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In-Silico Evaluation of Anti-Inflammatory Components of Propolis Against SARS-CoV2

by

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A thesis submitted in partial fulfillment for the
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I dedicate this thesis to the best teacher of the world which is without any doubt The Prophet Muhammad Peace Be Upon Him. After that this thesis is dedicated to my teachers, family and friends

CERTIFICATE OF APPROVAL

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Abstract

Propolis is known as bee glue and it is a resinous mixture in nature. It has been used for treating different diseases for a long time. The need for the exploration of the natural drugs is increasing due to many reasons such as drug resistance, emergence of new diseases, new drug targets and adverse drug reactions (ADR). COVID-19 is a deadly disease and it effects the respiratory system of the diseased person. This epidemic has caused the situation of chaos in the whole world. Genes causing inflammation in the humans, under the effect of COVID-19, were retrieved from COREMINE and further validated manually from the literature and related proteins were also retrieved, based on threshold 0.01, applied on COREMINE tool, two proteins were selected including ACE2 and TMPRSS2 and their PDB structures were retrieved from PDB. Similarly, propolis compounds were also retrieved from literature based on thir anti-inflammatory activity. The study tends to explore the compounds which could be used to lower the inflammatory response under the effect of COVID-19. COREMINE is a medical based database which helps in searching, updating and sharing the medical information. Physiochemical properties of retrieved proteins were investigated with the help of EX-PASSY PROTPARAM in order to make sure that the proteins were stable enough for the study. Furthermore, the validation of proteins was done with the help of PROCHECK and PROSA by generating Ramachandran plot and analyzing energy distribution of the selected genes respectively, to make sure that the proteins were good enough for the docking purpose. Moreover, the binding pockets of the proteins were located using DOGSiteScorer to see if their structure is complete or not. The molecular docking was performed with the help of AutoDock, in addition their pharmacokinetics and toxicological properties were also tested by Molinspiration and ADMET. The compounds were tested against the Lipinkis and Vebers rule to check if they are good enough to be used orally. The physiochemical properties of protein showed that the both the structures were stable for the test as they showed the instability index to be less than 45 and the theoretical pI showed the results to be greater than 5. Ramachandran plot showed that ACE2 had 93% amino acid residues in the favored region and TMPRSS2 had 87.9% amino acid

residues in the favored region. The Z-score of both the proteins were less than -9 which shows that the proteins were a good selection for the analysis. The vina score of ACE2 showed best result with acacetin having score -7.9 and in case of TMPRSS2 again acacetin showed the best result as -7.9 as well. All the compounds showed very good ADMET result. Based on the results of the test, Caffeic Acid, Chyrsin and Acacetin showed that they had the potential to be taken orally. These three compounds can be validated on the animal models to provide new cure for COVID-19.

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Abbreviations

ACE2	Angiotensin-converting Enzyme 2
ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
APE	Aqueous Propolis Extract
CAPE	Caffeic Acid Phenethylester
EEP	Ethanolic Extract of Propolis
ExPASy	Expert Protein Analysis System
HSCCC	High-Speed Counter current Chromatograph
H_2O_2	Hydrogen Peroxide
MERS CoV-2	Middle East Respiratory Syndrome Voronavirus 2
NFAT	Nuclear Factor of Activated T-cells
NO	Nitric Oxide
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SARS CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SSC	Squamous Cell Carcinoma
TMPRSS2	Transmembrane Serine Protease 2
TPSA	Topological Polar Surface Area

Chapter 1

Introduction

The name of "Propolis" originates from the word that was used by Greek philosopher Aristotle 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 is glue that is produced by the bee. This glue is resinous, gummy and a balsamic material that bees collect from the flowers. The scientific name of the bee is *Apis mellifera L.* that belongs to family named as *Apidae* and its genus name is *Apis*. 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's composition depends on the botanical region from which it is obtained. Bee propolis helps in the maintenance of the homeostasis, reduction of the vibration, maintenance of the air flow, helps in the prevention of the putrefaction and also from the squatter as well [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. The chemical diversity of the propolis has played the core role 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 to 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].

From at least 300 BC propolis is being known for its importance in medicine globally. Bee was considered as Holy material for the Egyptians and it has been used for the purpose of mummifying the corpses and as an antibiotic. It was considered as a God Jupiter that was converted into the Bee by the lady Mellisa for the Romans and it was used to cure the skin lesions [5]. It is considered as a God Jupiter that is converted into the Bee by the lady *Mellisa* for the Romans and it is used to cure the skin lesions. Greeks used propolis for its healing qualities and it has been used for the tissue regeneration and wound healing in the Boer war. It is still used as a medicine for the treatment of wounds and burns by the people in Balkan States. They also use it for the sore throat and stomach ulcer [6]. The ethanol that is being extracted from the propolis is being known for its anti-inflammatory effects for hundreds of years [7]. It is also being used as an immuno-modulatory agent for centuries [8]. From the 12^{th} century, bee propolis is being used as a medicine for the diseases of mouth, infections of throat and for the dental caries [9]. 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 [6, 10]. In recent times it is gaining popularity due to its pharmacological and phytochemistry properties. As the composition of the Bee propolis depends on many factors but there are 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 verified on various pathogens causing community acquired infections in organisms, specifically humans. Beside the well-known pathogens, confrontation has also been seemed in opportunistic microorganisms [12]. Propolis is moderately non-poisonous and shows an exten-

sive variety of antimicrobial activities against variety of microorganisms, parasites, and infectious agents [13]. Other organic and pharmacological properties have additionally been investigated for propolis. The therapeutic and antimicrobial properties of propolis have been generally revealed and have a long history [14]. 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 [15]. In recent studies propolis has been shown to play some role in treatment for cold disorder (upper respiratory tract infection, influenza and common cold). Due to its dermatological properties it also has been used in wound heal up, treatment of burns, genital is, acne, neurodermatitis and herpes simplex infecetions. Due to its antimicrobial, antitumor, anti-inflammatory, antioxidant and immunomodulatory activities it is being used in complementary medicines [16].

It is likewise utilized in toothpastes and mouth freshener and to treat gum disease and stomach. It is broadly utilized in beauty care products and in human being nourishment's 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 it is antioxidant, antiviral and antimicrobial characteristics, its broadly utilized in human being, medication of chemical, pharmaceutical and beauty care product [17].

A deadly virus known as coronavirus is creating COVID-19, a global pandemic designated by the World Health Organization. Various diseases of the coronavirus family, which is a wide family of viruses, infect birds and mammals [18]. Bats, birds, snakes, and other mammals are all carriers of the virus. The corona virus is made up of a non-living DNA protein from the orthocoronavirina subfamily, which belongs to the Coronaviridae family and the order Nedoviruson. The corona virus is a closed virus with a helical nucleus capsid balance and a trapped positive RNA genome [19]. Coronaviruses are spherical viruses with genomes ranging from 27 to 34 kb in size. It is the world's largest virus, by far. The name corona virus comes from the Latin word corona, which means "crown" or "halo" because of their unusual appearance. Because their level is covered in the club size spike protein, it looks like a crown or solar corona when seen under electron microscopy [20].

The virus infects humans through a respiratory infection. Infections that are typically mild yet have the potential to be lethal. The first vaccinations to protect against coronavirus-related severe disease are now available. Corona virus first arose in persons who ate fish in the entire cell market in China's Wuhan province. The Chinese health center authorities reported in November 2019 that the infection with the reported virus had indicated that persons experienced symptoms similar to the corona virus. [21].

According to recent research, the coronavirus family has been separated into two groups: SARS-CoV-2 and MERS-CoV-2. The Life cycle / Incubation period of COVID-19 is broken down into about three steps. The incubation period occurs with and without a detectable virus in stage one, which is asymptomatic. The virus is present in the second stage, which is known as the non-severe symptomatic period. The third stage has a symptoms of acute respiratory distress and a high viral burden. Because the Corona virus was a new virus to the human immune system when it first disseminated, there was no natural immunity against it at the time. This makes the general public more susceptible to this virus and also this virus creates a sense of havoc in the general public. As a result, the virus has a significant possibility of spreading quickly. The virus infects epithelial cells, resulting in mild to severe chest discomfort, tracheitis, bronchitis, and rhinorrhea, as well as the need for a ventilator in severe cases. In addition, in the severe cases the viral load also increases and the patients get the severest symptoms and this causes the major problems for the doctors to manage the symptoms. Doctors also have the concerns about that of the shortage of the ventilators and shortage of hospitals in the present era [22].

1.1 Problem Statement

As an apicultural product, propolis contains a wide range of biological qualities. Propolis has shown promise in the treatment of a variety of illnesses and pathological situations. In light of the increased interest in natural products as medications, it is necessary to investigate the anti-inflammatory role of propolis in humans and

to develop innovative and effective ways that will aid in the improvement of already existing COVID-19 treatments in clinical practice.

1.2 Aims and Objectives

Due to its therapeutic qualities, which include anti-inflammatory properties that have been proven by several scientific research, we attempted to conduct an in silico assessment of propolis to identify their effect on the COVID-19. For the evaluation of the anti-inflammatory properties by doing the molecular modelling.

- To perform molecular docking for exploring the interactions of propolis with particular protein targets involved in inflammation under the influence of COVID-19.
- To anticipate the pharmacokinetics and toxicological features of the selected compounds for oral bioavailable medications, the believability of the compounds is determined.

Chapter 2

Literature Review

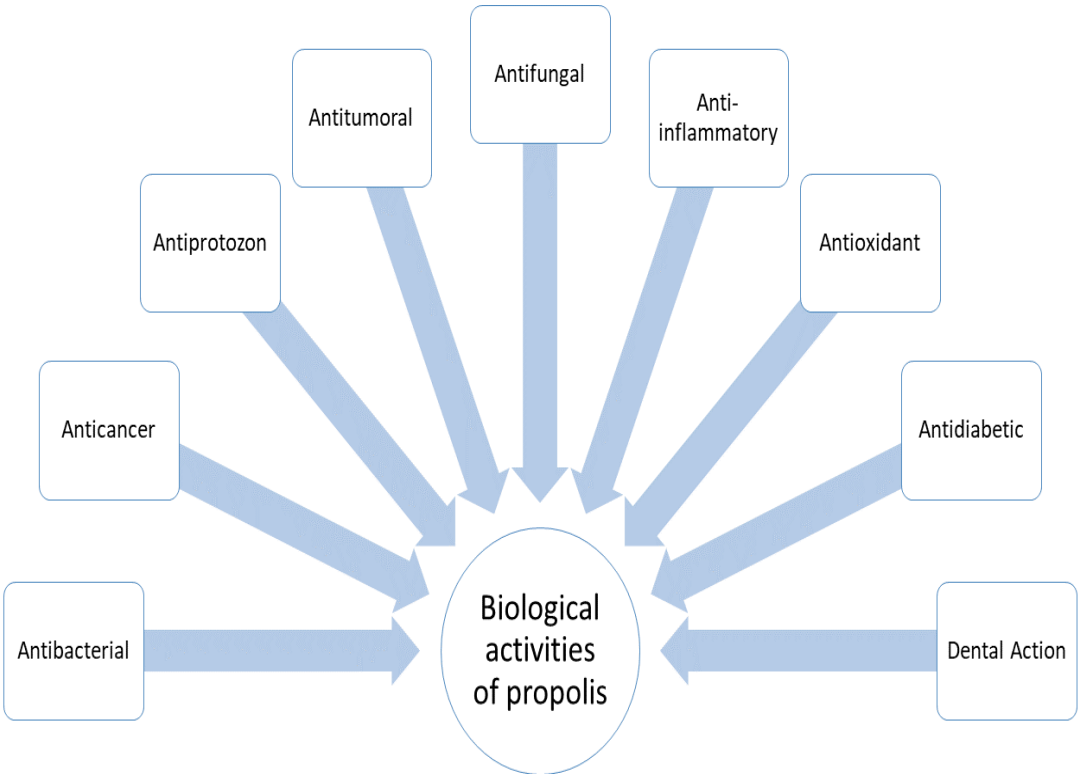

FIGURE 2.1: Biological activities of propolis

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 [23]. Ancient

Egyptians use the bees that make the propolis and use the propolis in many ailments and do the ornaments [24]. 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 [25]. By transporting dead bees from the hive, the bees protect their hive from the infections that can be caused by the decomposition of the carcass. Dervici et al. in 1960s shows the importance of the propolis I the reduction of the bacterial infections within the hive [26]. According to the ancient Jews, propolis is being used as the medicine and they use the word "tzori" that is Hebrew word [27]. 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 poplars that includes *P. nigra*, *P. balsamifera* and *P. gileaddensis* [28]. 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 day, 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 [29].

In past, propolis is used in conventional drug. Now, the propolis is used as a modern treatment methods as well ans scientific society is doing a lot of research in this regard. Solely rare documents about use of propolis are available. Some sources as of the twelfth century defined 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 [30]. 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". [31].

2.1.1 Propolis in Early Modern Times

The interest of European people developed into the propolis after the Renaissance theory of the ad fonts and it also brought the interest of the people in the teaching and medicine practices including the propolis. By the regard of the medical humanists, forgotten and old medical remedies were rediscovered and start to be used again. 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, bruises and squats [32]. During the seventeenth century, the propolis was included in the pharmacopoeias in England and it is used as a healing ointment and also the research on its more medical benefits starts as well [33]. 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 tempers the heat of wounds. It is used to dry up the milk of women's breasts when they have weaned their children" [34].

