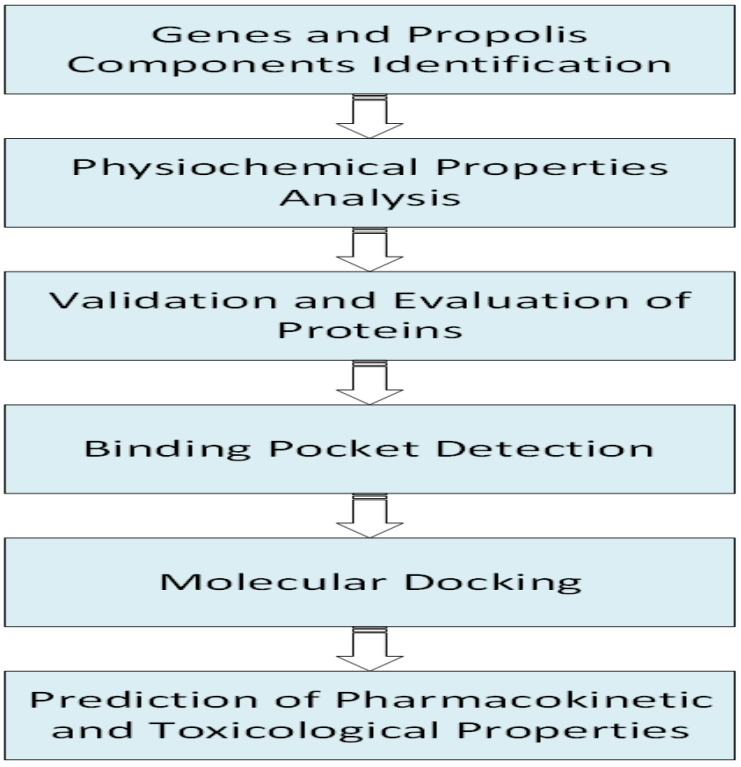

FIGURE 3.1: Prposed diagram

3.2 Identification of Genes and Propolis

Genes involved in ulcer and all the propolis components were identified through the literature review. The PubMed IDs of papers that contain information regarding our studies were used for literature review by putting in the Bioreader server (http://www.cbs.dtu.dk/services/BioReader/). Two human (ABCG2 and HLADQB1) and two bacterial genes (CagA and VacA) were selected that are found to be majorly associated with the gastric ulcers. Selected bacterial and human genes that were involved in the direct occurrence of ulcer or in the pathway that lead to the ulcer.

Table 3.1: Compounds name with their PubChem IDs

PubChem ID	Compounds Name
73170	Alpha-Amyrins
5280442	Acacetin
5281650	Alpha-Mangostin
5280443	Apigenin
5472440	Artepillin C
5281787	Caffeicacid Phenethyl Ester (CAPE)
5281607	Chrysin
444539	Cinnamic Acid
442126	Decursin
445858	Ferulic Acid
5281616	Galangin
370	Gallic Acid
5464078	Gamma -Mangostin
5495928	Garcinone B
5281666	Kaempferide
5280863	Kaempferol
5280445	Luteolin

640546	Methyl Pinoresinol
7296	Methylcyclopentane
5281672	Myricetin
439246	Naringenin
10228	Osthol
637542	P-Coumaric Acid
73202	Pinobanksin
68071	Pinocembrin
5316793	Pinostrobin Chalcone
72	Protocatechuic Acid
5280343	Quercetin
5280804	Quercetin-3-O-GLUCOSIDE
5320844	Quercetin-4-O-GLUCOSIDE
44259222	Quercetin-5-O-GLUCOSIDE
5381351	Quercetin-7-O-GLUCOSIDE
5280805	Rutin
92156	Beta-amyrins Acetate
73145	Beta-amyrins
10742	Syringic Acid
78738	Polyquaternium 37
13990811	Isosakuranetin Chalcone
6474310	Isochlorogenic Acid A

There were more than 300 propolis compounds that were known but only 40 compounds were used in this study. These 40 compounds were found to be used in the treatment of ulcer in recent studies. These 40 chemical compounds that are found in the propolis were taken and devised for the docking. Table 3.1 shows the compounds which were used in this study. Selected bacterial and human genes that were involved in the direct occurrence of ulcer or in the pathway that lead to the ulcer.

3.3 Physiochemical Properties Analysis

ProtParam tool of ExPASy (https://web.expasy.org/protparam/) was used to determine the physiochemical properties. Various physical and chemical properties like overall number of positive charged residues (Arg + Lys) and negative (Asp + Glu), Hypothetical pI, coefficients extinction, index of instability, aliphatic index and grand average of hydropathicity (GRAVY) were computed using ProtParam, a proteomics server [165].

3.4 Validation and Evaluation of Proteins

The 3D structures of all proteins were retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) database in PDB format. The 3D structure of identified proteins were analyzed using PyMOL for predicting the reliability and model surface loops of the predicted models and to do structural investigations. Program PROCHECK (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=index.html) is used to identify the effectiveness of proteins [165].

For the assessment of structure of protein backbone, Ramchandran plot was used to used that is also a two-dimensional geometrical plot that consist of phi and psi angles and it also depicts the information regarding the protein structure and its 3D conformation [166]. For the determination of the energy graphs, to check protein structure quality, ProSA (protein Structure Analysis) web server (www.prosa.services.came.sbg.ac.at /prosa.php) was used.

3.5 Binding Pocket Detection

For the docking simulation, MOE reduces the time for experimentation with great accuracy of the binding mode predictions. Active site residues of the proteins were

detected from the DOGSiteScorer (https://proteins.plus/) [167]. Pocket with the highest drug score were chosen.

3.6 Molecular Docking

Consequently docking contributed fundamental part in the rational drugs designing [168]. It helps in the detection of novel small molecular compounds, revealing the important properties, such as high binding interaction with target protein having reasonable absorption, distribution, metabolism and excretion (ADME) profile and drug likeness, which helps in selection of lead for the target [169]. Molecular operating environment tool was for molecular docking.

3.6.1 Receptor Preparation

3D protein structures were downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) and Protein Data Bank (http://www.rcsb.org) in .PDB format. Proteins were devised for docking in MOE tool by removing

- Water molecules
- Hetro-atoms
- Ligands

After removing water molecules, hetro-atoms and ligand, the hydrogen atoms were added and energy of the proteins was minimized. After minimizing the energies of proteins the 3D structure was save in .PDB format for further proceedings.

3.6.2 Ligand Preparation

After identification of components 40 through literature review, the 3D structures of all the ligands were drawn with *ChemBioDraw Ultra 11.0* along with their

geometry optimization. All the ligands were saved in MOL format to be further used by MOE. After that, ligands database was created in MOE and ligands were added into that database one by one by removing hydrogen atoms and minimizing the energy and saved in CDX or MOL format. These are the formats that are accepted by MOE. After adding the ligands in the database, the whole database was saved in MDB format which is accepted by MOE.

3.7 Docking Simulation

Molecular Operating Environment (MOE) was used for molecular docking. Active site residues of the proteins were detected from the DOGSiteScorer. The detected pocket of each protein was chosen which shows the highest drug score. Protein HLA-DQBI (5KSB) had 0.8 drug score with dimensions 1280.49, 1370.02 and 31.89(volume, surface and depth). CagA (5X7B) had 0.8 drug score with dimensions with 753.22, 1038.51 and 17.02 (volume, surface and depth). VacA (60DY) had 0.81 drug score with dimensions 1032.62, 1127.74 and 42.91(volume, surface and depth). ABCG2 (6VXI) had drug score 0.81 with dimensions 1759.89, 1839.8 and 38.12(volume, surface and depth).

3.8 Analysis and Visualization of Proteins

For the interpretation of docking results; interactions between ligand and active pocket of protein were calculated. After docking simulation, 5 compounds with the highest S-score were selected for each protein that collectively makes 20. The binding affinity or the S-Score is automatically calculated by the MOE and it is based on all the pairs of the atoms that move according to each other that help in the determination of the intermolecular and intramolecular interactions. Better the binding stability, the more the negative the binding affinity. So on these basis top 5 compounds for each protein were selected.

PyMOL, a desktop based visualization tool, was utilized to study these interactions. The PDB file of complex protein was uploaded in PyMOL. Complex proteins were visualized by PyMOL by selecting their interaction residue with the ligand. Distance was measured and AA residues were labeled that were present in the interaction complex.

3.9 Calculation of Pharmacokinetic Parameters

The Molinspiration online toolkit (http://www.molinspiration.com/cgi-bin/ properties) was used to predict the drug likeness properties of the compounds. To prove the pharmaceutical fidelity of, the orally active drugs should have utilized drug likeness properties. In this project multiple parameters were calculated such as the number of hydrogen-bond donors, miLogP, the number of hydrogen-bond acceptors, TPSA, molecular mass of the compounds and the number of rotatable bonds. Violations Lipinskis rule of five [170] was also calculated. Absorption rate percentage was also calculated through a previously described method [171]. The formula used for calculating the absorption rate percentage is given below:

$$\% \text{ ABS} = 109 - (0.345 \times \text{TPSA}).$$

3.9.1 Rule of Five Properties

For devising Rule of 5 a set of straightforward atomic descriptors utilized by Lipinski. The rules stated:

- The logP values of most drug-like molecules should be less than or equal to 5.
- Molecular weight should be less than or equal to 500.
- Maximum number of hydrogen bond acceptors should be less than or equal to 10.

- Maximum number of hydrogen bond donors should be less than or equal to 5.

Compounds disobeying more than one of these guidelines rules may be oral availability issues. Based on the Vebers rule, the number of rotatable bonds in the orally bioavailable drugs should be less than or equal to 10 and topological polar surface area (TPSA) value should be less and or equal to the 140.