Another book that was the Universal Herbal and was published in 1824 and known as the "Populus Nigra: Black Poplar Tree". In this book he said the young leaves of the plants acts as excellent ingredients for healing the hard and painful swellings. Moreover, the book also states that the propolis is also having a lot of importance in healing the wounds with great efficacy, it not only have this importance but many more as well. For plants,The buds of the white and black poplar are being pressed with the fingers that give a balsamic resinous substance that is known as a propolis and these buds looks very pleasant in the spring and the propolis is being extracted by the spirits of wine and it also smells as a storax [35].

With these composition propolis have a lot of medical importances, like anti-ulcer, anti-tumor, anti-inflammation, dental actions, gynecological care, dermatological health, gastrointestinal care, neurodegenerative and aging disease and many more.

Propolis is a sticky material and it is found in the bee hive, the bee make it to overcome the threat of any foreign material or pathogen ,like virus, bacteria, fungi spores, to enter into their hive and destroy the honey present in the bee hive.

2.2 Properties of Propolis

2.2.1 Melting Point

At 25 degree celsius to 45 degree celcius the propolis remains 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 degree celsius. At 60 degree celsius to 70 degree celsius, propolis becomes liquid but the melting point of some samples can be as high as 100 degree celsius [36].

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. Some of the most common solvents that are used for the extractions of propolis include water, ethanol, methanol, chloroform, ether, acetone and dichloromethane [36]. The inert materials those are soluble in water or alcohol is removed for the preservation of the desired compound. The chemical composition of propolis depends on the geographical regions and method used for extraction. Due to this reason the solvents must be chosen carefully [37].

2.2.3 Chemical Components of Propolis

The resin type of thing is called propolis and it 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 the resins in the propolis [38]. 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 [39]. Â–amylase [40] and some other polyphenolic compounds, flavones, esters, phenolic acid and fatty acids are present in propolis [41–44]. 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 addition 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 [21-22]. 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 [45, 46].

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 2.1. All the major properties of the propolis are described below one by one.

TABLE 2.1: Health Applications of Propolis

Health Benefits	**Propolis Activity**	**Type of Studies**	**References**
Reproductive care	Anti-oxidant	Animals	[47]
	Hormone balance	Animals	[48]
	Anti-oxidative agent	Animals	[47]
	Reduce premenstrual Syndrome	Humans	[49]
	Post-menopausal treatment	Humans	[50]
Neurodegenerative and aging diseases	Longevity promoting	Animals	[51]
	Alzheimer's diseases	Animals	[52]
	Mental illness	Humans	[53]
Wound healing	Fibroblast migration	Animals	[54]
	Collagen production	Human	[55]
GI Disorder	Vasodilatation	Human	[56]
	Antiparasitic	Human	[57]
	Antiulceration	Human	[58]
	Collagen metabolism	Animals	[59]

Table 2.1 continued from previous page

Health Benefits	Propolis Activity	Type of Studies	References
Gynecological care	Diabetic foot ulcer	Human	[60]
	Antifungal	Human	[61]
	Antifungal and antibiofilm	Human	[62]
	Antibacterial	Laboratory	[63]
Oral health	Daily mouth wash	Human	[64]
	Toothpaste disinfection	Laboratory	[65]
	Toothpaste against gingivitis	Human	[66]
	Oral therapeutic drug	Human	[63]
Oncology treatment	Anti-breast cancer	Human	[67]
	Antimelanoma cancer	Animals	[68]
	Anti-lung cancer	Human	[60]

2.3.1 Anti-Cancer Effects of Propolis

From the main chemical components of the propolis, two components have an anti-proliferative property and these compounds are the Caffeic Acid phenethyl Ester (CAPE) and chrysin. This property is due to the suppression of the com-

plexes of the cyclins and the arrest of cell cycle in the cancer cells by the effects of the CAPE or chrysin. 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 chemo-preventive anti-cancer drug. From all the cancers, 3rd number is the oral cancer in Saudi Arabia while lymphoma and leukemia are the first two cancer malignancies. It is considered this due to the formation of the second primary tumors in 3% to 7% population in the head and neck squamous cell carcinomas and they also have higher rates of the recurrence [69]. For the patients that have premalignant lesion or the head and neck squamous cell carcinoma, chemo preventive drugs acts as an appropriate therapy. 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 chemo-preventive agents. 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 chemo-preventive approaches. Another therapeutic effect of propolis also can be the induction of the apoptosis [70, 71]. 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 astaxanthin and the flavonoids that both are present in the propolis protect the cells from the β-Amyloid that is involved in the induction of the apoptotic death of the cells [71–75]. There are many other biological activities of the propolis that also includes immunostimulant activity. Against many environmental mutagens that includes 1-nitropyrene, 4-Nitro-O-Phenylenediamine, 2-amino-3-methylmidazao, benzo[a]pyrene and quinoline it shows antimutagenic effect [76].

The anti-tumoral action for propolis became reviewed. 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 provokes apoptosis of tumor cells, and their property to start macrophages to deliver causes in shape

for controlling the ability of B, T and NK cells, for my part. 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 chemo-therapeutic specialists or radiation in mice [77]. The mixes 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 [78]. Though the caffeic acid, An anti-metastatic activity, phenethyl esters (CAPE) from poplar propolis and Artepillin C from Baccharis propolis have been recognized as the greatest effective antitumor agent in various polyphenols [79, 80]. In human lymphocytes, anti-carcinogenic capability of propolis in vitro was discovered. Plasma checks had been acquired from 10 sound males, nonsmoking volunteers, which had been incubated and offered to increasing concentrating of propolis (0.01, zero.05, 0.1, 0.2, 0.5, zero.7, and 1.Zero mL) [81]. Suggested micronucleus quotes had been 1.4770.38 - 4.0270. 64 Mitotic record costs have been somewhere in the range of 19.4572.22 - 0.2870.33. The contrasts between the manipulate and uncovered cells were statically important (pp; 0: 05). In peripheral human being lymphocytes in vitro are acquaintance to various concentrations of propolis cannot produce a cancer-causing influence. Though, it showed that propolis might have a cancer-causing influence in high concentrations by increasing micronucleus (MN) rates [82]. Still if the propolis is used in a controlled manner than it have the capability to heal the cancerous part of the patient body. This is how the propolis have the anti-cancer effects on the humans.

2.3.2 Anti-Oxidant Effects 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 [83].
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, Alzheimer's disease and the Parkinson's disease [84–88]. The cellular levels of the H2O2 and the NO can be reduced by the propolis due to its anti-inflammatory properties [88]. 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 [89]. 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 [90] and humans [91] can be increased by the propolis during the in vivo studies and it leads towards the decreased peroxidation of the lipids [92, 93]. Hydrogen peroxide (H2O2) that induces the DNA damage in the cultured fibroblasts is also inhibited by the Turkish propolis [94].
A remarkable medical property of the ethanolic extract of propolis (EEP) is described by the Krol et al. that shows protection against the gamma radiations [11]. 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 experiment that the luminal H2O2 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 [95]. Apart from CAPE, the antioxidant behavior of another component the propolis known as the tecto-chrysin 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 by returning decreasing the production of the Malonaldehyde (MDA) [96].

2.3.3 Antibacterial and Antiviral Activities of Propolis

As there are various types of flavonoids in the propolis, whcih has shown the antibacterial and anti-inflammatory properties, [97] that could be used as powerful natural antibiotics [98]. The flavonoids play a very important role in the cure of respiratory disorders that can be common cold or the influenza viruses [99]. 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* [9]. Velikova et al. [100, 101] and Marcucci et al. [102] 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 [103]. 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 [1, 104]. 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, andsolvents that are being used for the extraction of propolis [105]. The growth of the *B. cereus* and *S. aureusis* inhibited by the 125-500 μg/ml propolis [106]. The polyphenols content plays a very important role in the antimicrobial activity of the propolis [107]. 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 [108]. Against the *Herpes smplex 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 [109]. 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 [14]. 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 [110]. 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.

By agar diffusion method, the antimicrobial activity of the propolis that is composed from Gujarat by agar diffusion method beside *Asparagus nigar*, *Staphylococcusaureus*, *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* didn't shows any action the yeast *C. albicans* presented the reasonable zone of inhibition. But, 40% was least the methanolic extracts [1, 111, 112]. Since, propolis have the anti-viral and anti-bacterial properties it can be used to treat the bacterial and viral infections in humans, and as COVID-19 is also a virus this makes the selection of propolis for this research an absolute choice. Furthermore, propolis also have anti-fungal properties, means it not only stops bacteria and viruses but also it have anti-fungal properties to cure the diseases caused by the fungi in humans as well as in animals and other living beings. Moreover, the propolis also have these anti-fungal properties to cure their own bee hive as well to maintain it's purity.

2.3.4 Antifungal 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 studies. The order of the sensitivity tests for the antifungal activity was in this order: *C. albicans C.tropicalis C. krusei C. guilliermondii*. Kovalik investigated 12 patients that were suffering from the chronic sinusitis that is caused by the *Candida albicans* [111]. 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 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 15-30mg/ml. The 0.25-2.0mg/ml concentration of the propolis is enough for the repressed growth of the *A.sulphureufor* 10 days [113]. 38 strains of fungi and 60 strains of yeast [112] and *Aspergillus parasiticus* strain NRRL 2998 [114] 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* [115] lethal towards the *Trichomonas vaginalis* [116].

2.3.5 Anti-Inflammatory Effects 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. Not just these but many other compounds are seen to have the anti-inflammatory properties as well including Chrysin, Acacetn and many other as well. As in the onset of the many inflammatory diseases, T-cells play a key role so Márquez et al. [48] examines the immunosuppressive activity in the T-cells. The results show that the phenolic compound plays 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 examining the 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 [117].

Irritation is the composite biological reaction of vascular tissues to destructive stimuli, such as free radicals, pathogens, damaged cells and irritants. The key influence of the host resistance method is an Anti-inflammatory action [82]. The action of propolis has been looked into by Almeida and Menezes. NADPH-oxidase ornithine decarboxylase, Myeloperoxidase movement, tyrosine-protein kinase, and hyaluronidase from guinea pig pole cell shave inhibitory properties of propolis. Through the existence of flavonoids dynamicand cinnamic acid by products the anti-inflammatory action can be described [49]. The former comprises of naringenin, quercetin, andacacetin; the later contains caffeic acid (CA) and caffeic acid phenyl ester (CAPE) [82]. Previous incorporates, naringenin, quercetin, anda cacetin the last includes caffeic corrosive (CA) and caffeic corrosive phenyl ester (CAPE) [81]. Galangin and CAPE, being average famous propolis components, showed anti-inflammatory 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 Caffeic corrosive, quercetin, and naringenin were a less intense modulator of arachidonic corrosive digestion than CAPE [49, 82].

2.3.6 Anti-Ulcer Activity

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* [50]. 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 disc. Ethanol was used as a controlling mechanism in this study. The effect of propolis is 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* [51]. According to the Tossoun et al. for the management of the chronic skin ulcers

[52]. Another study was done by M. Kucharzewski et.al. to find out the effect of propolis ointment towards the healing of the chronic venous leg ulcers. This study showed very good results showing positive response from the users. For this study, 56 patients were considered and divided into two groups. 28 patients include into the group I with ulceration area of 6.9 to 9.78cm2 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 9.4cm2 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 Unna's boot compression only. Propolis' gastroprotective properties protect the gastric mucosa from the harm caused by NSAIDs. The antisecretory, antioxidant, and cytoprotective properties of propolis and the phenolic chemicals included in its chemical make-up were linked to gastroprotection [53].

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 marketer's catalysts, that is, catalase, superoxide dismutase, glutathione S-transferase, and glucose 6-phosphate dehydrogenase, became

moreover reestablished correspondingly closer after propolis organizationto control. Arrival of serum transaminases, lactate dehydrogenase, soluble phosphatase, and γ-glutamyltranspeptidase become basically reestablished closer to control after propolis remedy. Results propose that propolis increases the cancer prevention agent protect in opposition to mercury-actuated poisonous first-class and gives proof that it has remedial ability as hepatoprotective specialist [54].

For the protection of the liver of rats from the injury of carbon tetrachloride, the aqueous propolis Extract (APE) shows its properties. This is 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 [55]. 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 cytotoxity of AA is significantly decreased by the pre-treatment with the PP (1, 10, 100, 200 and 400 μ/ml, 24 h) in the rat hepatocyte culture. The method was dose-dependent. The mortality and the incidence of the severe hepatic 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 [56].

2.3.8 Cardio-Protective Effects of Propolis

In rats, the antihypertensive effect of propolis is also shown [57]. 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 as 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 elimination of the free radicals in the diabetic rats [58].

2.3.9 Dental Actions

Five propolis samples were collected from the four different regions and their antimicrobial activities were tested against nine anaerobic strains that were *Prevotella melaninogenica*, *Peptostreptococcus anaerobius*, *Prevotella oralis*, *nucleatum*, *Veillonella parvula*, *Peptostreptococcus micros*, *Actinomyces naeslundii*, *Porphyromonas gingivalis*, and *Fusobacterium Lactobacillus acidophilus*. The results were evaluated by using the agar diluation method for determining the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC). All the strains show susceptibility against the propolis and their MIC values ranged from the 4 to 512 mg/ml. The most effective behavior is known by the Kazan-Ankara' Propolis on the MIC values that ranged from 8 to 512 mg/ml. Within the 4h of incubation, death was observed for the *peptostreptococcus anaerobius*, *Lactobacillius acidophilus*, micros and *Actinomyces naeslundii* and for the *Prevotella oralis*, *Prevotella melaninogencia* and *Porphyromonas gingivalis* it is 8h while it is 12 h for the *Fusobacterium nucleatum* and for *Veillonella parvula* it is 16h [62]. Samples of propolis show more positive effect against the Gram-positive anaerobic bacteria as compared to the Gram-negative bacteria. It can be used in the diseases associated with the oral cavity because it contains flavonoids [62].