3.10 Prediction of Toxicological Properties

Drug toxicity is defined as the level of damage that a compound has an ability to cause to an organism [172]. Toxicity is the most important factor for any drug and AdmetSAR 2.0 (http://lmmd.ecust.edu.cn/ admetsar2/) was used to predict the toxicological properties of the selected compounds AdmetSAR 2.0 that is an online toolkit. Toxicity, carcinogenic properties, rat acute toxicity, acute oral toxicity of the ligands and their inhibitory effects on the proteins were predicted through this tool.

Chapter 4

Results and Discussion

4.1 Retrieval of Identified Proteins and Propolis

After identification of targeted proteins, the 3D structure two human (ABCG2 and HLADQB1) and two bacterial proteins (CagA and VacA) were downloaded from RCSB database in PDB format. Through literature 40 propolis components were selected and their structures were drawn with *ChemBioDraw Ultra 11.0* along with their geometry optimization. Fig 4.1 shows the 3D structure of proteins retrived from RCSB PDB database.

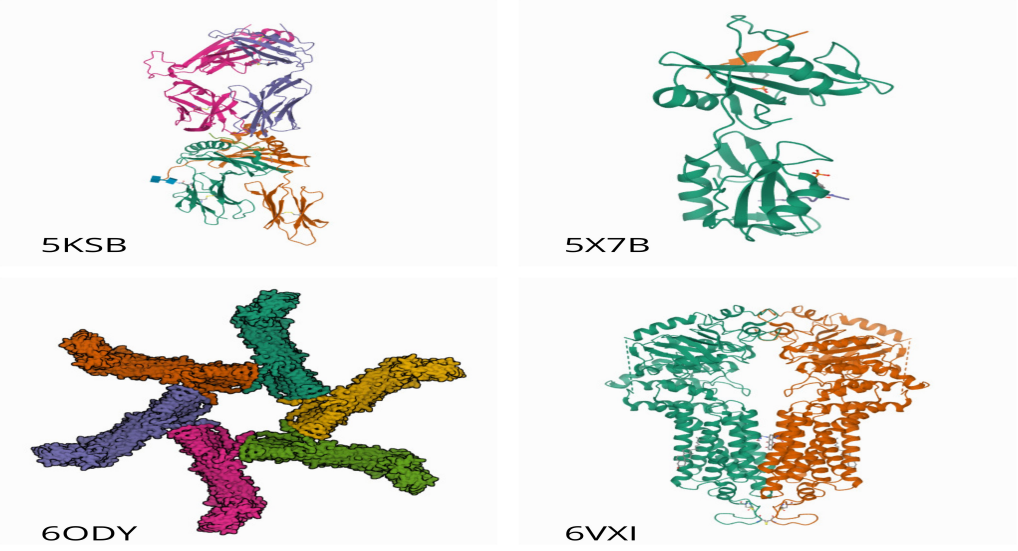

FIGURE 4.1: 3D structure of proteins with their PDB ID

4.2 Physiochemical Characterization of Proteins

Protein binding behavior is governed by physiochemical properties and these properties are determined by the analogous properties of amino acids that are present in it. ProtParam is most commonly used to calculate the physiochemical properties of sequence, plays influential role to determine the function of a protein. Physiochemical properties of the target for ABCG2 (6VXI), HLA-DQB1 (5KSB), CagA (5X7B) and VacA (6ODY) proteins, were figured out via the ExPASys ProtParam server includes hypothetical pI (isoelectric point), molecular weight, overall negative R and positive +R amino acids, extinction coefficient (EI), instability index (II) and aliphatic index (AI). PI denotes protein net charge. The calculated pI can be useful for developing buffer system for purification by using familiar isoelectric focusing method. The computed pI value (pI is greater than 5) point towards its basic character [173]. Extinction coefficient indicates the light absorption capacity, its values for all four proteins shows in table 4.1, which signifying the Tyr and Trp high concentration occurrence. Instability index results (II is less than 40) categorized that proteins is probably stable in test tube condition except 5KSB. The AI is characterized as the comparative volume of a protein taken by aliphatic side (alanine, leucine, valine and isoleucine). Increase in AI denotes increased thermostability of the globular proteins [174]. The exceptionally high AI for all proteins concludes that these proteins might be stable at wide collection of temperature [175]. The T-pI, AI, II and extinction coefficient values of three proteins that include VacA (6ODY), CagA (5X7B) and ABCG2 (6VXI) are in range. The VacA protein shows best physiochemical protperties among all four proteins.

Table 4.1: Physiochemical properties of proteins

Protein	T-pI	-R	+R	ECp	ECr	II	AI
5KSB (DQB1)	7.30	25	16	32680	32430	46.20	98.18
6VXI (ABCG2)	8.91	51	62	61030	60280	29.26	98.85

5X7B (CagA)	8.82	163	175	64750	64750	21.58	74.77
6ODY (VacA)	9.05	96	110	136155	136030	20.40	79.15

- T-pI Theoretical pI,
- -R overall negative charged amino acids (Asp + Glu),
- +R overall positive charged amino acids (Arg + Lys),
- ECp extinction coefficient (all pairs of Cys residues from cystines),
- ECr- extinction coefficient (assuming all Cys residues are reduced),
- II instability index,
- AI aliphatic index,

4.3 Validation and Evaluation of Proteins

Ramachandran plot of all four proteins were attained by using PROCHECK [176]. In the good quality model, it is expected that it would have over 90 percent amino acid residues in favoured region [177]. G score must be in between -0.5 to -1.0. If a protein does not have values that lie between in this range is not considered as a good quality model [178].

Ramachandran plot of 5KSB showed 1282 amino residues are present in the core region represented by red color that is a favorable region, 135 amino residues are present in the allowed region represented by brown color, in the allowed regions that are represented by yellow color are 5 and in the disallowed region that represented by off white are only 5 amino residues shown in Fig 4.2. The overall G-factor is -0.09 shown in Table 4.2. On the whole 99.5 percent of the amino acid

residues were originates in favorable and allowed regions, 0.5 percent in generously allowed and disallowed regions, which verify the good quality of homology model.

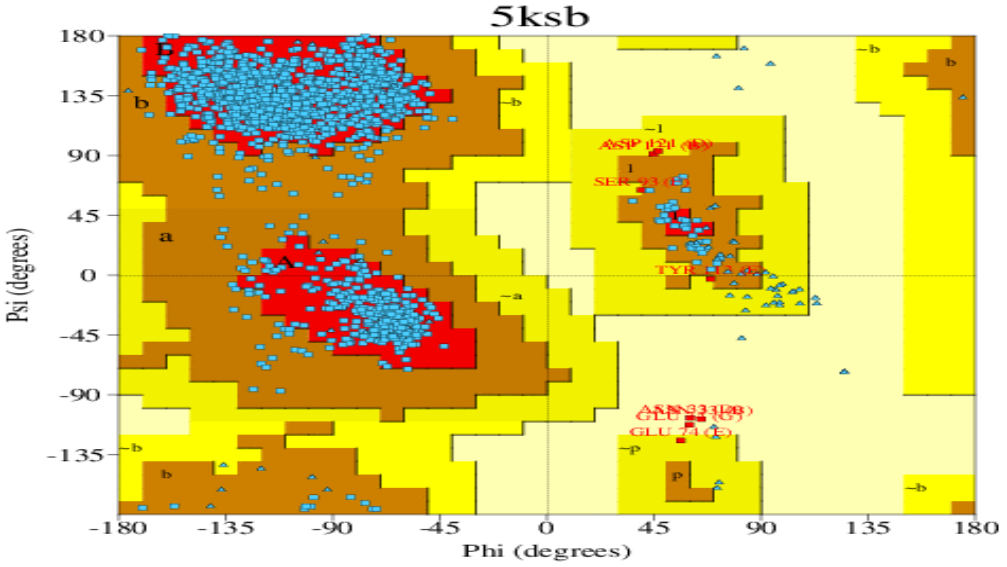

FIGURE 4.2: Validation of 5KSB protein by PROCHECK

Ramachandran plot of 6VXI shows there were 829 amino residues are present in the favorable region that is represented by red color and is core region, in allowed region there are 167 amino residues that represented by brown color and in disallowed region there are 0 amino residues (off-white) shown in Fig 4.3. The overall G-factor is -0.09 shown in Table 4.2 . On the whole 100 percent of the amino acid residues were originates in favorable and allowed regions.

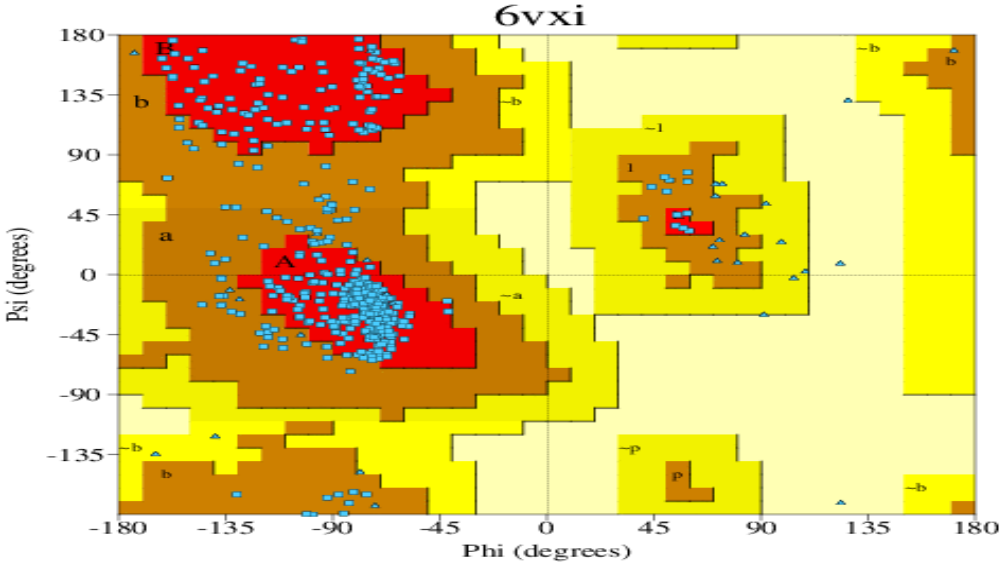

FIGURE 4.3: Validation of 6VXI protein by PROCHECK

Ramachandran plot of 5X7B shows there were 159 amino residues are present in the core region that is favorable region that are represented by red color, in the allowed region that is represented by brown color are 26 amino residues, 0 residues are present in the disallowed region that is represented by off white color shown in Fig 4.4. -0.02 is the overall G-factor shown in Table 4.2. On the whole 100 percent of the amino acid residues were originates in favorable and allowed regions.