2.3.10 Anti-Diabetic Effects of Propolis

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 profile, 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. Contrasted with the manage everyday rats, diabetic rats had higher blood glucose, BNU, createnine, 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. Oral business enterprise of propolis separate in measurements of one hundred, 2 hundred, and three hundred mg/kg better the frame and kidney weights, serum glucose, lipid profile, MDA, and renal capacity exams. Renal GSH, SOD, and CAT had been altogether increased whilst MDA turned into significantly decreased [63]. 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 [64].

2.4 Corona Virus

The virus starts to spread in the community with the help of very limited immune response and it starts to spread with the help of coughing sneezing, this in-turns enters the respiratory pathways and its reproduction starts. Within the respiratory tract, the virus confronts a very strong immune response. This is the stage where the disease becomes clearly visible clinically, as, the immune response starts to secrete several natural cytokines, the presence of these beta and lambda cytokines makes it clearly visible [65]. In 80% of the cases the disease remains moderate and remains in the upper chest pathways only [66]. Initially, the look after for these patients can be provided at their homes and this therapy is known as symptomatic therapy. Yet, only 20% of the cases infected with the COVID-19 tends to develop

the pulmonary insufficiency and some of those cases are prone to develop severe illness [67].

As per an epidemiological study conducted by the Chinese CDC's including the 292 cases from Wuhan, 49% of the patients tends to die because of the severe illness. Many researchers doing their work on COVID-19 shows that severely sick patients tends to show many symptoms including hypertension, heart diseases, chronic obstructive pulmonary disease, malignant tumors and chronic kidney diseases. From these 292 cases 145 were acute patients and 90.2% among those were 60 years of age, 40% of the deaths have these underlying disease [68].

As per the structural and functional studies which are linked with the increase in ACE2 for SARC CoV-2, in the heart, lungs, ileum, bladder and kidneys ACE2 is found in high amount. The most strong expression of the ACE2 can be seen on the epithelial cells of the lungs [60]. Researchers are conducting their researches to find out, if the virus is also linked with another target after the spike proteins are cleaved with the help of proteases [118]. For the activation of the SARS CoV-2 and MERS CoV-2 a two-step protease cleavage model is proposed, which includes the breakdown S1 and S2 crack site in the spike proteins [59].

2.4.1 Targeting the Life Cycle of Virus

A corona virus must navigate a number of challenges before it can penetrate the host cells. For intruders, cellular membranes act as barriers. All animal viruses must initially break through the plasma membrane as their primary defense. For some viruses that copy their DNA in the nucleus, the nuclear membrane serves as the second defense. After breaking all of these barriers the corona virus works to capture the host genetic machinery to replicate itself. As shown in Figure 2.2, the initial and the most important thing regarding the development of the active vaccine is its capabilities to neutralize the specific antibodies which are produced by the immune system to stop COVID-19. Different researchers in different parts of the globe are trying to develop different vaccines using different procedures to stop COVID-19. Two main antibodies developed for rats, non-human primates, pigs and rabbits are CD8+ T cells and the D614G, yet, clinically it is also tested

that both of these antibodies also provides the patients with the protection against the COVID-19. With the help of the B memory cells, these monoclonal antibodies are extracted and these specific antibodies can be exerted on to the COVID-19. Researchers are also working on other antibodies which will have the ability to protect both mice and non-human primates [119]. During the second wave of this pandemic, it's critical to keep an eye on the availability of blood. The neutralize assays are utilized in the screening procedure, and now FDA approval have been acquired to use them. We employed particular antibodies to degrade the virus with less affinity due to competing attachment of cells with ACE2. COVID-19 is genetically inhibited in human tissue at an ACE2 binding site. SSAA09E2 is a suppressed COVID-19 binding site that targets the interaction between ACE2 and COVID-19. Two gene expressions are employed to reduce binding site suppression. These two gene expressions include ACE2 and TMPRSS2. These are the main genes which allows the entarence of the virus in the host cell [120].

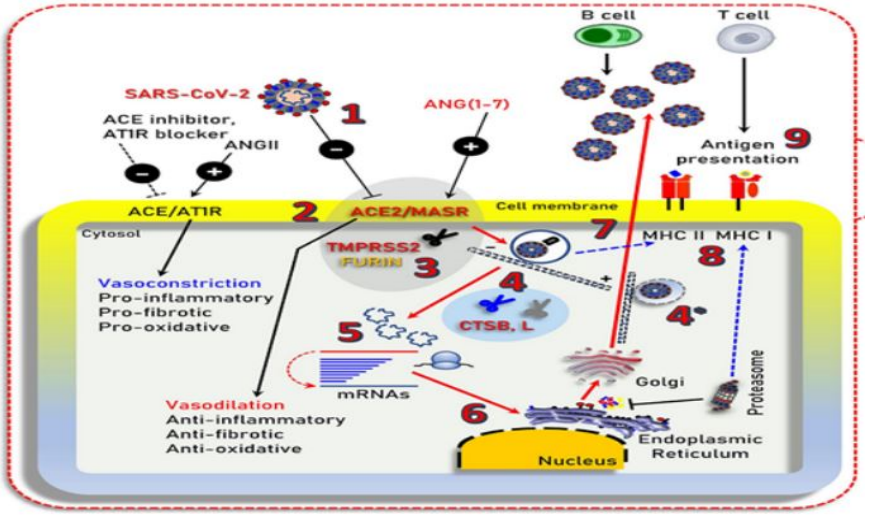

FIGURE 2.2: Cell Signaling Cascade for COVID-19 [121]

It is very interesting to find out that the TMPRSS2 gene and ACE2 gene can suppress the genes of COVID-19. Presently, a drug named comostat mesylate have showed its effect in reducing the severity of the COVID-19 genes. Hence, multiple studies are now going to test its capacity if this drug can do it or not, and this will be done on the initial sites of COVID-19 [122]. The tropism of this

virus can be reduced with the help of TMPRSS2 gene. The COVID-19 virus can force the host to enhance its antiviral humoral activity. COVID-19 is also expected to bind to the cell membrane with the help of a mediated inhibition of the endocytosis. Various other pathways can be used for the mediation of the inhibitors including cathrin inhibitor, dynasore inhibitors, and latrun-culin B inhibitors. A drug named as depolymerizing drug are used on all of these inhibitors. For stopping the translation of the viral RNA in the COVID-19, two specific drugs can be used namely Mpro and its own RNA and this will hinder the process of the transcription of the genes of the COVID-19 virus. A study shows that this drug have the capacity to bind with the COVID-19 RdRp with great strength [123]. Many of these drugs are clinically approved to be used against the viruses. The RdRp have a very positive effect on the patients which are suffering with acute COVID-19. Most of the drugs provides their assistance in very early phase of the disease. For the inhibition of the lysosomal compartmentalization in early phase of infection both hydroxychloroquine and lysosomotropic can be used as drugs [124].

2.4.2 COVID-19 Pathogenesis and their Route

In the second phase of the disease, ARDS and cytokine storm have an alarming relationship, which can lead to system damage. The peptide and non-peptide will protect COVID-19 in the inflammatory signaling MASR pathway in response to ACE2 depletion. Two peptides, ANG (1-7) and ANG, cause a mediated injury in cell inflammation, and these peptides stimulate insulin action while also protecting against atherosclerosis and heart failure [125].

The cause is an overabundance of imbalanced reactive oxygen and nitric oxide ratio in the vessel wall, which causes endothelial dysfunction. Nuclear factor erythroid 2, which works as a radicle scavenger, is one of the medications used to preserve this disinfection. According to research, the participation of the host inflammatory response in most of the body's organs acts as an endothelitis. This inflammatory response is also linked to venous thromboembolism and intravascular disseminated coagulation. Anticoagulant medications are used to treat this [126].

Systemic inflammatory cells generate a significant amount of cytokine that causes immunological discomfort, disinfection, and coagulation, resulting in an uncontrolled response. COVID19 transcription profiling is done using the IL-6 signaling pathway. Since, cytokine expression patterns vary from person to person and even within the same person over time, we propose a road map for managing COVID-19 that takes an individualised approach rather than concentrating on the blockbuster procedure (providing one cure for most of the patients, if it does not cure all). The fundamental science of the infection cycle, in particular the proteins and their physiological conditions linked to the stages of the disease's development, must be outlined in order to comprehend the difficulties and customize treatment for each patient if these patients are to be dealt with new, potentially curative options.

2.4.3 Genome Variation in SARS-CoV-2

SARS-genome CoV-2's is said to be 80 percent similar to that of the preceding human corona virus. Four structural genes code for four structural proteins. ORFIab, one of the biggest genes in SARS-CoV-2, encodes the ppIab protein as well as 15 non-synthetic proteins. The ppIa protein, which contains 10 non-structural proteins, is encoded by the ORFIa gene [127]. SARS-CoV-2 is closely related to the SARS-Coronavirus group, according to the evolutionary taxonomy tree [128]. Between SARS-CoV and SARS-CoV-2, there are a few major differences. Mutations in SARSCoV are caused by the absence of protein 8a and variations in the amount of amino acids in proteins 8b and 3c. Further research has revealed that homologous recombination has replaced Wuhan's spike glycoprotein. SARS-spike CoV-2's protein (S) is a combination of glycoprotein bat SARS-CoV. The utilization of fluorescence experiments also reveals that COVID-19 employs the same ACE2 receptor and host cell entrance mechanism as SARS-CoV and this induce the entrance of the COVID virus into the host, this also helps the virus to incorporate its genome into the host cell as well which takes the charge of host machinery. [129]. The genome variation in the COVID-19 is very crucial to study to contain the spread of this disease.

2.4.4 Replication of Corona Virus

COVID-19 uses the same cellular entrance for receptor ACE2 as SARS-CoV, which is present in the lower region of the human respiratory system and controls human-to-human transmission. Severe Acute Respiratory Syndrome (SARS-CoV2) was isolated from a COVID-19 patient's bronchoalveolar lavage fluid (BALF) [130]. Corona virus S-glycoprotein can attach to ACE2 receptor cells on the surface of human cells. S glycoprotein has two subunits, S1 and S2. The essential domains carry out their tasks. The first RBD is used to identify the S1 virus engaged in the cellular trapezium and host range, whereas the second domain uses virus S2. These two tandem domains are Heptad Repeats 1 (HR1) and Hepated Repeat 2 (HR2), which promote cell membrane fusion [131].

After membrane fusion, the viral genome RNA is released into the cytoplasm, and the non-coated RNA is translated into two polyproteins. PP1A and PP1AB are two polyproteins that encode non-structural proteins. The double membrane vesicle's encoding can create a replication complex (RTC) [132].

RTC repeats and creates a domestic series of sub genomic RNA on a regular basis, which encodes auxiliary and structural proteins. In the endoplasmic reticulum (ER) and Golgi, nucleocapsid proteins (N) and envelope glycoproteins (E) unite to create viral particle buds, which mediate freshly generated genomic RNA [133]. When the virus fuses with the vesicular plasma membrane, it is finally liberated. Because the interaction of the SARS-CoV-2 spike (S) glycoprotein and the ACE2 receptor is so important for virus entry, the virus receptor's binding affinity is being investigated in a variety of methods. Human cells expressing ACE2 increased SARS-CoV-2 intake, according to systematic identification of SARSCoV-2 receptors, although human Dipeptidyl peptidase-4 (DPP4) and APN (Amino peptidase N) increased SARS-CoV-2 consumption [134].

According to studies, the SARS-CoV-2 spike protein's cryo-EM structure revealed in perfusion confirmation that the S-protein and ACE2 binding effect is 10 to 20 times stronger than SARS-CoV-2. Cathepsins in cell surface-associated Tran's membrane proteases Serine 2 (TMPRSS2) and SARS-CoV-2 were responsible for the discovery of the trimmer S protein [135].

2.4.5 Covid and Host Gene Interaction

Catechol L, encoding an accurate gene (SIGMAR) sigma. 1 receptor that was recently identified to be regulated in vitro, was one of the highest scoring genes. The ACE2 receptor and effective anti-SARS-CoV2 medicines are two well-known host genes implicated in SARS-CoV-2 spike protein binding and entry.

Advanced genes were discovered in a range of protein complexes, including vascular ATPs retromer and endosomes, ARP 2/3, PI3K, and others. The relevance of genes and molecular pathways involved in COVID-19 [136] is highlighted within each route of viral pathogenesis and variety.

RAB7A, PIK3C3, NPC1, CCDC22, ATP6V1A, and ATP6 AP1 are a collection of six genes involved in cholesterol production [137]. These genes play a key role in the interaction of comparable transcription signup regulatory hosts in pathway regulation. The deletion of these 6 genes using CRISPR resulted in a rise in cellular cholesterol. Some of the six genes are already implicated in LDL cholesterol control. For example, Rab7a deficiency leads LDL to accumulate in endosomes, whereas NPC1 knockout cells reduce cholesterol levels in the outer plasma membrane and accumulate more in the late endosome/lysosome compartments [138]. According to viral infections, the pathway of cholesterol production is negatively controlled by SARS-CoV-2 infection and can be stabilized by pharmaceutical treatments that upgrade the same pathway [139].

Changes in lipid content have been demonstrated to have a direct effect on SARS-CoV-2 virion development, and this infection has also been proven to occur before hepatitis C and influenza A [140]. Amlodipine, a calcium channel antagonist, has been proven to raise cholesterol levels and reduce COVID-19 infections [141].

Furthermore, recent research has shown that patients taking amlodipine or other dihydropyridine calcium channel blockers have a decreased COVID-19 death rate. Understanding the link between SARS-CoV-2 and cholesterol production pathways is an essential area of future research [142].