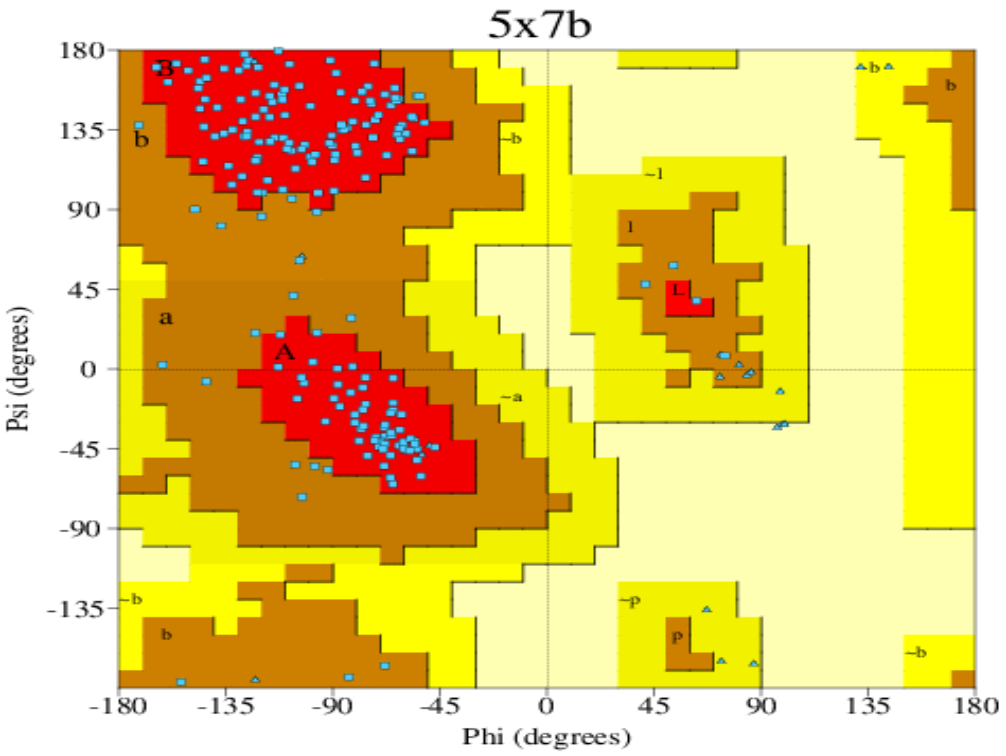

FIGURE 4.4: Validation of 5X7B protein by PROCHECK

Ramachandran plot of 6ODY shows there were 2149 amino residues are present in the favorable region that is a core region that is represented by red color, in the allowed region that is represented by brown color there are 1602 amino residues, 8 residues are present in the generously allowed region that is represented by yellow color and disallowed region is represented by disallowed region contains 3 amino residues shown in Fig 4.5. -0.02 is the overall G-factor shown in Table 4.2. On the whole 99.7 percent of the amino acid residues were originates in favorable and allowed regions, 0.3 percent in generously allowed and disallowed regions, which verify the quality of homology model.

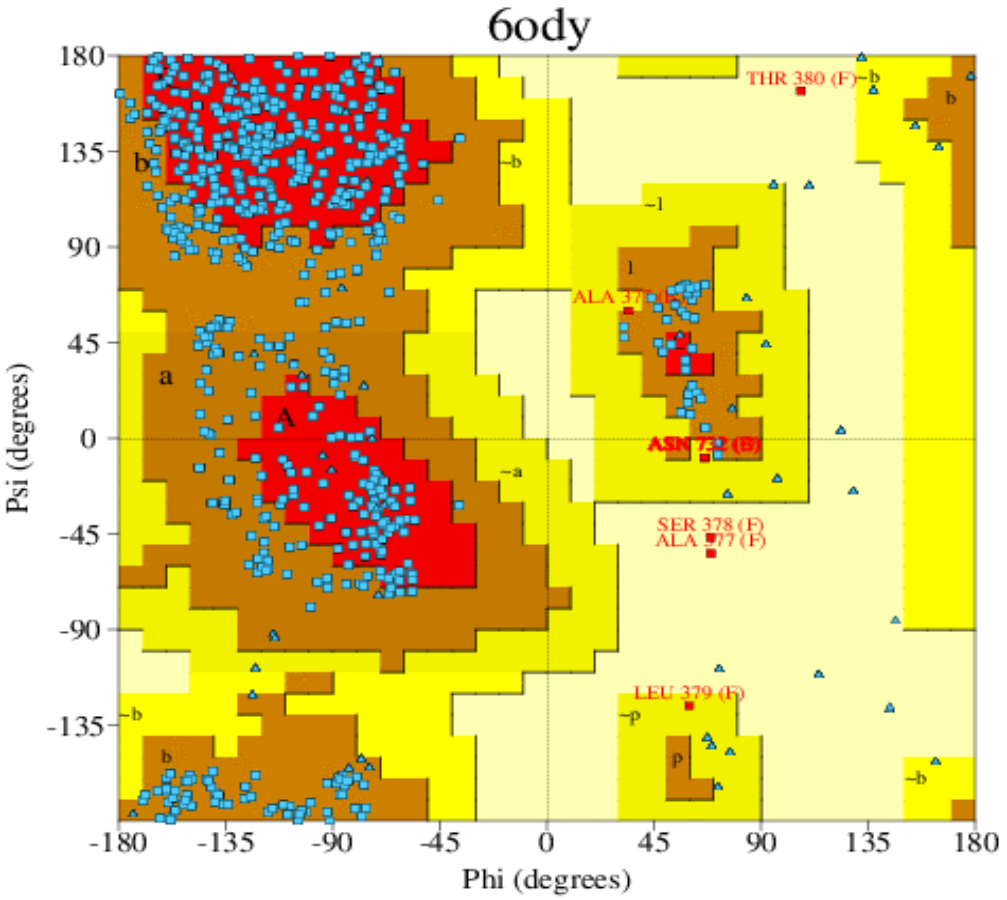

FIGURE 4.5: Validation of 6ODY protein by PROCHECK

Only 5KSB protein from all the four protein have 90 percent amino residues in the favoured regions that means it has the characteristics of good quality model.

For the examination of Z-scores and energy plots, ProSA was used. Overall quality score for a particular query structure were computed by ProSA program [179]. The Z score of 5KSB, 6VXI, 5X7B and 6ODY calculated are -5.74, -8.18, -7.81 and -6.42 respectively shown in Table 4.2 represent the overall model quality.

The Z-score of the input structure is checked by ProSA that whether it lie within the range of scores that are found for native proteins of same size. The divergence of overall energy of the structure and energy distribution due to the random conformations is quantify by Z-score. If the values are positive that will be related to the challenging and invalid parts of the query structure [180]. Figs 4.6, 4.7, 4.8, 4.9 shows the z score and energy plot of proteins.