Human transcriptome profile of alveolar adenocarcinoma cells A549 infected with COVID-19 to create human viral intercom [143]. This network topological analysis finds fifteen SARS-CoV-2 targets, all of which are members of the interferon-

stimulating genes (ISGs) gene family [144].

These six interferon stimulating genes, IFITMI, ISG15, OAS2, IFITI, IRF7, and MXI, are necessary for the discovery and treatment of Covid-19 as possible therapeutic drugs. TLR3 agonists and ISGs have been connected. This interaction demonstrated that TLR3 agonists play a key role in Covid-19's potential as a therapeutic medication. Controlling the congenital response appears to be a promising technique for dealing with COVID-19, according to the findings of this investigation [145]. COVID-19's primary cell receptor is angiotensin converting enzyme 2 (ACE2), which plays a critical role in the virus's contact with the cell and the progression of the infection. In a recent study, we looked into the underlying mechanism of ACE2 in the lungs in Silico and found that the novel COVID-19 medication was effective [143]. A gene that represents ACE2 and is made up of a network of protein-protein interactions. Genes that interact with ACE2 are shown to be involved in sterol biosynthesis, adenosylhomocysteine activity, trial kylsulfonius activity, CoA ligase activity, and acetate-CoAA metabolism [146].

The interaction between the COVID-19 protein and the human protein is shown using the accumulation of space network properties on the first two layers. Upregulated genes are linked to the substantial function of genes expressed by other researchers and the PPI in the SARS-CoV-2 human network [146]. In response to all of these viral infections, a complete investigation of the gene expression of a virus termed Calu3 and IAV infected with either COVID-19 or MER-CoV-2 was developed. Antiviral interferon signaling was shown to be upregulated in these infections. However, the autophagy process's regulation of cytokine inflammation, mitochondrial organization of down-regulation, and the process of respiration and disposal were all seen. SARS-CoV-2 is present in infected cells but not in IAV-infected cells [147].

In the COVID-19 patient, the regulation of the inflammatory process was accompanied by signs of COVID-19 lung gene expression and inflammatory symptoms. The PPI sub-network of genes in mitochondrial and inflammatory processes that are independently related with up and down regulation in SARS-CoV-2 infected cells was also identified using the expression analysis network [148].

The Cold Dump Plague Formula (CDPF) may have a pharmacological action

against COVID-19 at the molecular level through multi-target multi-pathway multi-components, according to the KEGG Pathway Study. Inflammatory and antiviral immune regulatory pathways account for the majority of locked pathways. The receptors by which COVID-19 enters host cells and is selected for molecular docking and demonstrate good binding activity are provided by two main hub targets, ACE2 and IL6 [149].

Cystic fibrosis is caused by a mutation in the CFTR gene. Patients with cystic fibrosis are known to have a more severe viral respiratory tract infection than the normal population. SARS-CoV-2 infection did not worsen in people with cystic fibrosis, according to another study. This finding is interesting because corona virus infection has been related to a variety of disorders, including pre-existing lung disease. The majority of recent investigations have shed light on why SARS-CoV-2 cannot cause more severe cystic fibrosis symptoms. The first two genes, ACE and ACE2, he claims, play a critical role in COVID-19 [150].

mRNA for TMPRSS2 and airway epithelial cells is high in cystic fibrosis, whereas mRNA for ACE2 is low, a serine protease is reduced, and an increase in ACE2 is thought to boost cell binding to COVID-19. However, an increase in ACE2 will speed up the conversion of anti-inflammatory angiotensin II to inflammation-free angiotensin 1-7, reducing the lung damage and inflammation produced by SARSCoV-2. SARS-CoV-2 penetration into airway epithelial cells is reduced when TMPRSS2 is reduced. Second, most cystic fibrosis patients are given azithromycin, which inhibits the function of the cerebral prosthesis by suppressing viral infections and inflammation in the lungs. The most abundant serine proteases in the lungs are syringe and acetaminophen cystic fibrosis. The ability of TMPRSS2 to allow SARS-CoV-2 to infiltrate airway epithelial cells is likely to be limited as a result of these modifications. As a result, a variety of factors in cystic fibrosis contribute to reduce the severity of severe COVID-19 [150].

Human leukocyte antigen (HLA) plays a critical part in the immune system's normal operation. Three gene classes make up the HLA system. These three genes are denoted by the letters I, II, and III. HLA Class I genes are responsible for presenting peptide antigens from the cytoplasm to T lymphocytes at the cell level. From a traditional standpoint, the development of autoimmune illnesses is pathologically

activated the immune system in a patient with a genetic abnormality, and the Class II gene implicated in the human leukocyte antigen gene represents peptides of the antigenic cell. Further investigations have suggested that specific variants of the HLA gene may produce cytokine storms in COVID-19 patients [151]. Human leukocyte antigen (HLA) offers a variety of infectious pathogens. The development of new therapeutics for COVID-19 could be aided by understanding the host regulatory mechanisms that limit viral infection and pathogenesis. Newly validated published experimental data on protein-protein interactions and host responses related to host microRNA collaboration with host and viral genes are being used. There are 311 host genes and 2,197 human microRNAs, also known as mRNA, that could be used as targets. MicroRNAs play a role in viral replication, immune system differentiation, and T cell activation and differentiation, among other biological activities [151].

One of the most common symptoms of COVID-19 is a change in olfactory function, however the source is unknown. Cell types that express in Severe Acute Respiratory Syndrome (SARS-CoV-2) have been found in the olfactory epithelium and olfactory bulbs, which allow cell entry, according to a study. In the majority of sequences discovered in mouse, non-human primate, and human vulvo vaginal mucosa, two key genes for CoV-2 entrance, ACE2 and TMPRSS2, are involved. ACE2 is also expressed in support cells, stem cells, and perivascular cells, rather than nerve cells, according to other studies. The presence of ACE2 protein in mouse epithelial cystecticular cells and olfactory bulb parasites was confirmed by immunostaining, which demonstrated extensive expression. These findings imply that COVID-19 infection of non-neuronal cell types results in anosmia, as well as an odour problem in patients [152].

2.4.6 Variation in Other Genes Associated with COVID-19 Susceptibility and Severity

Two studies were recently conducted among two European populations, and these investigations are referred to as independent genome wide association studies. In both investigations, the loci 3p21.31 and 9q34.2 have been shown to be associ-

ated with COVID-19, and their severity has been determined. ABO's role in COVID-19 has been revealed in genetic and non-genetic studies. The majority of prior research have found that people with blood group A are more susceptible to COVID-19 infection than people with blood group O, who are less susceptible. The majority of investigations found that ABO is already very susceptible to infection from diseases such as malaria, influenza, schistosomiasis, and COVID-19. According to recent research, viruses, bacteria, and parasites all have blood groups that operate as receptors or co-receptors, and these blood antigens play a part in intracellular uptake signal transduction. With all of this, it's also worth noting that natural antibodies linked to blood groups may play a role in the virus's innate immune response. It's also becoming more important to describe ABO's significance in COVID-19 [153]. It was discovered through pedigree analysis that a gene on the locus plays a function in the IL-1 signaling pathway and is linked to the severity of COVID-19. TMEM189 and UBE2V1 are the genes in question. This investigation also demonstrated that there is a clinical difference in sibling outcomes. A substantial correlation was discovered in some loci and with COVID-19 patients in another study conducted in the United Kingdom. These genes are part of a cluster that encodes the antiviral restriction enzyme activator. OASI, OAS2, and OAS3 are the genes found in this cluster. These genes have a strong link to COVID-19 and have a lot of genetic diversity [154].

The severity of COVID-19 is influenced by cytokine storms, which have lethal consequences. Inflammatory responses are uncontrollable, which may be due to both hereditary and non-genetic causes. With COVID-19 as a co-variable adjustment link, genetic markers are used to identify cytokine genes. Early cytokine storm control is critical for improving the patient's health in COVID-19 patients. East and south Asian populations are less susceptible than Africans due to differences in ACE2 and TMPRSS2. However, there is diversity in both, and neither has offered genetic information about COVID-19 susceptibility. Further research revealed that the genes found in COVID-19 encode a protein, and that this protein has a link to other populations. Although there is conflicting data to support this, polymorphisms in certain patient variables including the TMPRSS2 and ACE2 genes have been associated to an increase in unfavourable outcomes. [151].

A biological pathway of the disease contains multiple genetic variations that are linked to Covid-19 susceptibility and severity. Due to these variations in the genes of the virus as well as the patient the virus gains the power to overcome the host's cell replication machinery and by this power the virus replicates itself. These genetic indicators are closely related with COVID-19, and they serve a critical role in identifying sensitive individuals and developing therapy options. Without knowing these variations in the virus and the humans it is very difficult to create the newer and more effective cure strategy. It is very important for the scientific society to know these variations. A change in the genetic sequence of the virus known as SARS-CoV-2 virus when compared to a reference sequence like Wuhan-Hu1 (the first genetic sequence identified) or USA-WA1/2020 is referred to as a mutation (which also refers to be the as mutation or genetic mutation). [155].

2.4.7 Gene associated with the Activation of Inflammatory Pathways

Coronavirus spreads through the human body in a variety of ways, including direct/indirect contact, respiratory droplets, fecal-oral transmission, and many other as well. In the early days of infection, the virus reaches the upper respiratory tract, here the key role is of ACE2 for pathogen entry. The virus then releases its RNA genomic material into the nucleus after its entry in the host cell, this takes the charge of the host replication machinery and this takes the charge of that machinery for its own use ands its own replication. Afterwards, the replication begins in lower respiratory tract, leading to viremia and this also causes the inflammatory response as well. Because the patients shows asymptomatic behavior at this stage, the disease is very easily treatable, if the disease moves to the next stage then the load is difficult to contain [156]. Macrophages, dendritic cells, and respiratory epithelial cells emit chemokines and cytokines during this stage to trigger immune responses and eliminate the pathogen from the body as shown in 2.3. However, once the inflammatory pathways are activated, an increase in the severity of infection can be seen, leading to cytokine storm or cytokine storm syndrome (CSS) [157].

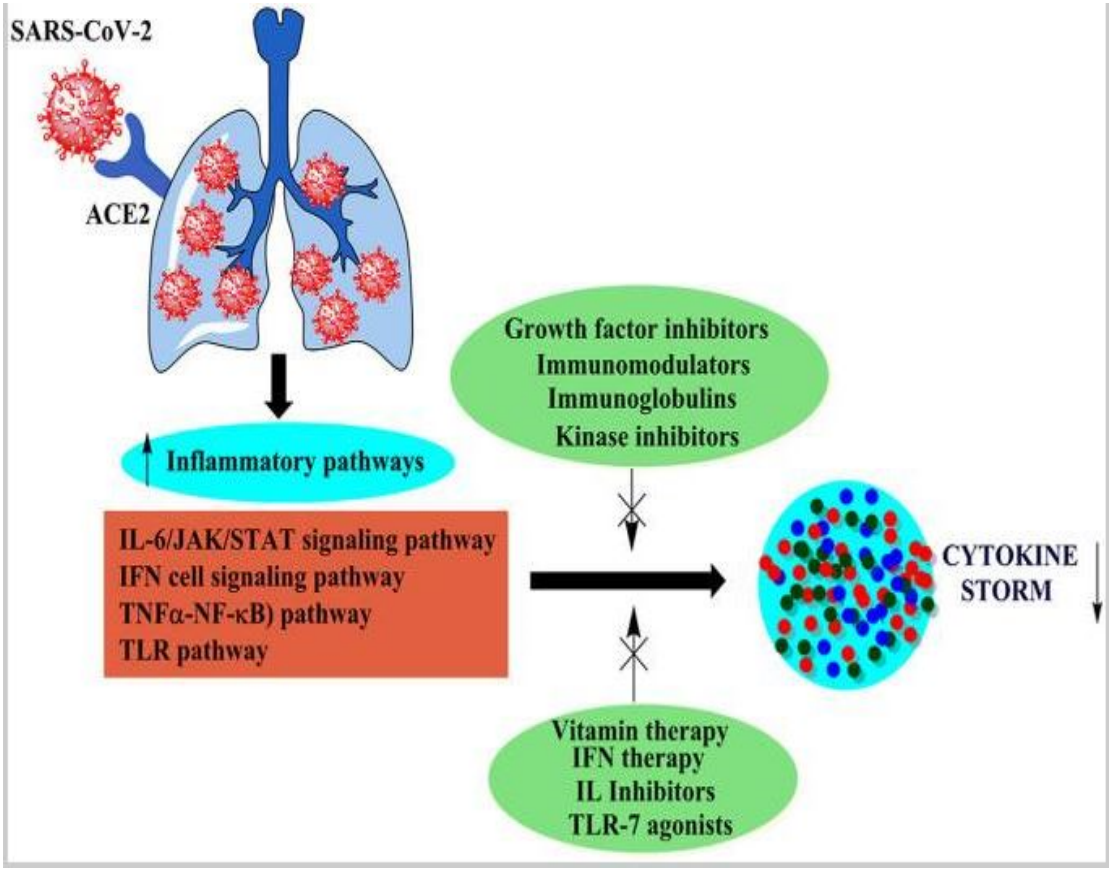

FIGURE 2.3: Inflammatory pathways induced against SARS-CoV-2, therapies, growth inhibition and cytokine storm [158]

2.4.8 Inflammatory Signaling in COVID-19

After the human body come in contact with SARS-CoV-2, the pathways of inflammation like the Janus kinase/interleukin-6/ STAT (JAK/IL-6/ STAT) signaling pathways [16, 159], interferon (IFN) cell signaling pathway, tumor necrosis factor—nuclear factor-kappa (TNF-NF-B) pathway, toll-like receptor (TLR) pathway, T-cell receptor various immune cells produce modest quantities of antiviral IFNs and high levels of proinflammatory cytokines (IL-1, IL-2R, IL-6, IL-7, IL-8, IL-17, and TNF-) and cytokines and chemokines. These discharges from the cells acting as pro-inflammation causing and cause an inflammatory response of unregulated nature, which plays a major role in COVID-19 development and exacerbates infection. In the sections below, we present a brief overview of the several inflammatory pathways that play a role in the pathophysiology of COVID-19 [158].

Chapter 3

Methodology

3.1 Block Diagram

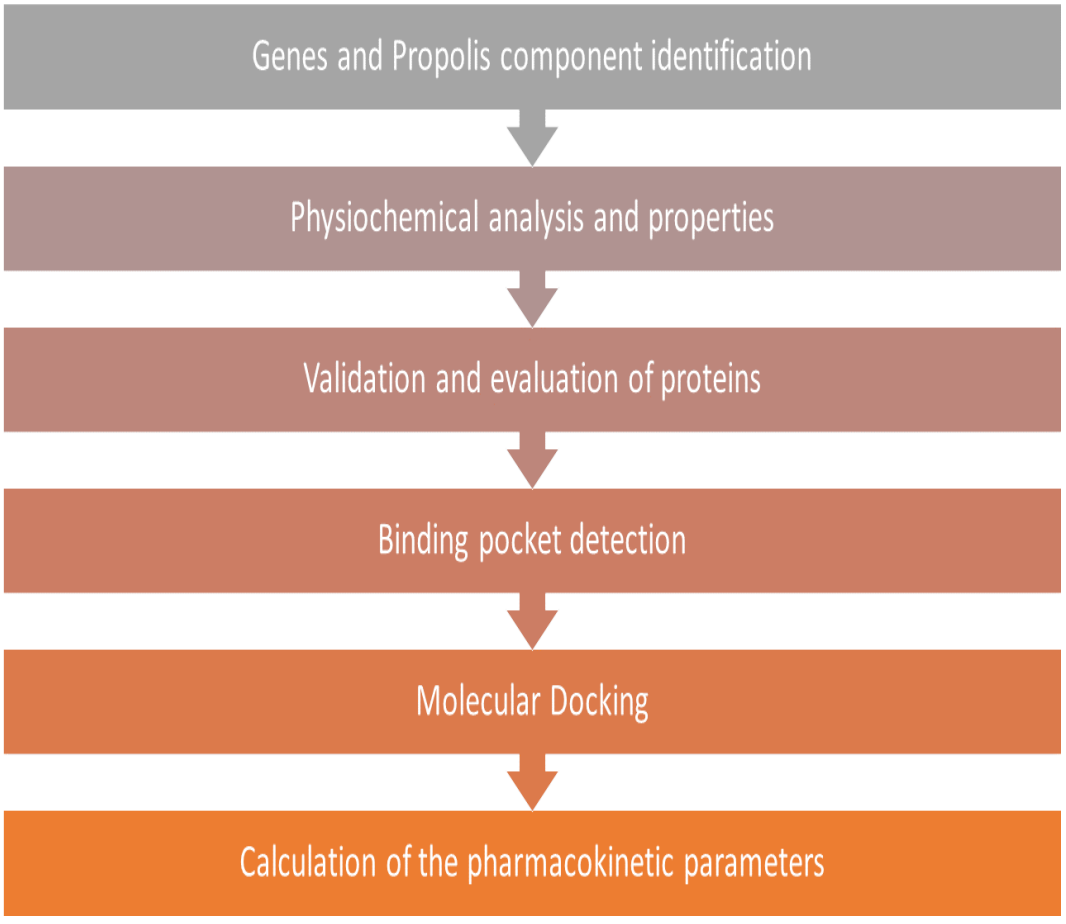

FIGURE 3.1: Block diagram describing the research methodology opted for the study.

3.2 Genes and Propolis Compounds Identification

The candidate genes which shows a connection with the provocation of inflammation under the effect of COVID-19 were explored using the tool COREMINE https://coremine.com/medical/. It uses many sources to address the query of the user including PubMed, OMIM, Drug Bank and Swiss-Prot. By using patented text-mining algorithms one can find articles based on official terms and their synonyms and advance queries helps as well. Compounds of propolis having anti-inflammatory responses were selected with the help of literature based on their anti-inflammatory activity and were retrieved by using the database ZINC https://zinc12.docking.org/.

3.2.1 Physiochemical Properties Analysis

The physiochemical properties were determined using ExPASy's ProtParam https://web.expasy.org/protparam/ tool. Various physical and chemical properties were calculated using ProtParam, a proteomics server which includes the overall number of positively charged (Arg + Lys) and negatively charged (Asp + Glu) residues, Hypothetical pI, coefficients of extinction, index of instability, aliphatic index, and grand average of hydropathicity (GRAVY) [160].

3.2.2 Validation and Evaluation of Proteins

All protein's 3D structures were obtained in PDB format from the Research Collaboratory for Structural Bioinformatics (RCSB) database. PyMOL was used for the analysis of the 3D structure of identified proteins in order to predict the reliability and model surface loops of predicted models as well as conduct structural investigations. The Ramachandran plot, which is a two-dimensional geometrical plot consisting of phi and psi angles and depicting information on the protein structure and its 3D conformation, was used to examine the structure of

the protein backbone [161]. The ProSA (protein Structure Analysis) web server (www.prosa.services.came.sbg.ac.at /prosa.php) was used to determine the energy graphs and to assess protein structure quality.

3.2.3 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/ [162]. Pockets showing the highest drug score are chosen for further analysis.

3.2.4 Ligand Retreival

The ligands were retrieved from the ZINC database, as these are the compounds and not proteins or anything like that. The ZINC database https://zinc.docking.org/ is a very well-known database from where any chemical compound can be downloaded in SDF format for the docking.

3.2.5 Molecular Docking

As a result, docking played an important role in the development of rational medications [87]. It aids in the discovery of novel small molecular compounds by revealing important properties such as high binding interaction with target protein, a reasonable absorption, distribution, metabolism, and excretion (ADME) profile, and drug likeness using SWISSADME http://www.swissadme.ch/index.php, all of which aid in the selection of a lead for the target [163]. The molecular docking tool was part of the molecular operating environment. Molecular docking is done with the help of an online but credible tool namely, CB-Dock, here is the link of that particular tool http://clab.labshare.cn/cb-dock/php/index.php. The CB-Dock is a tool which access five binding pockets and it gives the result for these five binding pockets. After that it is upto the user to select the best result having least vina score which is usually the first one out of these five results.

3.2.6 Docking Simulation

For docking online server of CB-Dock was used and for the result simulation PyMol was used from where the binding positioning of the ligand with the protein was assessed and also the number and nature of bonding was found.

3.2.7 Calculation of the Pharmacokinetic Parameters

SWISS ADME was used for the calculation of the pharmacokinetic parameters. The number of hydrogen-bond donors, miLogP, the number of hydrogen-bond acceptors, TPSA, the molecular mass of the compounds, and the number of rotatable bonds were all estimated as part of this effort. Lipinski's rule of five [164] was also used to calculate violations. A previously known approach [165] was used to calculate the absorption rate percentage.

3.2.7.1 Rule of Five Properties

Lipinski used a collection of simple atomic descriptors to come up with Rule of 5 as shown below:

- Most drug-like compounds should have logP values of less than or equal to 5.
- A molecular weight of less than or equal to 500 is required.
- The maximum number of hydrogen bond acceptors should not exceed ten.
- The maximum number of hydrogen bond donors should be 5 or less.

Oral availability difficulties may arise when compounds contravene more than one of these regulatory regulations. The number of rotatable bonds in orally accessible medications should be fewer than or equal to 10 and the topological polar surface area (TPSA) value should be less than or equal to 140, according to the Vebers rule.

Chapter 4

Results and Discussion

4.1 Extraction of Genes from Coremine Tool

The link given ahead https://coremine.com/medical/ is a CORMINE utility. The genes-protein associated to the concept words COVID19 (mesh) and SARS-CoV-2 (mesh) were investigated. Within the time period of 2019-22, COVID-19 (mesh) found 31866 connected chains. For COVID-19 from the year 2019, the Core-mine system gives a list of 2403 genes discovered in literature. Validation of the genes and their association with the COVID-19 was assessed, for the assurance of their accuracy. The file was saved as an excel spreadsheet. The information in the file includes association, name, type, and significance value. The strongly linked genes were chosen using a 0.01 threshold, yielding a total of 59 candidate genes. These genes had a different functions in the human body and serves different functions under the effect of the COVID-19. The genes were further validated with the help of literature. These genes were reported to have the inflammatory effect in the human body both under the effect of COVID-19 and without the effect of COVID-19.After putting the threshold, two genes were shortlisted from this list of genes, namely, ACE2 and TMPRSS2, these genes are showing on top of the list as well. After shortlisting these genes the ACE2 and TMPRSS2 are being used in the research. These two genes are the most significant genes out of these 59 genes. The genes found with the help of core-mine are given in table 4.1.

TABLE 4.1: Genes along with their normal functions and functions in case of SARS-CoV2

Symbol	**Function of Gene**	**Function in Covid-19**
ACE2	The ACE2 protein-coding gene has been related to a variety of disorders, including SARS-COV2.	ACE2 has been linked to the entry of coronavirus receptors into human cells, including the (SARS-CoV2), which causes COVID-19.
TMPRSS2	Endothelial cells in the digestive and respiratory tract express a cell surface protein termed TMPRSS2	COVID-19 is disseminated by an endothelial cell surface protein called TMPRSS 2 that allows the virus to enter the cell. Also, it is responsible for generating inflammatory response in the lungs.

Table 4.1 continued from previous page

Symbol	Function of Gene	Function in Covid-19
CDSN	Corneodesmosin is a human protein that is encoded by the CDSN gene. Cornfield squamous epithelial cells in the epidermis of humans produce a protein called corneodesmasin, which is encoded by the corneodesmasin gene.	Binding and misfolding of the protein
CRP	Inflammatory cytokines are generated from the liver in response to the gene CRP. This reaction increases rapidly in infection, trauma, and inflammation, and it decreases as the disease improves.	C-reactive protein regulates the host protein's innate immunity. These proteins are connected to COVID-19 and are very beneficial for guiding and mechanical ventilation.
FURIN	FURIN is a protease enzyme found in both animals and humans. FURIN is the gene that encodes this gene.	In human cells, it plays a significant role in the increase of SARS-CoV-2.

Table 4.1 continued from previous page

Symbol	Function of Gene	Function in Covid-19
VTN	The factors discovered in cell adhesion by vitro section in serum and tissue	Involved in the detection of serum
SH2D3C	Adapter protein that has a role in cell adhesion and is implicated in the cell signaling pathway. Tissue organization and migration are controlled by the immune system.	SARS
F2	Coagulation factor II is a protein-making factor that is involved in sending instructions to the F2 gene.	F2 is a thrombophilia biomarker that studies pneumonia and severe Coronary Artery Disease patients.
IL6	A gene known as IL-6 stimulates the acute phase response of protein synthesis and neutrophils.	Inflammatory multisystem disease
ACE	Angiotensin converting enzymes (ACE) are made using instructions provided by the ACE gene.	Inflammatory multisystem disease

Table 4.1 continued from previous page

Symbol	Function of Gene	Function in Covid-19
TP53	TP53 is a protein that gives a gene instructions on how to make protein. Cell division is uncontrollably governed by a protein that functions as a tumor suppressor gene, which keeps cell growth and division in check.	The innate immune response regulates cell division and survival by acting as a gatekeeper.
EGFR	EGFR stands for epidermal growth factor receptor, which gives instructions on how to make protein. On one side of the cell, this protein is still present.	Multiple viruses, including coronavirus, can enter through this hole.
BCL2	BCL2, a gene that regulates apoptosis, is known. By preventing programmed cell death, it helps cells to live longer.	Important in viral replication and in the process of apoptosis.
IL1RN	IL1RN reduces the binding activity of interleukin-1 and prevents the core receptor from forming a signaling association.	Acting as an inhibitor causes severe lung damage in Covid-19 patients as a result of cytokine release.

Table 4.1 continued from previous page

Symbol	Function of Gene	Function in Covid-19
CASP3	CASPASES are the genes that are involved in inflammation, apoptosis, and necrosis, as well as a signaling pathway.	Suppression of CASP3 keeps the host's immunological response under check.
CAT	CAT refers to the instructions for generating an enzyme that are delivered by a gene. Catalase is an enzyme. Four subunits make up a functional enzyme. The haeme group is the name given to these components.	Play an important role in the successful binding of ACE2.
BMND7	The BMND7 genetic locus is involved in bone mineral density and osteoporosis.	Antiviral immunity and lung inflammation are aided by this substance.
BMND8	The BMND8 genetic locus is involved in bone mineral density and osteoporosis.	Antiviral immunity and lung inflammation are aided by this substance.

Table 4.1 continued from previous page

Symbol	Function of Gene	Function in Covid-19
BSG	A gene known as BSG codes for 147 proteins in humans. This gene produces an extracellular matrix that allows cells to differentiate. These proteins are contained in the OK blood group system, which is used to identify blood groups.	In human zygote, act as a host receptor for viral entrance.
SH2D3A	SH2D3A is the name of a protein-coding gene. This gene is linked to Acute Respiratory Insufficiency.	Immune system and respiratory organs are weakened.
APP	Retrobulbar Neuritis APP known as the amyloid precursor protein is produced by the provision of the instruction to a gene known as APP.	The protection of the immune response and immune system.