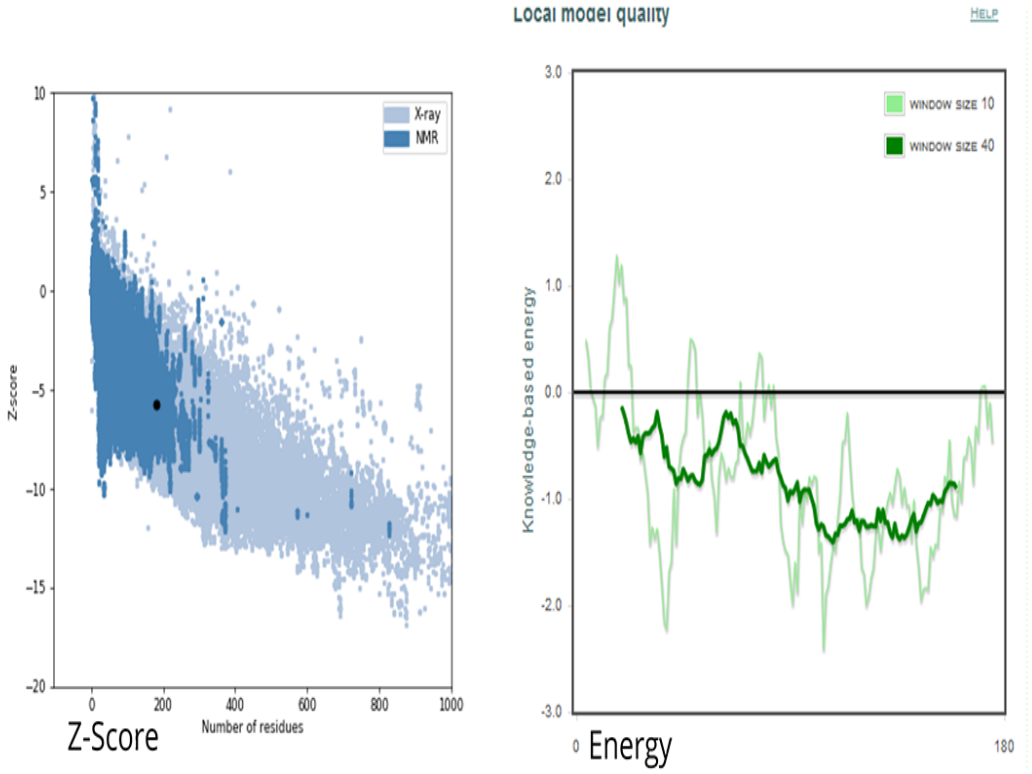

FIGURE 4.6: Z score and energy plot of protein 5KSB

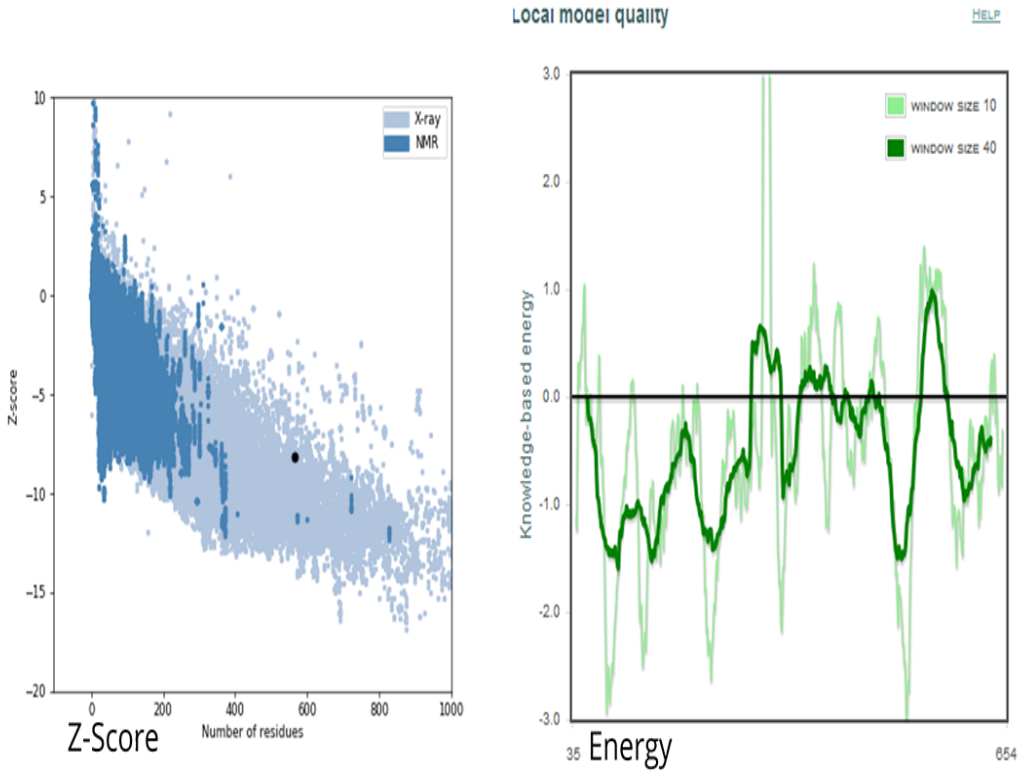

FIGURE 4.7: Z score and energy plot of protein 6VXI

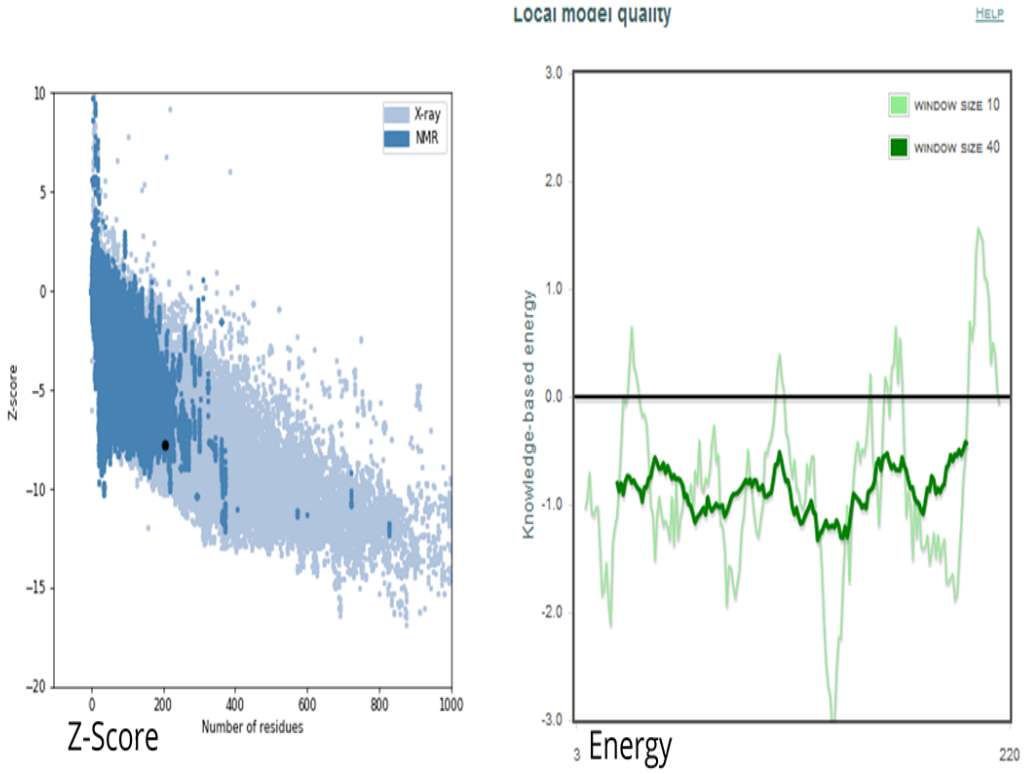

FIGURE 4.8: Z score and energy plot of protein 5X7B

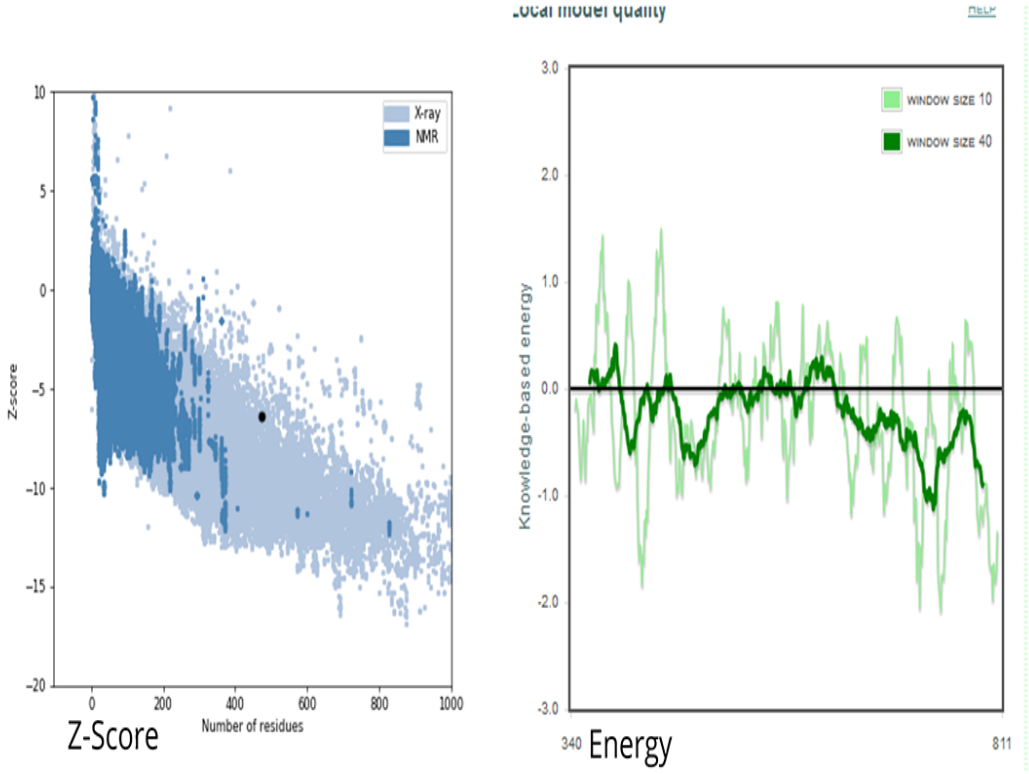

FIGURE 4.9: Z score and energy plot of protein 6ODY

Based on the z score we have best protein that is 5KSB and after that we have 6ODY, 5X7B and 6VXI respectively.

Errat showed overall quality factor of proteins, which confirmed the validity of better predicted model [181]. The overall quality factors of four proteins shown in table 4.2. Generally accepted range is greater than 50 for a high quality model [182]. Figs 4.10 and 4.11 shows the Errat (Overall quality factor of proteins).

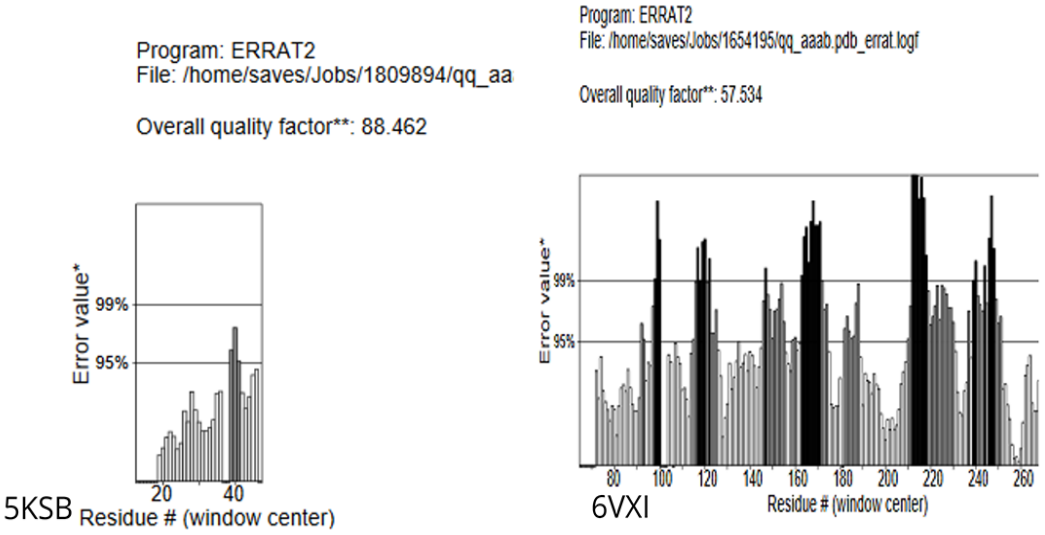

FIGURE 4.10: Errat (Overall quality factor of proteins)

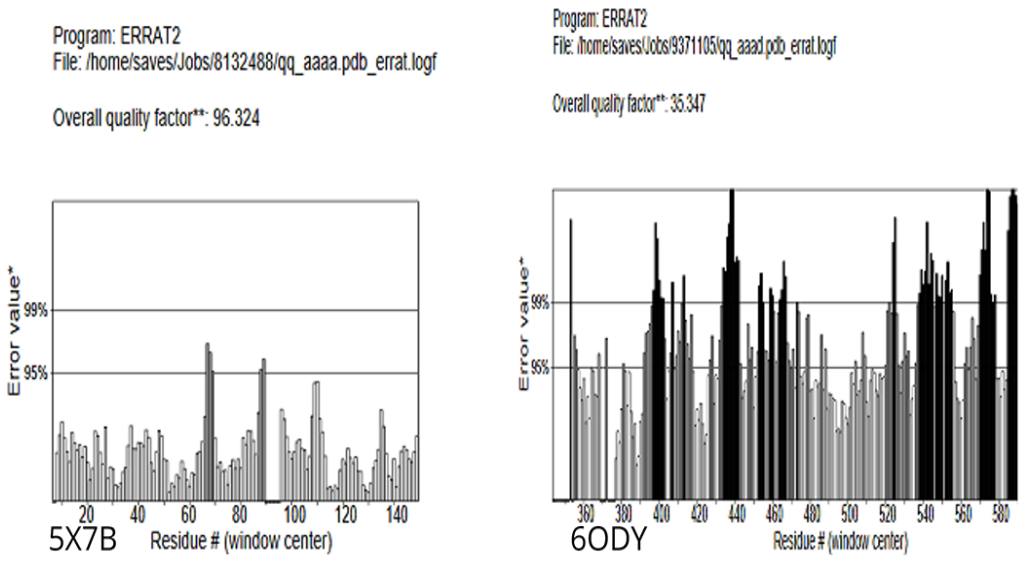

FIGURE 4.11: Errat (Overall quality factor of proteins)

TABLE 4.2: Properties of Proteins

Protein	Ramachandran plot results					ProSA	Errat
	MF	AR	GAR	DR	G score	Z-score	Overall Quality factor
5KSB (DQB1)	90	9.5	0.4	0.1	-0.09	-5.74	88.462
6VXI (ABCG2)	83.2	16.8	0	0	-0.09	-8.18	57.534
5X7B (CagA)	85.9	14.1	0	0	-0.02	-7.81	96.324
6ODY (VacA)	57.1	42.6	0.2	0.1	-0.21	-6.42	53.347

- MF - most favored,
- AR - allowed region,
- GAR - generously allowed region,
- DR - disallowed region,
- G score - Overall Average

4.4 Binding Pockets of Proteins

Before performing docking against ligands prediction of active site is requisite, which lessen the search space on receptor surface of proteins. Typically, active sites are eminent by crystal of the target proteins where ligand bound; computational methods can be used for prediction of these sites. Docking studies of all four proteins were performed on the binding pockets predicted by DOGSiteScorer server

as shown in Fig 4.12. The binding pocket must have less number of residues that acts as an interaction with the remaining protein structure and from all the other binding sites it must have highest drug score. Because highest drug score means better binding affinity between ligand and protein.

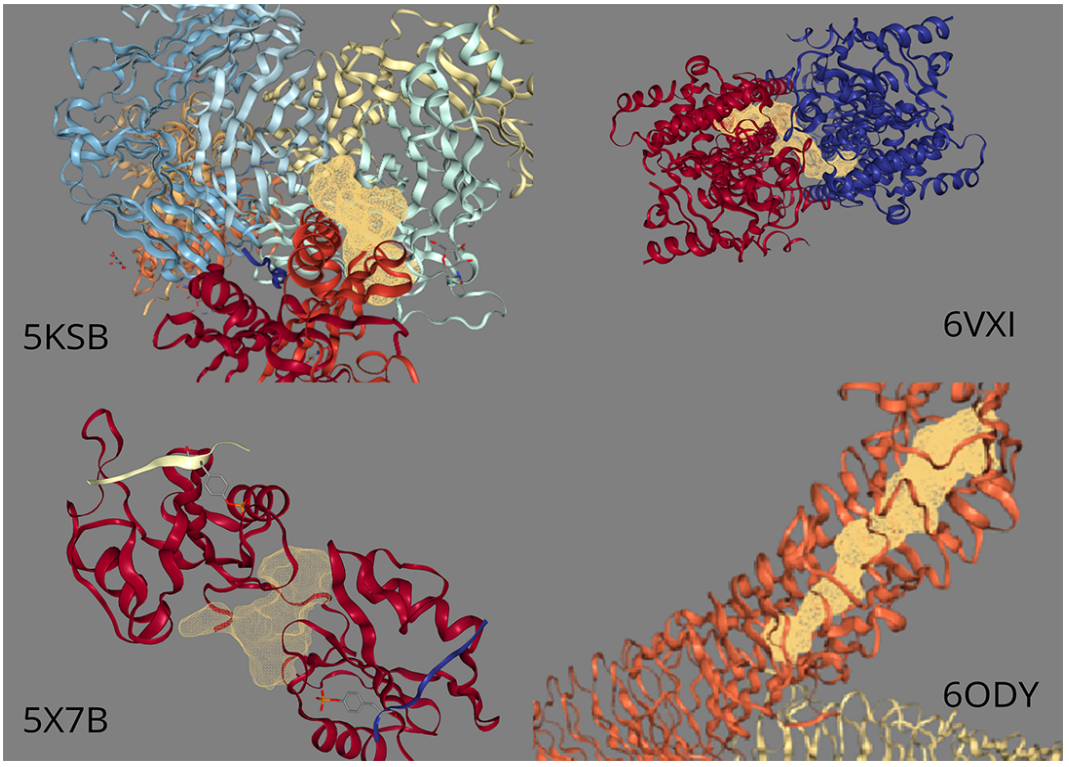

FIGURE 4.12: Binding pockets predicted by DOGSiteScorer

4.5 Molecular Docking

Molecular docking simulations were performed for the purpose of understanding the mechanisms of ulcer causing proteins inhibition by propolis compounds and to find out the binding interactions between protein pockets and the ligands. All the selected ligands were docked against all the four selected proteins that are reportedly found to be associated with the gastric and peptic ulcer.

Ligands are shown in table 4.3 along with their pubChem ID and componenets name. Ligands that show best associations with proteins on the basis of binding affinity score were selected and went for further study.

TABLE 4.3: Component names that shows best association on the basis of binding affinity.

PubChem ID	**Name**
5281672	Myricetin
5280343	Quercetin
5464078	Gamma Mangostin
4166098	Methyl Pinoresinol
5381351	Quercetin-7-O-Glucoside
44259222	Quercetin-5-O-Glucoside
5320844	Quercetin-4-O-Glucoside
92156	Beta Amyrins Acetate
10742	Syringic Acid
5316793	Pinostrobin Chalcone
5281616	Galangin
439246	Naringenin
5472440	Artepillin C
5280805	Rutin

All the resulted ligands are shown in Tables 4.4, 4.5, 4.6 and 4.7 with their respective proteins along with their S score or binding affinity and their interactions with the specific residues.

These are top 5 ligands for each protein that were selected on the basis on the S score or binding affinity. The S score is considered as the drug score. Some of ligands show strong associations with more than one protein such as Myricetin, Gama mangostin, Quercetin-5-O-glucoside.

TABLE 4.4: Selected compounds with their binding affinity and predicted hydrogen bonds for the 6VXI protein

Compound	Binding Affinity	Interactions Residue / Hydrogen Bond
Artepillin C	-11.99999	ASN 436

Gamma -mangostin	-11.60828	GLN 398 SER 440
Myricetin	-11.23474	GLN 393 GLU 446 THR 542
Rutin	-10.73596	ASN 436 GLU 446 ALA 394
Quercetin-5-O-GLUCOSIDE	-10.40701	ASN 436 THR 542

ABCG2 (6VXI) gene encodes ABCG2 protein with 655 amino acids that is a half transporter belongs to the ABCtransporters superfamily. ABCG2 protein uses energy from the ATP hydrolysis like all other proteins of this family for the transport of substrates. This protein is present in the internal lining of the brain, blood-brain barrier, ovaries, prostate, placenta, testes gastrointestinal tract and adrenal gland. It functions as a protective agent that eliminates the xenobiotics from the cells into the extracellular environment. ABCG2 is localized in the apical membrane cells of the digestive tract that confirm its protective role that limits the accumulation of harmful xenobiotics in cells/organs. If it lost its function than it will result into the development of gastric ulcer [183].

ABCG2 (6VXI) protein shows binding affinity ranges from -11.9999 to -10.40701. It shows best association with Artepillin C (-11.99999) greater than gamma mangostin (-11.60828) greater than Myricetin (-11.23474) greater than Rutin (-10.735 96) greater than Quercetin-5-O-glucoside (-10.40701). Artepillin C shows hydrogen bonding with ASN436. Gamma mangostin shows 2 hydrogen bonds with GLN398 and SER440 while myrecetin shows 3 hydrogen bonds with GLN393, GLU446 and THR542. Rutin also have 3 hydrogen bonds with ASN436, GLU446 and ALA394. Quercetin-5-O-glucoside shows two hydrogen bonds with ASN436

and THR542. Table 4.4 is showing all the compounds with their binding affinities and hydrogen bonds and Fig 4.13 is showing the 3D structures of all the interactions.

This protein is present in the internal lining of the brain, blood-brain barrier, ovaries, prostate, placenta, testes gastrointestinal tract and adrenal gland. It functions as a protective agent that eliminates the xenobiotics from the cells into the extracellular environment.