Table 4.1 continued from previous page

Symbol	Function of Gene	Function in Covid-19
CD274	CD274 is a gene that regulates microtubule stabilization.	Different immune inhibitors have been deregulated.
VEGFA	Permeabilization of blood vessels causes cell migration and inhibits apoptosis. VEGFA, a gene found in endothelial cells, stimulates cell proliferation.	Spike protein inhibits the activation of sensory neurons.
ERBB2	The ERBB2 gene is involved in microtubule peripheral stability and outgrowth control.	Promotion of oncogenesis
PSMD1	Protein homeostasis is critical for maintaining or eliminating misfolded and damaged proteins. Proteins with cellular functions are eliminated.	Helps in the elimination of the damaged proteins.
MTOR	A gene called MTOR provides instructions for the production of protein that is found in several types of cells throughout the body, particularly in the brain.	Helps regulating the process of apoptosis and also regulates cell proliferation.
CTSL	In glioma U25I cells, a human protein termed CTSL mediates cell invasion and migration	Involved in the cleavage of viral proteins and also strengthen the activities performed by the virus.

Table 4.1 continued from previous page

Symbol	Function of Gene	Function in Covid-19
CDH1	The CDHI gene instructs the production of epithelial cadherin protein.	A mutation in a cancer-predisposing gene is the root of the problem.
INS	A gene called INS gives instructions to manage the glucose level in the blood produced by the insulin hormone.	Insulin resistance is a chronic condition that is triggered by this substance.
DPP4	In the metabolism of glucose, the DPP4 gene plays a vital role in the breakdown of incretin GLP-1.	Causes the inflammatory bowel diseases
BDNF	BDNF is a neurotropic factor found in the brain and spinal cord that provides instructions for producing protein.	Plays a part in both neurodegeneration and neurodevelopment.
MMP9	The MMP9 gene, which plays a role in inflammation and cardiovascular disease, regulates pathological remolding.	Neutrophil activation and inflammation are mediated by neutrophils.
NFE2L2	The antioxidant response element, expressed by the gene NFE2l2, interacts to a transcription factor.	Helps in the mediation of the metabolic pathways

Table 4.1 continued from previous page

Symbol	Function of Gene	Function in Covid-19
GABPA	GABPA refers to three GA binding protein subunits that act as binding sites for DNA.	Activation of the mitochondrial genes that aid in the expression of certain genes.
TSC1	Hamartin is a protein that is created as a result of a gene called TSCI giving instructions.	Involved in cancer regulation
AGT	The angiotensinogen proteins are produced by a gene known as AGT. The AGT helps in salt concentration which in turn helps in the regulation of the blood pressure in the human body.	Along with TMPRSS2 it also helps the virus to enter the host cell.
BRAF	With the protein expressed by this gene the signaling of cell nucleus and the outside of the cells are mediated and it also helps in more expression of BRAF gene.	Participate in viral propagation as well as cell apoptosis.
VIM	In the cytosol, vimentin plays a critical role in anchoring and sustaining the position of organelles.	Plays a vital role in the formation of bones.

Table 4.1 continued from previous page

Symbol	Function of Gene	Function in Covid-19
NOS2	Nitric oxide is a messenger molecule that performs functions all over the body (NO)	Play a important role in the enhancement of the immune system and vascular disinfection
KRAS	A gene called KRAS plays a function in the signaling system for generating protein instructions.	Cause different mutations in the developing embryonic organs.
PTEN	PTEN is a tumor suppressor that uses the action of the phosphatase protein gene.	Anti-apoptotic protein levels rise when nuclear translocation is involved.
STAT3	STAT3 is a protein that regulates cell development and division, as well as cell self-destruction and a variety of other biological functions.	Plays an important role in the production of a pro-inflammatory response as well as the unbalancing of the immune system
TXK	Agammaglobulinemia is a disease linked to TXK.	Regulate the expression of genes
MMP2	Gives instructions for manufacturing an enzyme matrix termed metallopeptidase 2 by the MMP2 gene.	Play a part in the physiology of the lungs

Table 4.1 continued from previous page

Symbol	Function of Gene	Function in Covid-19
IL6R	Responses that leads to an acute-phase reactions are implicated in the IL6R-controlled immune response's regulation.	Causes the inflammatory bowel diseases
CDKN1A	A gene called CDKNIA is implicated in cell growth regulation and cell response to DNA damage.	By inhibiting DNA replication, they contribute to DNA damage.
LEP	By employing the gene LEP, it is engaged in the regulation of body weight and provides information for hormone production.	Encourage the body's natural and adaptive responses.
IGHV3-	Antigen recognition of the heavy chain in the V region of the variable domain of immunoglobin by the gene IGHV3-53	Participate in the regulation of the immune responses.
CLEC4M	CLEC4M is a receptor trans membrane gene that is expressed in lymph node endothelial cells.	It possesses a polymorphism in its encoded region and has a role in receptor binding.

Table 4.1 continued from previous page

Symbol	Function of Gene	Function in Covid-19
KLK3	Prostatitis and Urethral Stricture are two diseases linked to a protein coding area regulated by KLK3.	Play an important role in the prostate cancer susceptibility
ACHE	Acetylcholine and other choline esters are broken down by this enzyme.	Act as a stimulus for the nerves.
SYT1	SYT1 is the gene that allows neurotransmitters to be released in the human brain.	Plays a critical role in the susceptibility of the migraine.
PDCD1	The immune system, which aids tumor survival, is aided by inhibitor-mediated pathways, which are controlled by PDCD1.	Production of the tumor cells in the body.
PARP1	PARP1 genes are first responders that can detect DNA damage and then provide a choice for repairing the pathways.	Mitochondrial metabolism dysfunction

Table 4.1 continued from previous page

Symbol	Function of Gene	Function in Covid-19
RELA	RELA aids in the biding of DNA with the frail binding site of the DNA.	Mimics the role of microRNA in the diseases
WNK1	This gene gives instructions for creating different variants of the WNK1 protein.	Plays a very critical role in the coagulation of blood in the body.
DECR1	DECRI keeps prostate tumor cells safe from Ferro ptosis by regulating PUFA levels for survival.	Produces lag in proinflammatory and migratory responses in body.
CDKN2A	The p16 and the p14 proteins are the most investigated, and the gene contains instructions for the generation of multiple proteins in the body.	Act as a biomarker for the ageing.
ADIPOQ	AMPK phosphorylation activates skeletal muscle, which improves glucose utilization and fatty-acid combustion in the liver.	Lag formation in the process of angiogenesis.

4.2 Selection of Genes and Proteins

The genes which are involved in the causation of inflammation in human lungs under the influence of SARS-CoV2 were found by using COREMINE and than validated manually with the help of literature. In addition, the genes retrieved from COREMINE was set on a threshold of 0.01 and two genes were shortlisted which were causing the inflammation in humans under the influence of COVID-19, namely ACE2 and TMPRSS2 because they were the top most query in under the threshold, furthermore, their protein structures are also retrieved from protein data bank PDB.

4.3 Selection of Propolis Compounds

There were more than 300 compounds in propolis but only 13 are known for having anti-inflammatory effect in case of human but only 3 are selected for this study after lot of literature survey and most repeated compounds having the anti-inflammatory responses, these includes the acacetin, apigenin, caffeic acid, phenethyl ester and chrysin which are clearly shown in table 4.2. Out of these five most repeated compounds found in the propolis having the anti-inflammatory responses three were selected for the study and these were selected after finding out that these were not previously used by the scientific society.

TABLE 4.2: Retrieved compounds of propolis having anti-inflammatory activity.

Compound Name	Activities	References
Acacetin	Anti-Inflammatory	[155]
Apigenin	Anti-Inflammatory	[166]
Caffeic Acid	Anti-Tumor Anti-Inflammatory	[167]
Phenethyl Ester	Anti-Inflammatory	[168]
Chrysin	Anti-Tumor Anti-Inflammatory	[169]

4.4 Retrieval of Identified Proteins and Propolis Compounds

Once the targeted proteins were identified, the 3-dimensional structure of two human genes ACE2 and TMPRSS2 were downloaded from the web database of PDB known as RCBS database https://www.rcsb.org/ in PDB format. However, the propolis compounds were downloaded from the web server of ZINC database https://zinc12.docking.org/. In the figure below the 3D structure of the selected proteins are shown in Figure 4.1:

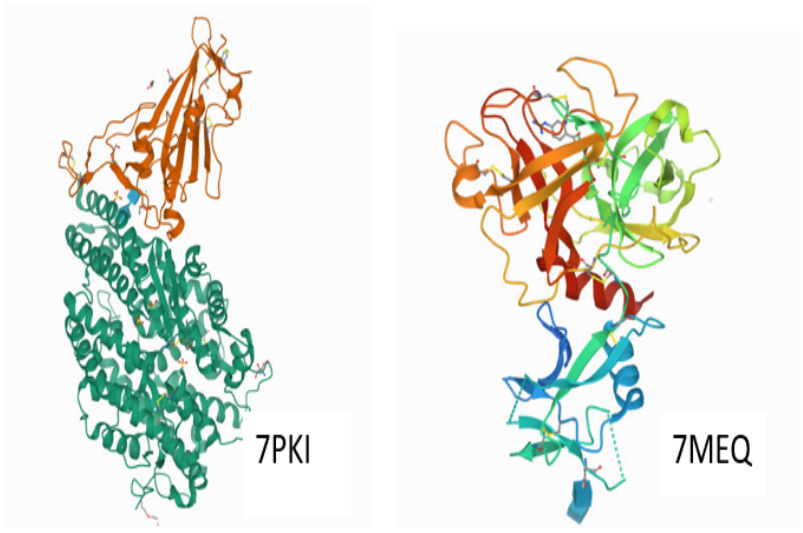

FIGURE 4.1: 3D structure of proteins along with their PDB ID.

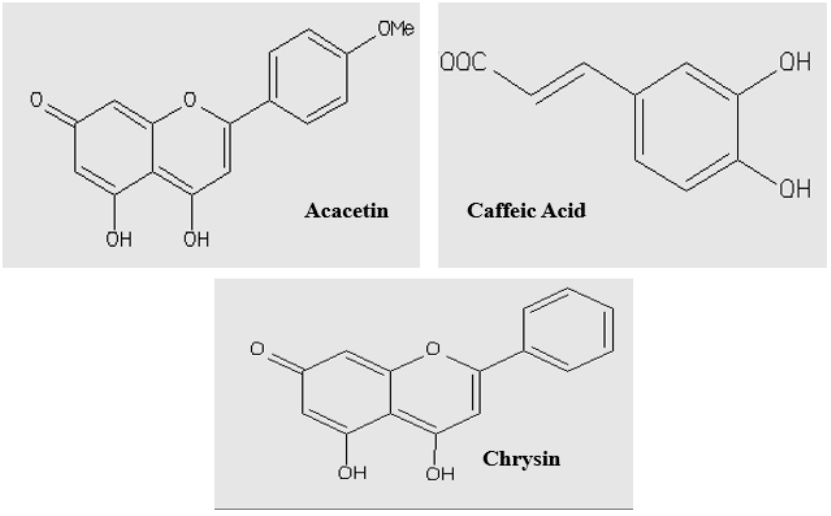

FIGURE 4.2: 2D structure of propolis compounds.

4.4.1 Physiochemical Properties of the Protein

Physiochemical qualities influence protein binding behavior, and these properties are determined by the corresponding properties of amino acids contained in it. ProtParam, which is most typically employed to calculate the physiochemical properties of sequences, is important in determining a protein's function. The ExPASys ProtParam server was used to determine the physiochemical parameters of the target for ACE2 and TMPRSS2 proteins, which included hypothetical pI (isoelectric point), molecular weight, overall negative R and positive +R amino acids, instability index (II), and aliphatic index (AI). Protein net charge is denoted by the letter PI. The computed pI can be used to create a purification buffer system utilizing the well-known isoelectric focusing approach. Its basic character [170] is indicated by the estimated pI value (pI is greater than 5). The results of the Instability Index (II) (less than 45) indicate that proteins are likely stable in test tube conditions. The AI is defined as the proportional volume of a protein taken from the aliphatic side (alanine, leucine, valine and isoleucine). Improved AI indicates increased globular protein thermostability [171]. The extremely high AI for all proteins suggests that they may be stable at a wide range of temperatures [172].

TABLE 4.3: Physiochemical properties of proteins (i.e. ACE2 and TMPRSS2)

Protein	**T-pI**	**-R**	**+R**	**E-Cp**	**E-Cr**	**II**	**AI**
ACE2	5.04	79	52	152220	151720	42.29	75.12
TMPRSS2	6.23	38	32	100475	99350	33.18	70.30

- -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,

4.4.2 Validation and Evaluation of Proteins

Ramachandran plot were generated during this study for the validation of the proteins and for this PROCHECK was used [161]. If the residues in the favored region are above 90%, the protein is tagged as a good protein for the docking purpose. G-Score should remain between the range of 0.5-1.0 if the protein used have less than 0.5 or above than 1.0 are not considered as a good model for the process of docking.