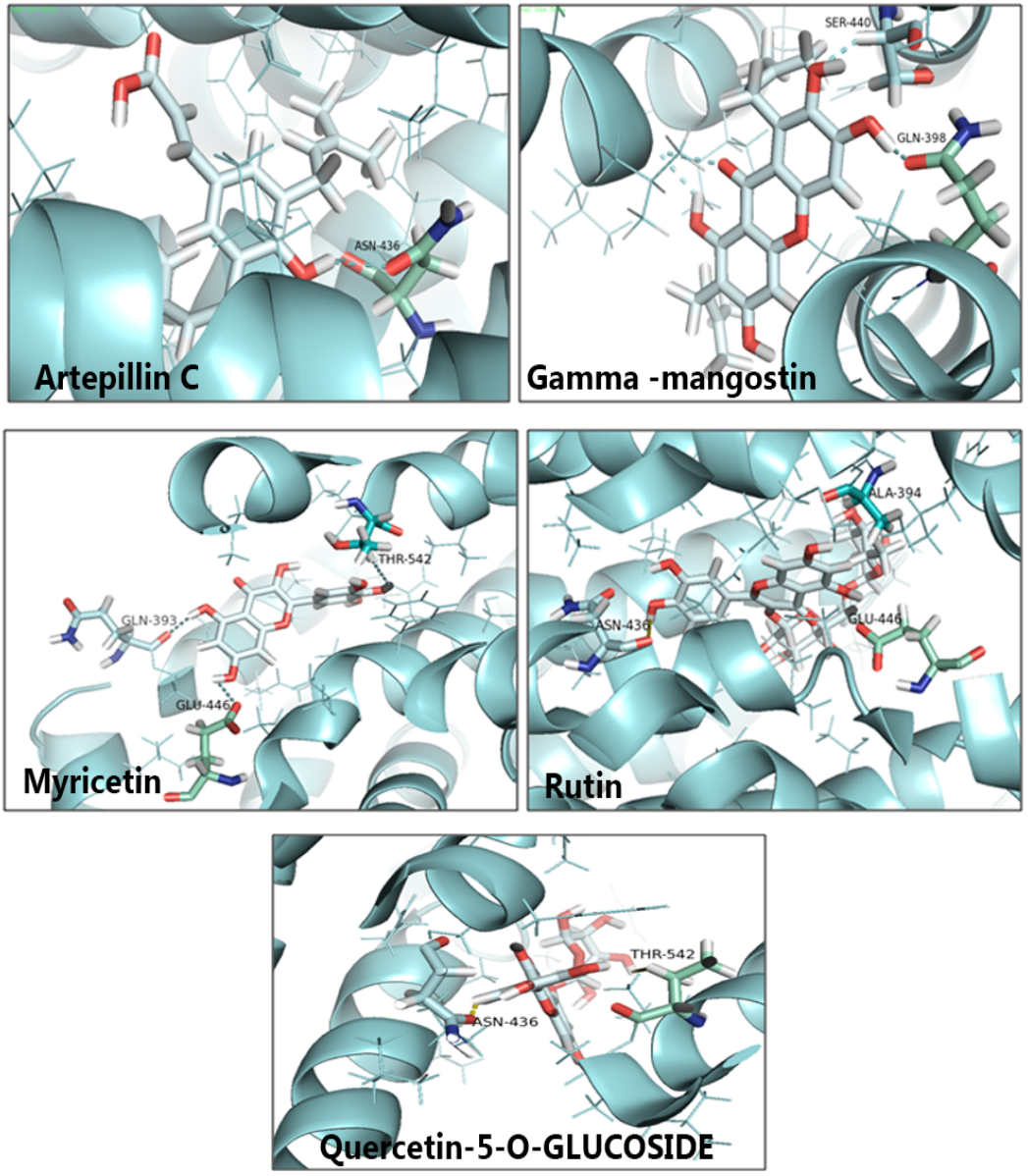

FIGURE 4.13: 3D visualize images of the selected ligands of the 6VXI that includes Artepillin C, Gamma-mangostin, Myricetin, Rutin and Quercetin-5-O-GLUCOSIDE

TABLE 4.5: Selected compounds with their binding affinity and predicted hydrogen bonds for the 6ODY protein.

Compound	Binding Affinity	Interactions Residue / Hydrogen Bond
Myricetin	-14.6582	GLU 720 THR 737 ASN 775 LYS 780
Syringic acid	-10.52881	GLU 724 LYS 774 ASN 775 LYS 780
Pinostrobin chalcone	-10.17305	LYS 723 LYS 774 ANS 775 LYS 780
Galangin	-10.16203	LYS 723 SER 779 LYS 780
Naringenin	-10.15973	LYS 723 LYS 780

VacA (60DY) is produced by the *H.pylori* and it is involved in the occurrence of gastric tissue damage and also causes massive cellular vacuolation. It secretes a VacA toxin that binds to the host cells that causes vacuolation. Vacuolation is the accumulation of large vesicles. Apart from this, it also affects mintochondrial functions [184]. VacA (60DY) protein shows binding affinity energy ranges from -14.6582 to -10.15973. It shows best associations with Myricetin (-14.6582) greater than Syringic acid (-10.52881) greater than Pinostrobin chalcone (-10.17305) greater than Glangin (10.16203) greater than Naringenin (-10.15973). Different compounds show different hydrogen bonds with different residues such

as myrecetin shows 4 hydrogen bonds with GLU 720, THR 737, ASN 775 and LYS 780. Syringic acid also shows 4 hydrogen bonds but with different residues that include GLU 724, LYS 774, ASN 775 and LYS 780. Pinostrobin chalcone mads 4 bonds that are LYS 723, LYS 774, ASN 775 and LYS 780. Glangin made 3 bonds with LYS 723, SER 779and LYS 780 while narigenin made only two hydrogen bonds with LYS 723and LYS 780. Table 4.5 is presenting all these bindings while Fig 4.14 is presenting the 3D structures of all the selected ligands.

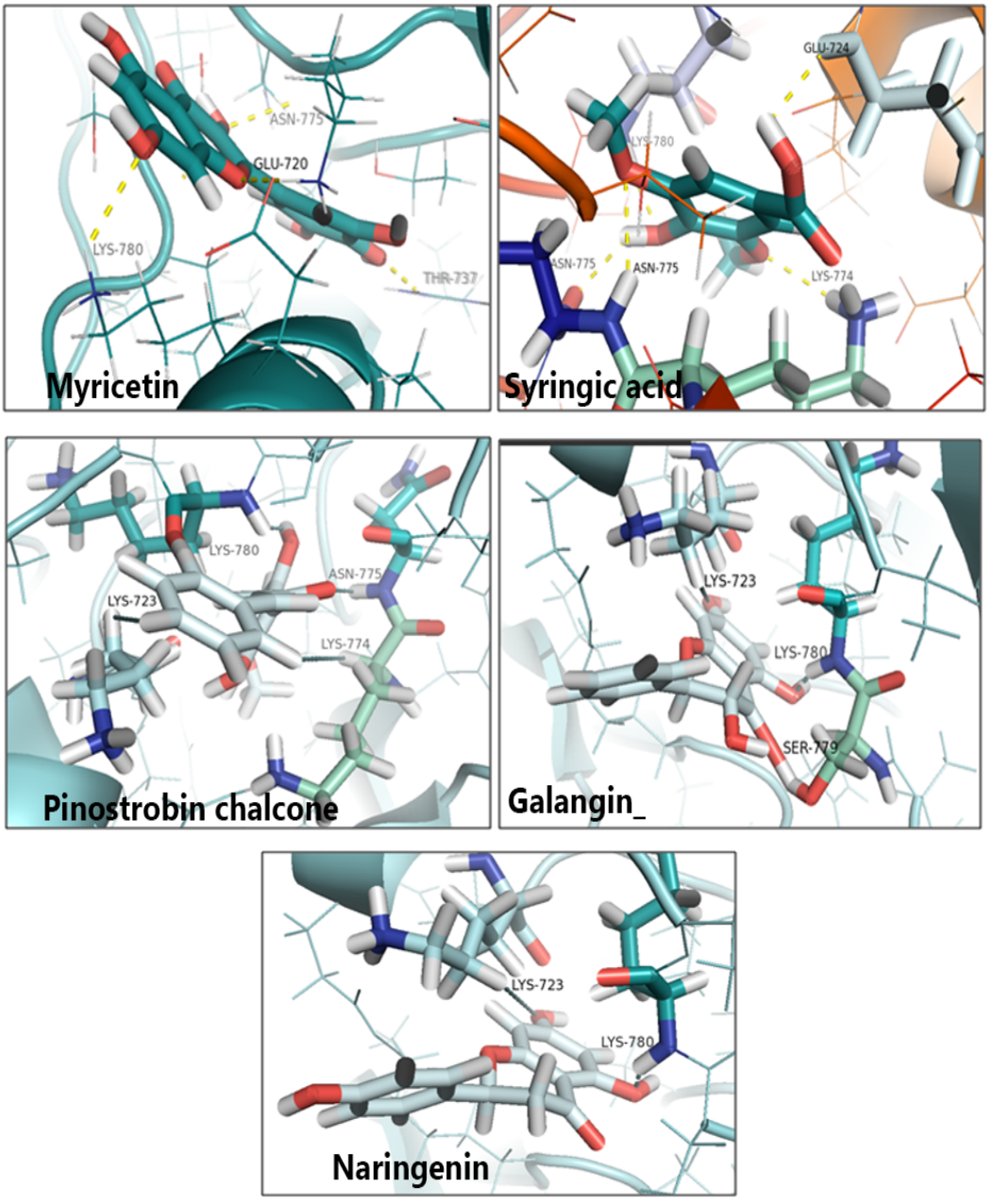

FIGURE 4.14: 3D visualize images of the selected ligands of the 6ODY thatincludes Myricetin, Syringic acid, Pinostrobin chalcone, Galangin and Naringenin

TABLE 4.6: Selected compounds with their binding affinity and predicted hydrogen bonds for the 5X7B protein.

Compound	Binding Affinity	Interactions Residue / Hydrogen Bond
Quercetin-5-O-GLUCOSIDE	-15.05985	ARG 138 GLN 141
Myricetin	-14.31787	SER 3 ASN 10
Quercetin-4-O-GLUCOSIDE	-13.52168	SER 3 HIS 8 LEU 177
Gamma -mangostin	-13.04374	ASN 10 ALA 105 THR 179
Beta-amyrins Acetate	-12.9312	SER 3

CagA (5X7B) is a major virulence factor of the *H.pylori* that is involved in the hastric pathologies. This is the strongest risk factor involved in gastric cancer. Through bacterial type IV secretion, the cagA gene-encoded CagA protein is delivered into the gastric epithelial cells where it undergoes tyrosine phosphorylation. It acts as a non-physiological scaffold that interacts with multiple signaling molecules of host.Transgenic expression has confirmed the oncogenic potential of CagA [185].

Binding affinity of the protein CagA (5X7B) with the best 5 ligands ranges from-15.05985to-12.9312. According to binding affinities, the compounds ranked as Quercetin-5-O-glucoside (-15.05985) greater than Myricetin (-14.31787) greater than Quercetin-4-O-glucoside (-13.52168) greater than Gamma mangostin (-13.043 74) greater than Beta-amyrins acetate (-12.9312). Quercetin-5-O-glucoside made 2 hydrogen bonds with ARG 138 and GLN 141. Myricetin form 2 hydrogen bonds with residues Ser 3 and ASN 10. Quercetin-4-O-glucoside form hydrogen bonds with SER 3, HIS 8 and LEU 177. Gamma mangostin binds with 3 residues that

include ASN 10, ALA 105 and THR179 while beta-amyrins acetate only binds with the SER3.

Table 4.6 is presenting all these bindings while Fig 4.15 is presenting the 3D structures of all the selected ligands.

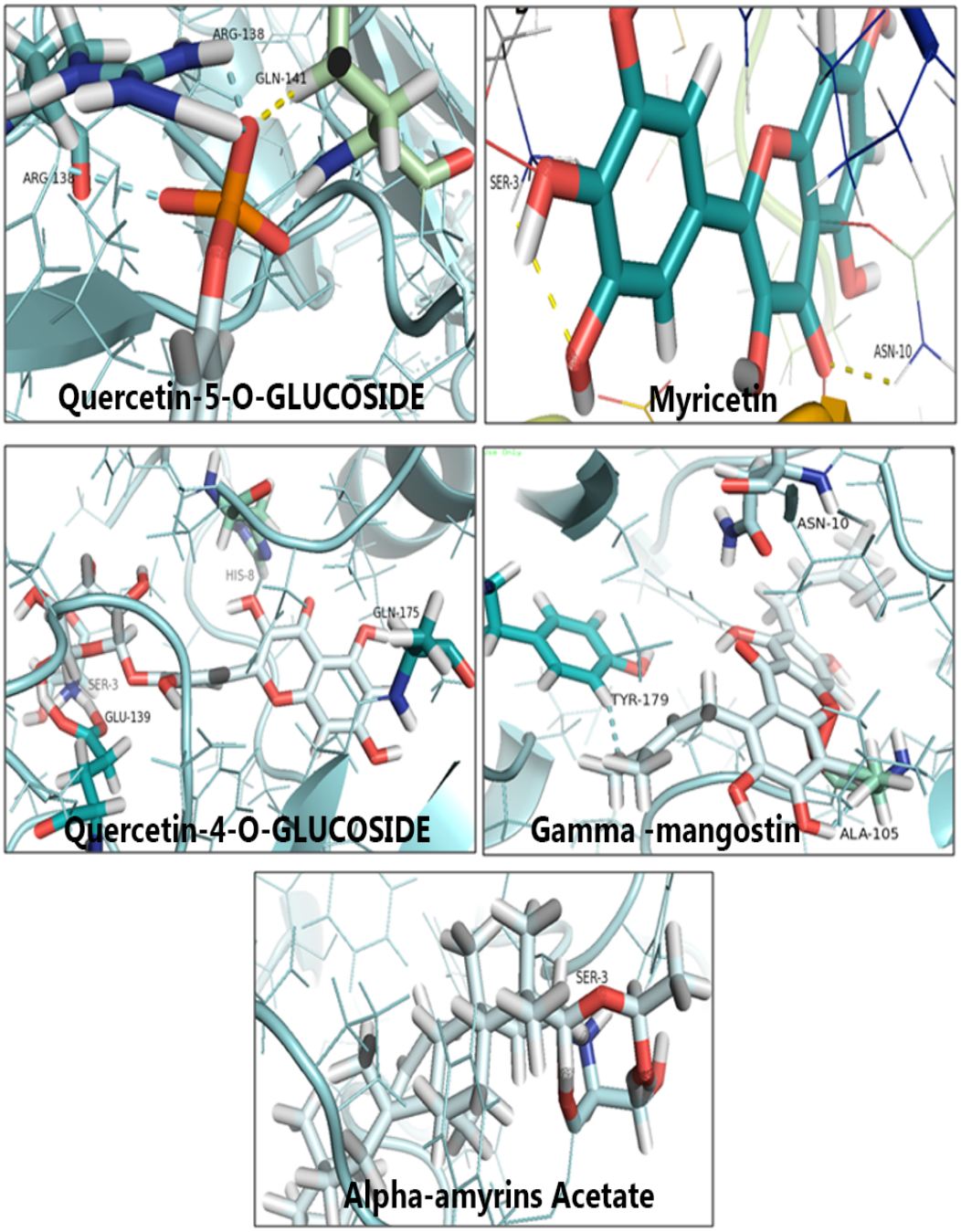

FIGURE 4.15: 3D visualize images of the selected ligands of the 5X7B protein that includes Quercetin-5-O-glucoside, Myricetin, Quercetin-4-O-glucoside, Gamma mangostin and Beta- amyrins acetate

When a ligand binds with the protein it will inhibits or decrease its function by blocking the significant amino acid residues present at the pocket or drug site.

TABLE 4.7: Selected compounds with their binding affinity and predicted hydrogen bonds for the 5KSB protein.

Compound	Binding Affinity	Interactions Residue / Hydrogen Bond
Myricetin	-14.31514	ARG 70 THR 115 ARG 26
Quercetin	-13.5425	ARG 26 THR 115 GLU 66
Gamma -mangostin	-12.74535	GLN 6
Methyl pinoresinol	-12.15555	GLN 6
Quercetin-7-O-GLUCOSIDE	-11.25713	GLY 47 PRO 120 VAL 168 THR 185

HLA-DQBI (5KSB) belongs to the MHC II class that is involved in the immune system function. A protein binds with another protein known as HLA-DQBA1 that is produced by another MHC class II. A functional protein complex is formed that is known as antigen-binding DQ heterodimer and it displays foreign peptides to the immune system that trigger the immune response of body [186].

For protein HLA-DQBI (5KSB), binding affinity ranges from -15.05985 to -12.9321. It shows best associations with Myricetin (-14.31514) is greater than Quercetin (-13.5425) is greater than Gamma mangostin (-12.74535) is greater than Methyl pinoresinol (-12.15555) is greater than Quercetin-7-O-Glucoside (-11.25713).

Myricetin binds with the ARG 70, THR 115 and ARG 26. Quercetin form 3 hydrogen bonds with the residues ARG 26, THR 115 and GLU 66. Gamma

mangostin and methyl pinoresinol both forms only one hydrogen bond and that is with residue GLN6. While Quercetin-7-O-Glucoside binds with 4 residues that include GLY 47, PRO 120, VAL 168 and THR 185. Table 4.7 is presenting all these bindings while Fig 4.16 is presenting the 3D structures of all the selected ligands.