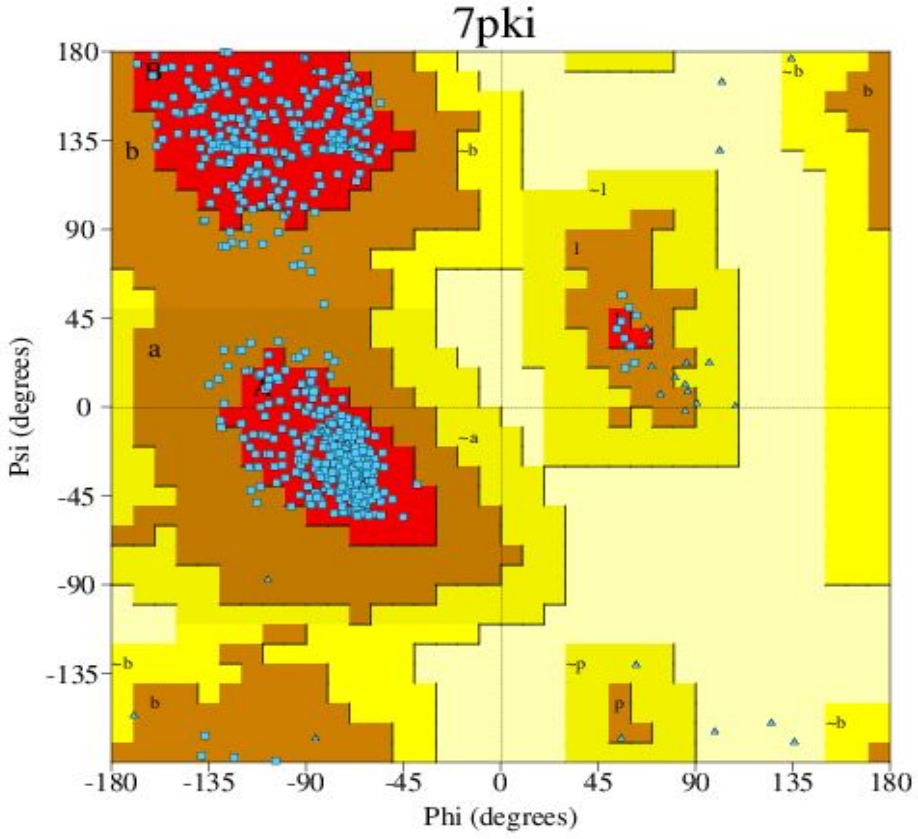

FIGURE 4.3: Validation of 7PKI (ACE2) protein using PROCHECK

The Ramachandran plot of ACE2 (7PKI) showed that it had 792 total number of amino acid residues in the red region which represents the core of it. In the most favored region it had around 655 amino acid residues which showed the percentage

of 93%. In the region termed as additionally allowed region the total number of residues were 49 which made around 7% of the protein. Average G-Score was around 0.25. Overall the 93% of the residues in the favored region showed that the model was good for the homology modelling.

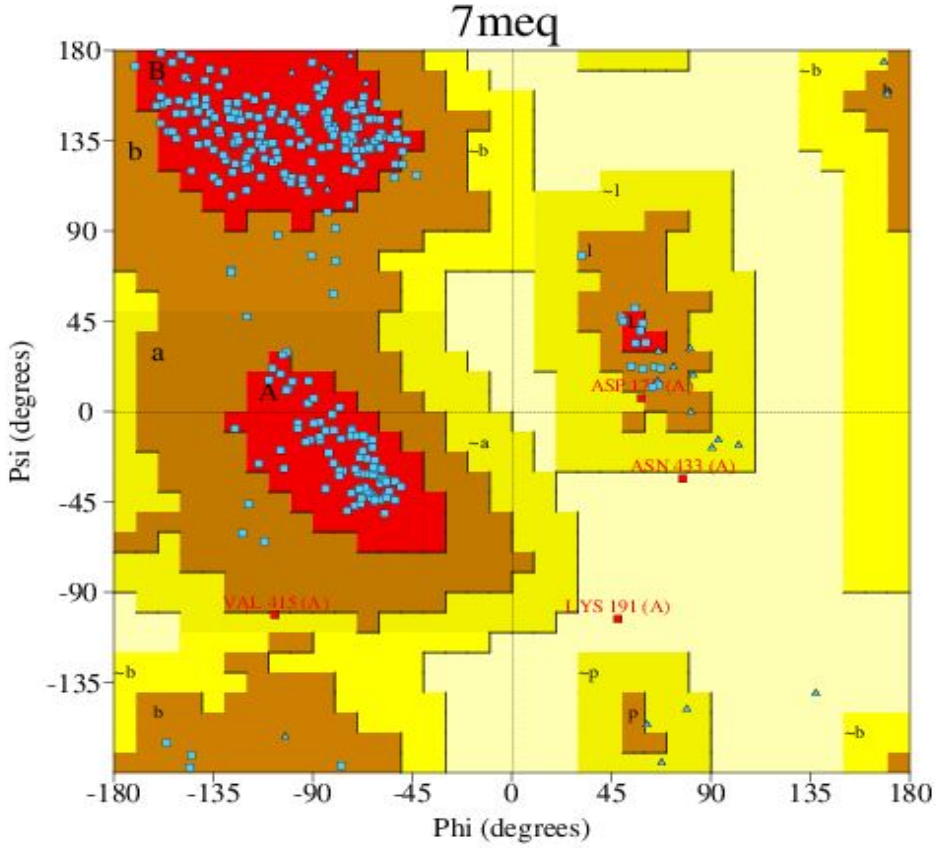

FIGURE 4.4: Validation of 7MEQ (TMPRSS2) protein using PROCHECK

The Ramachandran plot of TMPRSS2 (7MEQ) showed that it had 326 total number of amino acid residues in the red region which represents the core of it. In the most favored region it had around 240 amino acid residues which showed the percentage of 87.9%. In the region termed as additionally allowed region the total number of residues were 29 which made around 10.6% of the protein. Average G-Score was around 0.05. Overall the 87.9% of the residues in the favored region showed that the model was good for the homology modelling. ProSA checks the Z-score of the input structure to see if it falls within the range of scores found for native proteins of the same size. The Z-score measures the divergence of the structure's overall energy and energy distribution due to random conformations.

If the values are positive, this indicates that the query structure is difficult and invalid.

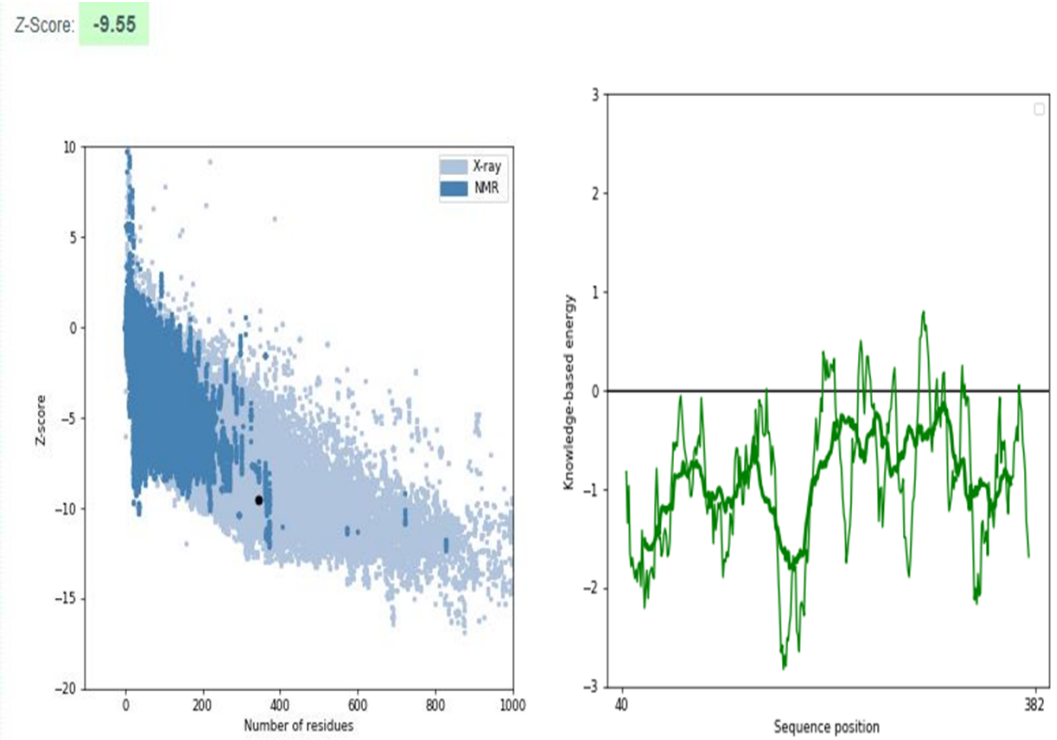

FIGURE 4.5: Z-Score and energy plot of protein TMPRSS2

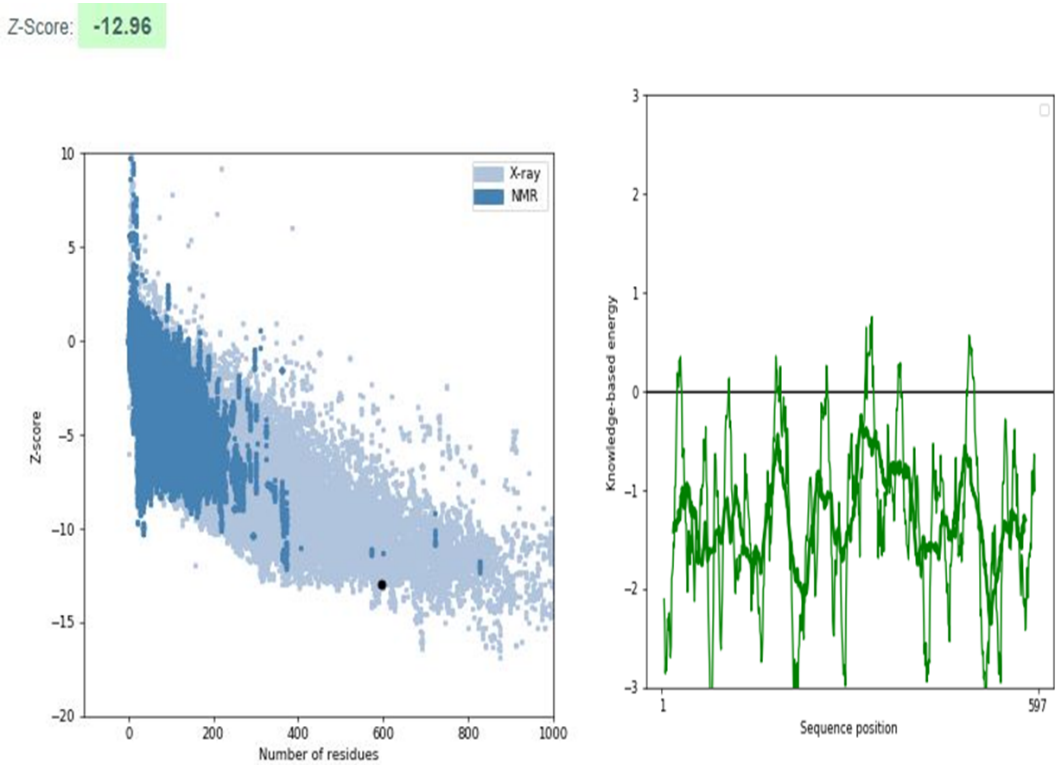

FIGURE 4.6: Z-Score and energy plot of protein ACE2

On the basis of Z-Score it can be said that 7PKI showed that it was best for the modelling followed by 7MEQ.

TABLE 4.4: Properties of selected Proteins

Protein	**Ramachandran Plot Result**					**PROSA**
	MF	AR	GAR	DR	G-Score	Z-Score
7PKI						
	93.0	7.0	0.0	0.0	0.25	-9.55
(TMPRSS2)						
7MEQ						
	87.9	10.6	0.7	0.7	0.05	-12.96
(ACE2)						

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

4.4.3 Evaluation and Visualization of Binding Pockets of Proteins

Prediction of active site is required before docking with the ligands, it reduces the space to be searched on receptor surface present on the proteins structure. Active sites are typically visible in crystals of target proteins where ligands are bound; computational methods can be used to anticipate these sites. As illustrated in Fig, docking studies of both proteins were performed on the binding pockets predicted by the DOGSiteScorer service. The binding pocket must have the fewest number of residues that interact with the remaining protein structure, and it must have the highest drug score of all the binding sites. Because a higher drug score indicates

a higher ligand-protein binding affinity.

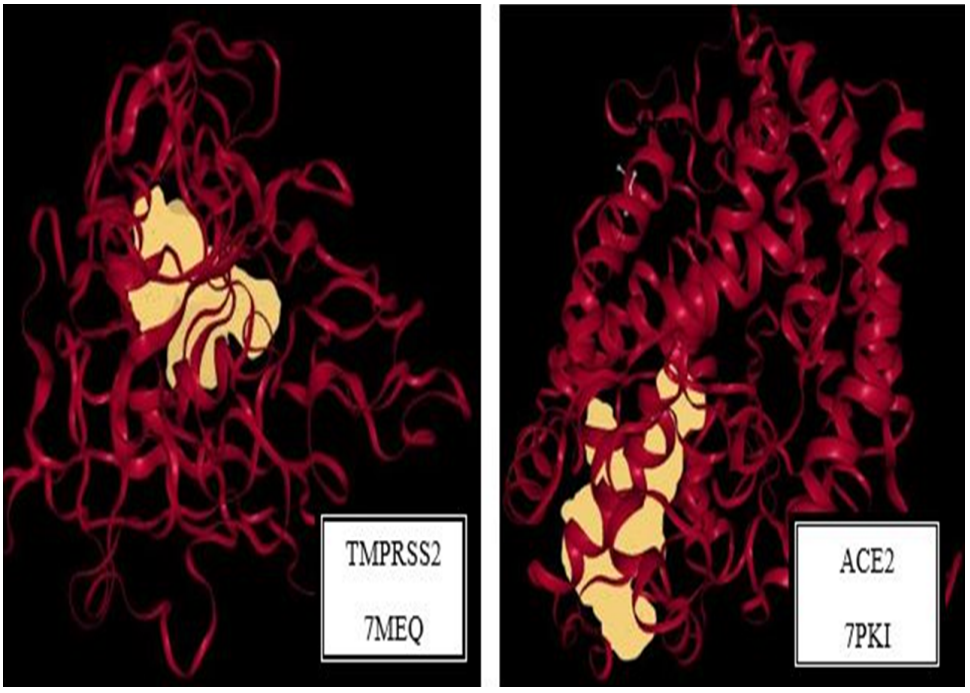

FIGURE 4.7: Predicted Binding Pockets using DogSiteScorer

4.4.4 Results Obtained after Molecular Docking

The docking done between ACE2 and the selected compounds of propolis showed very good affinity. The docking result also provided that the binding between Acacetin and ACE2 showed -7.9 vina score while having the cavity size of around 391. Similarly, between Caffeic acid and ACE2 the score was -5.9 and the size of the cavity was 318. Furthermore, between chrysin and ACE2 the score was -7.6 and the size of the cavity was 318.

In-addition, the docking between the TMPRSS2 and the selected compounds also showed very good results. The docking result also provided that the binding between Acacetin and TMPRSS2 showed vina score -7.9 while having cavity size of around 273.Similarly, between Caffeic acid and TMPRSS2 the score was -6.6 and the size of the cavity was around 347. furthermore, between chrysin and TMPRSS2 the score was -7.8 and the size of the cavity was 347. The binding pockets also shows that this is the most important binding pocket of the given

proteins, as it is evident that a protein can have multiple binding pockets in it.

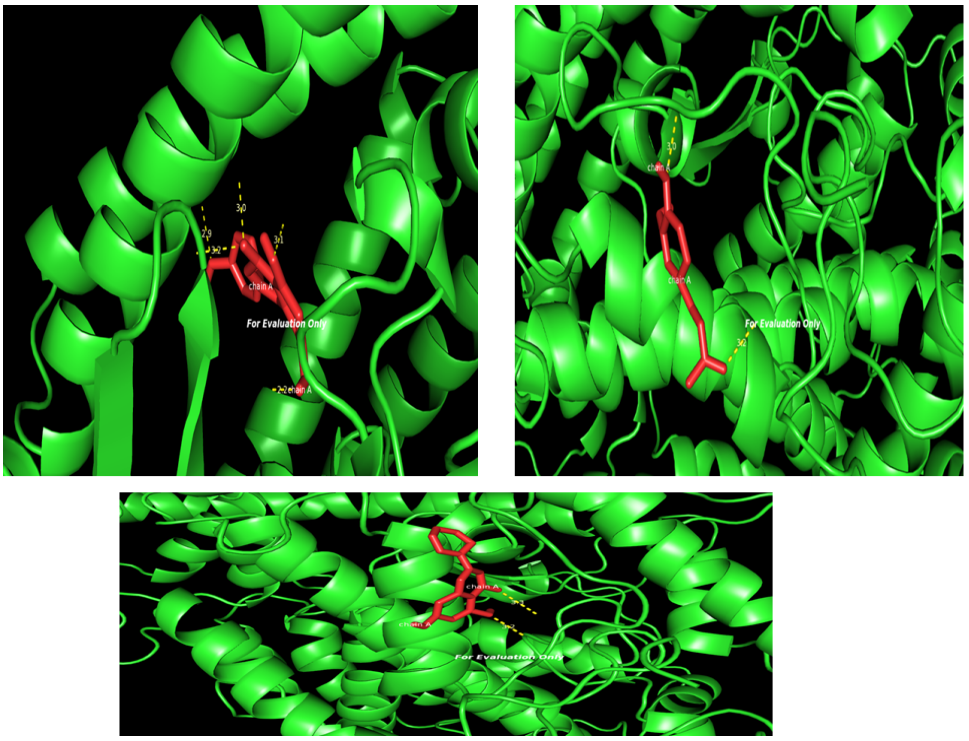

FIGURE 4.8: 3D visualization of the Protein (ACE2)-Ligand (Acacetin, Chrysin and Caffeic Acid) binding complexes

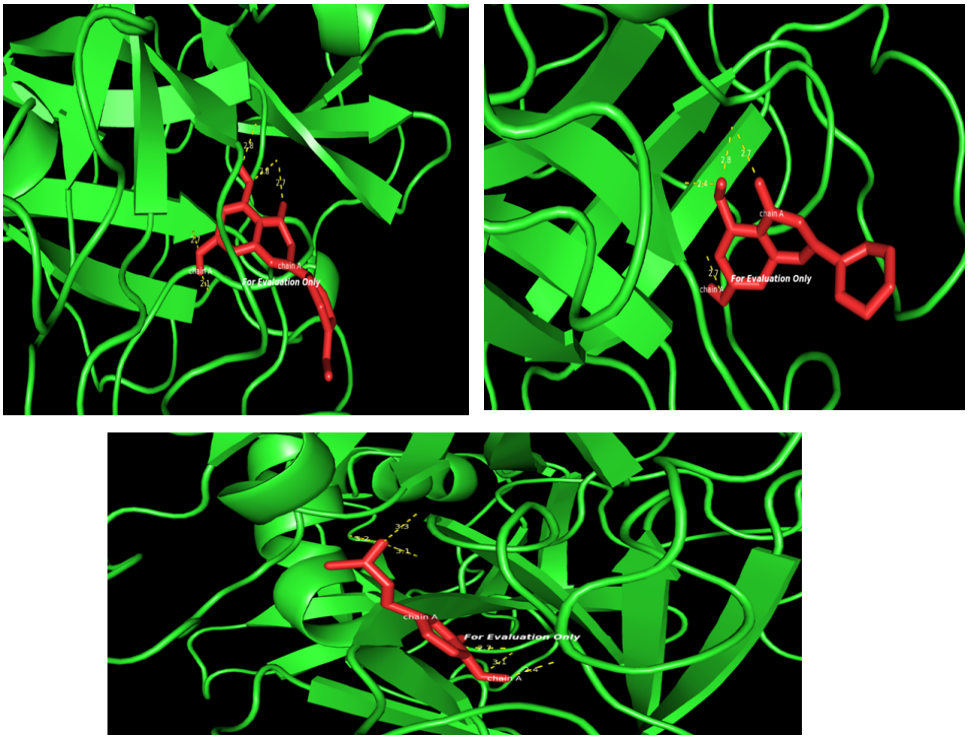

FIGURE 4.9: 3D visualization of the Protein (TMPRSS2)-Ligand (Acacetin, Chrysin and Caffeic Acid) binding complexes

Table 4.5: PDB-e-PISA summary chart for individual Docked interfaces

S. No	Structure 1				Structure 2				Interface	G	
	Range	Nat	Nres	Surface Area2	Range	Nat	Nres	Surface Area2	Area2	Kcal/mol	P-value
					ACE2-ACACETIN						
1	A	12	6	25712	LIG(A:1)	2	1	181	67.1	-0.6	-1.000
					ACE2-Chrysin						
2	A	10	4	25712	LIG(A:1)	2	1	138	62.7	-0.3	-1.000
					ACE2-Caffiec Acid						
3	A	17	7	25712	LIG(A:1)	2	1	192	114.1	0.6	-1.000
					TMPRSS2-ACACETIN						
4	A	13	6	16121	LIG(A:1)	2	1	181	82.8	-0.5	-1.000
					TMPRSS2-Chrysin						
5	A	12	5	16121	LIG(A:1)	2	1	139	58.3	-0.3	-1.000
					TMPRSS2-Caffeic Acid						
6	A	14	6	16121	LIG(A:1)	2	1	201	88.4	-0.0	-1.000

Table 4.6: Summary chart of PDBe PISA analysis parameters

S. No	Parameters	Details
1	Range	The selected range for the relevant interface structure is displayed by Range. For entire monomeric networks, the range is just a chain ID as provided in the corresponding PDB or user-uploaded file. - is used to represent whole chains without a chain ID. [R] The ligand is represented by the formula C:N, where R is the name of the residue (ligand), C is the chain ID and N shows the numbers of the residue.
2	Nat	In a particular structure, the number of interacting atoms.
3	Nres	The number of interacting residues with the relevant structure.
4	Surface Area2	Total number of area that can be accessed by the solvent in the area of a square Angstroms.

Table 4.6 continued from previous page

S. No	Parameters	Details
5	iG kcal/mol	Divide the difference between the total accessible surface areas of isolated and interface structures by two to get this. At the interface, the solvent-free energy gain is given in kcal/M. In isolated and interacting structures, the difference in total solvent energy is employed to calculate the value. Negative values are equivalent to hydrophobic interfaces or positive protein affinity. The effect of finished hydrogen connections and salt bridges over the interface is not included in this diagram.
6	iG P-value	Without solving, it reflects the measured energy gain P-value. When atoms of the interface are picked at random from a protein surface, such as the observed interface area, the P value analyses the possibility of getting a lower observed value. The P-value is an energy surprisingness metric that measures how energy surprising an interface is.

Table 4.6 continued from previous page

S. No	Parameters	Details
7	NHB	The total number of hydrogen connections available across the interface is displayed. For each hydrogen connection, the free energy of protein binding is around 0.5 kcal/mol (precise value depends on calibration technique and may change with version).
8	NSB	Over the interaction, there are likely to be a number of salt bridges. Every salt bridge adds roughly 3 kcal/mol to the free energy of protein binding (precise quantity depends on calibration technique and may vary with version).
9	NDS	It depicts the potential for disulfide bonds to form across the contact. Each salt bridge raises the free protein binder energy by about 4.0 kcal/mol (precise value varies depending on calibration process and may change with version number).

The results showed in the Table 4.4 showed that the binding affinity and all other parameters which are to be taken in discussion are well in limits and by no mean the contradicts with the needed results. The vina score should be below 2 and all the compounds showed the result under this threshold. The vina score showed by ACE2 with all the compounds against which it was docked are -7.9, -5.9 and -7.6 for Acacetin, Caffeic acid and Chrysin respectively. Similarly, the vina score showed by ACE2 with all the compounds against which it was docked are -7.9, -6.6 and -7.8 for Acacetin, Caffeic acid and Chrysin respectively. The result shows that the binding affinity of the protein and ligand is very good and the protein and the ligand shows very strong relationship with each other.

TABLE 4.7: Binding affinity of the protein-ligand after docking

Proteins	**Compounds**	**Affinity**
ACE2 (7PKI)	Acacetin	-7.9
	Caffeic Acid	-5.9
	Chrysin	-7.6
TMPRSS2	Acacetin	-7.9
	Caffeic Acid	-6.6
	Chrysin	-7.8

4.5 Toxicological Properties and Pharmacokinetic Analysis

Pharmacokinetic and the toxicological properties (PKs) are very important to determine in the process of drug development as they helps in the determination of the characteristics of effective compounds which can be used for the successful oral drug development, such as complete absorption from the gastrointestinal tract, proper distribution to the site of action, proper metabolism, and appropriate elimination from the diseased person and that does not show any of the

harmful effect. Drugs that do not pass the PKs in a clinical trial are not commercialized. The chemical descriptors of the molecules determine these features. To estimate the absorption, metabolism, distribution, excretion, and toxicity of novel chemicals with the potential to become medications, a variety of computational techniques are being explored. The Molinspiration online toolbox is utilized to assess pharmacokinetic parameters, while ADMET is used to check toxicity profiling of the selected 3 compounds following the docking simulation, which leads to further investigation. The Lipinkis rule of five [193] is used to determine pharmacokinetic properties.

All the compounds which are to taken orally should have the molecular wieght equal to or less than 500 amu, similarly, the LogP score should be equals to or less than 5. Furthermore, the donor sites for the hydrogen bonds should be 5 or less than 5, lastly, the acceptor sites for the hydrogen bonding should also have the same value as hydrogen donor sites [194].

As per the rules of Vebers, for the drugs which are available for oral usage the value of rotatable bonds should be 10, in addition the value of TPSA should not be in any case above 140 which stands for topological polar surface area, it is important to measure as it describes the transport of the drug across the membrane. All the medicines should have all of these qualities because the absence of even one can raise the issues in the bioavailability of the drug [205].

Table 4.8: Toxicological Properties and Pharmacokinetics Analysis by using ADMET.

Compound	**TPSA**	**MW**	**LOGp**	**HBD**	**HBA**	**n-ROT**
	<= 140	<500	<= 5	<5	<10	<= 10
Caffeic Acid	70.67	180.16	0.97	3	4	2
Chyrsin	78.60	254.24	2.27	2	4	1
Acacetin	79.90	284.26	2.56	2	5	2

Chapter 5

Conclusion and Future Direction

The purpose of the study was to identify the genes which are causing heavy inflammation in the case of COVID-19 and to find out the components in Propolis which is commonly known as to have anti-inflammatory effect in general. After that dock them together and see if they show good binding affinity and good ADME score, so that the compounds found in propolis can be used as a drug to lower the inflammation in the body. For this purpose two inflammatory genes were chosen and three compounds of propolis having antagonist effect against the prior discussed protein were chosen.

After the docking was performed, the physiochemical properties of the selected compounds were analyzed using the Lipinski's rule of five and the Vebers rule. After that, toxicological properties analysis was performed on the remaining targets that passed the Lipinski's rule of five and the Vebers rule, and one chemical was excluded from the candidates for medications for gastric ulcer that which still shows the toxicity. All of these pharmacological targets were put through their paces in terms of pharmacokinetics.

This in-silico investigation will aid in reducing the time and effort required to generate medications that can be tested on animals or people. All of these lead targets have shown promise in silico and meet all of the criteria for an orally accessible medication. This will not only provide us with the best therapeutic procedures, but it will also aid in the development of new pharmaceuticals for human benefit.

All of the substances that are created must be tested on animal models. Selected propolis chemicals must be developed as a medication and tested in clinical trials after successful application on animal models.

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