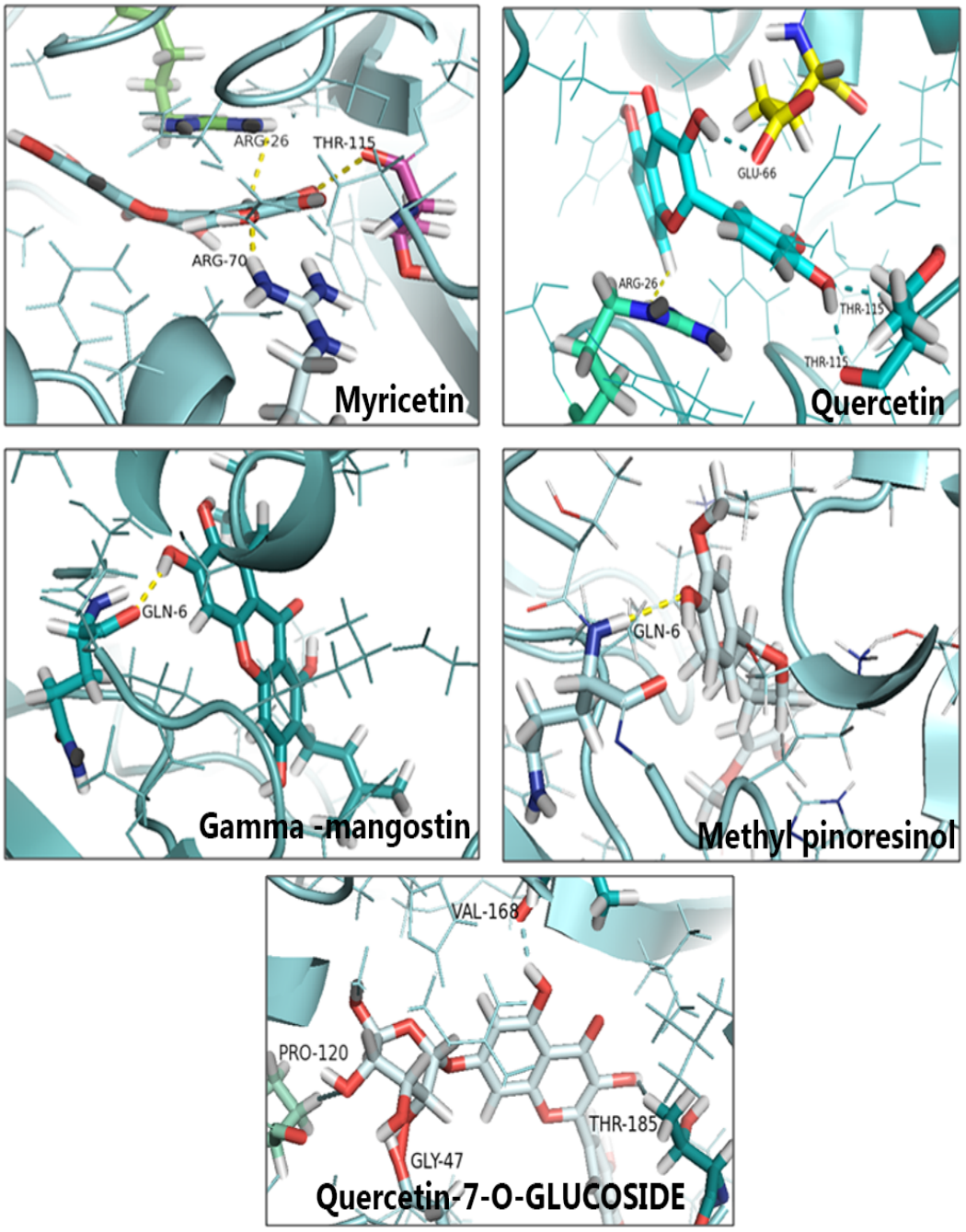

FIGURE 4.16: 3D visualize images of the selected ligands of the 5KSB protein that includes Myricetin, Quercetin, Gamma mangostin, Methyl pinoresinol and Quercetin-7-O-GLUCOSIDE

4.6 Pharmacokinetics and Toxicological Properties

In the drug development, pharmacokinetic properties (PKs) are considered as very important because they helps to determine the characteristics of the successful compounds that can be successful oral drugs as they should be completely absorbed from the gastrointestinal tract, proper distribution to the site of action, done a proper metabolism and should be eliminated from the body in a suitable manner that does not result into a harmful effect. Drugs that fail the PKs during a clinical trial are failed to commercialize. These properties depend upon the chemical descriptors of the molecules.

There are multiple computational approaches that are being used to determine the absorption, metabolism, distribution, excretion, and toxicity of the new compounds that have the potential of becoming drugs. Pharmacokinetics properties are determined by the Molinspiration online toolkit while ADMET is used for checking toxicity profiling of the selected 14 compounds after the docking simulation that lead towards further scrutiny. Pharmacokinetic properties are determined on the basis of the Lipinkis rule of five [165].

According to this rule, all the potential oral drug candidates must have molecular weight less than 500 amu, value of LogP is less than or equal to 5, hydrogen-bond donor sites must be five or less than five, and hydrogen-bond acceptor sites should be ten or less than ten [166].

Based on the Vebers rule, the number of rotatable bonds in the orally bioavailable drugs should be less than or equal to 10 and topological polar surface area (TPSA) value should be less and or equal to the 140 that is considered as a good descriptor for suitable drugs as it is involved in the passive molecular drug transport through membranes. ABS percentage should be ranged from 72.01 - 78.99% [187]. If any drug, is violating any of the above given rule than it will have problems regarding bioavailability. Table 4.8 is presenting the results of physiochemical properties of

the compounds. The compounds are tested against both Lipinkis rule of 5 and vebers rule.

TABLE 4.8: Physiochemical properties of the compounds good for oral bioavailability.

Compound	% ABS	TPSA	MW	LogP	HBD	HBA	n-ROTB	L
Result	72.01-78.99%	<= 140	< 500	<= 5	<5	<10	<=10	2
Quercetin-5-O-glucoside	72.62	210	464	-0.36	8	12	4	1
Myricetin	52.29	151	318	1.39	6	8	1	2
Quercetin-4-O-glucoside	72.62	210	464	-0.33	8	12	4	1
Gamma mangostin	38.33	111	396	6.05	4	6	4	0
Beta-amyrins Acetate	9.07	26	468	8.55	0	2	2	0
Quercetin	45.31	131	302	1.68	5	7	1	0
Methyl pinoresinol	26.69	77	358	2.59	2	4	6	2
Quercetin-7-O-glucoside	72.62	210	463	-0.10	8	12	4	0
Syringic acid	26.22	76	198	1.20	2	5	3	2

Pinostr-obin chalcone	23.03	66	270	3.70	2	4	4	0
Galangin	31.35	90	270	2.65	3	5	1	0
Naringenin	30.01	86	272	2.12	3	5	1	0
Artepillin C	19.785	57	300	5.26	2	3	6	1
Rutin	92.95	269	610	-1.06	10	16	6	3

Some compounds were screened out after these tests that do not follow these both rules. Quercetin-5-O-glucoside, Quercetin-4-O-glucoside, Quercetin-7-O-glucoside and Rutin were deleted because their TPSA is greater than 140 i.e. 210.50, HBD is also greater than 5 i.e. 8, HBA is also greater than 10 i.e. 12 and L violation is also greater than 1 i.e. 2. Rutin was also removed from the list because its TPSA is greater than 140 i.e. 269.43, molecular weight is greater than 500 i.e. 610.52, HBD is greater than 5 i.e. 10, HBA is greater than 10 i.e. 16 and L violation is also greater than 1 i.e. 3. No compound violating any of the part if rule can be promoted further for the next step of drug formation because these all are the things that make a oral biovaialable drug perfect without any side effects.

Toxicological properties are a major concern for all the oral bioavailable drug compounds and admetSAR server was being used for the prediction of the toxicological properties of all the screened out compounds that does not fulfill the physiochemical properties. The toxicological properties are shown in Table 4.9.

TABLE 4.9: Toxicological properties of the compound

Compounds	Parameters			
	AmesToxicity	Carcinogens	Acute oral Toxicity	hERG

Myricetin	Non-AMES toxic	Non-Carcinogens	II	Weak Inhibitor
Gamma - mangostin	Ames Toxic	Non-Carcinogens	III	Weak Inhibitor
Beta-amyrins Acetate	Non-AMES toxic	Non-Carcinogens	III	Weak Inhibitor
Quercetin	Non-AMES toxic	Non-Carcinogens	II	Weak Inhibitor
Methyl pinoresinol	Non-AMES toxic	Non-Carcinogens	III	Weak Inhibitor
Syringic acid	Non-AMES toxic	Non-Carcinogens	II	Weak Inhibitor
Pinostrobin chalcone	Non-AMES toxic	Non-Carcinogens	III	Weak Inhibitor
Galangin	Non-AMES toxic	Non-Carcinogens	II	Weak Inhibitor
Naringenin	Non-AMES toxic	Non-Carcinogens	II	Weak Inhibitor
Artepillin C	Non-AMES toxic	Non-Carcinogens	III	Weak Inhibitor

From all these compounds gamma mangostin compounds was screened out because it shows Ames toxicity. The compounds that show the presence of Ames toxicity have 90% chances of becoming a carcinogen. Due to these reasons gamma-mangostin was removed from the potential drug targets for gastric ulcer. After toxicity studies there were nine ligands or compounds that showed best pharmacokinetic profile minimum toxicity risks. These properties are very important because they show the credibility of any drug and how it will affect the body can be find out through these drugs.

4.7 Lead Identification

The docking score and binding interactions of all ligands have been analyzed. Out of 40 ligands; top 20 ligands which showed high S-score, hydrogen bonding interaction were selected for ADME properties prediction and toxicity risk investigation through admetSAR server was done to find out its effectiveness. The nine ligands (Myricetin , Naringenin, Quercetin [188] Beta-amyrins Acetate [189], Methyl pinoresinol [190], Syringic acid, Pinostrobin chalcone [191], Galangin, and Artepillin C [192]) has been selected as lead compounds as it has been identified as the most active from all molecules shown highest docking score, strong hydrogen and interactions with the target receptors. These compounds fulfill all the requirements that are required for an oral bioavailable drug.

Chapter 5

Conclusion and Future Direction

The purpose of this project was to find out the competent drug targets for the gastric ulcer that include two human genes i.e. 5ksb and 5x7b and two bacterial genes i.e. CagA and VacA. Physiochemical properties of each protein were checked and validation was performed by Ramachandran plot and Z score. 40 propolis compounds mined from the literature were docked for each protein and based on binding affinity 5 best compounds were selected for each protein. Binding affinity and the bonding between ligand and protein is directly proportional to each other. Due to this reason compounds with the highest binding affinity were selected..

After the docking results, on the selected compounds, physiochemical properties analysis was performed that is based on the Lipinskis rule of five and Vebers rule. After this, on the remaining targets that passed the Lipinskis rule of five and Vebers rule, the toxicological properties analysis was done and after that one compound was further removed from the candidates of the drugs for the gastric ulcer that shows the presence of toxicity. All these drug targets were tested against the pharmacokinetics properties.

The remaining compounds that include Myricetin, Beta amyrins Acetate, Querc etin, Methyl pinoresinol, Syringic acid, Pinostrobin chalcone, Glangin, Naringenin and Artepillin C are considered as the competent for the oral bioavailable drugs for the gastric ulcer. These are the compounds that pass each test and rule that is

important for the formation of any drug such as absorption, toxicity, metabolism and excretion.

This in-silico study will help to minimize the effort and time for developing drugs that can be further tested on animals or humans. All these lead targets show promising results in-silico and fulfill all requirements that an orally bioavailable drug should have. This will not only give us the best therapeutic techniques but also helps us to develop new drugs that can be used for human betterment. All the resulted compounds must be validated on animal models. After successful application on animal models, selected propolis compounds must be formulated as a drug and tested for clinical trials.

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