CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

In Silico Analysis of Turmeric as Anti-inflammatory Agent against ACE2 Receptor

by

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A thesis submitted in partial fulfillment for the degree of Master of Science

in the

Faculty of Health and Life Sciences
Department of Bioinformatics and Biosciences

2021

I dedicate this thesis to Allah Almighty, Prophet Muhammad (SAW) my parents, my husband Muntazir Abbas and my supervisor Dr. Sahar Fazal who are the source of inspiration and motivation for me.

CERTIFICATE OF APPROVAL

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Acknowledgement

All praise be to almighty ALLAH who has been bestowing me with his great bounties and enabled me to complete my dissertation. I am grateful to some people, who worked hard with me from the beginning till the completion of the present research particularly my supervisor Dr. Sahar Fazal, who has been always generous during all phases of the research. A lot of people helped and assisted during my research project and in the compilation of this document. I am grateful to all of them, and wish them the best in their endeavours. However, there are some, the contributions of whom make them worthy of thanking. I will start with Shumaila Azam, PHD scholar in CUST. I thank her for the time she spent discussing theories with me, and assisting in working out different angles to each obstacle. I would like to take this opportunity to say warm thanks to all my beloved friends, who have been so supportive along the way of doing my thesis. I also would like to express my wholehearted thanks to my family for the generous support they provided me throughout my entire life and particularly through the process of pursuing the MS degree. Because of their unconditional love and prayers, I have successfully completed this thesis.

(Namal Khan)

Abstract

Turmeric (*Curcuma longa*) is a rhizome containing perennial plant of ginger family. Turmeric is used for its medicinal properties in almost all the diseases. With increasing drug resistance problem, the interest to explore the natural products with medicinal properties is increasing. The purpose of this research work is to find out the compounds from the turmeric that can be used as anti-inflammatory agents. These compounds of turmeric were find out from the literature that reported their presence in the treatment of inflammation. Protein PDB ID is 1R42. 1R42 was selected from studying its role in inflammation in humans for this research work. Protein three dimensional structure was prepared for molecular docking. Molecular docking was performed for this purpose and after that selected compounds of turmeric for ACE2 protein, were tested against the pharmacokinetics properties. Selected turmeric compounds that pass Lipinski's rule for oral bioavailable drugs for inflammation are Curcumin, Demethylcurcumin,1-(3,4-Dihydroxyphenyl)-7-(4-Hydroxy-3-Methoxyphenyl) Hepta-1,6-diene-3, 5-Dione, (E)-Ferulic Acid, Vanillic Acid, Carvacrol, (E)-Carveol, E-4-(4-Hydroxy-3-Methoxyphenyl)-3-Buten-2-One, Vanillin, (Z)-Ferulic Acid, Thymol and Terpinen-4-Ol. These 12 compounds can be further validate on animal models to provide new treatment for inflammation in body.

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Abbreviations

ACE	Angiotensin Converting Enzyme
ACE2	Angiotensin Converting Enzyme 2
ADAM-17	A Disintegrin and Metalloproteinase-17
ARBs	Angiotensin Receptor Blockers
AT1R	Angiotensin Type 1 Receptor
AT2R	Angiotensin Type 2 Receptor
GIT	Gastro Intestinal Tract
RAS	Renin Angiotensin System
RAAS	Renin Angiotensin Aldosterone System
rhACE2	Recombinant human angiotensin converting enzyme 2
SARS-CoV-2	Severe Acute Respiratory Syndrome Corona Virus 2

Symbols

α	Alpha
γ	Gamma
κ	Kappa

Chapter 1

Introduction

1.1 Introduction

Angiotensin Converting Enzyme 2 (ACE2) is a glycoprotein that is from the family of zinc metallopeptidases [3]. ACE2 is predominantly present in major parts of body like in heart tissues, kidneys, and lungs and a lot of tissues at lower levels of colon [4]. ACE2 is present on chromosome Xp22 and it is a 40 kb gene, apart from ACE gene that is found on the chromosome 17. The human ACE2 protein is basically composed of 805 amino acids [5]. ACE2 is about 40% similar to the sequence of Angiotensin converting Enzyme, while it contains only a single catalytic zone. Remarkably exons of ACE are very closely related with the 18 exons of ACE2. The genetics of ACE2 shows several new polymorphism of ACE2, with specified geographical distributions. It has been described and linked with hypertension and heart diseases [5]. ACE2 is about 40% similar to the sequence of ACE, while it contains only a single catalytic zone. Residues of active site, include Glu-Tyr-Met-Gly-His-Try and (Zn) binding motifs, are highly sustained [6]. ACE2 protein is orientated with the catalytic site facing the extracellular space and N-terminus, circulating peptides can metabolized by catalytic site. There are number of possible regulatory sites in the small C-terminal cytoplasmic domain. The Angiotensin Converting Enzyme (ACE) protein is identical to ACE2 topology. Cytoplasmic

domains of ACE2 share similarities with the renal and trans-membrane protein collectrin [6]. The genetics of ACE2 shows several new polymorphism of ACE2, with specified geographical distributions. It has been described and linked with hypertension and heart diseases [5].

Worldwide Cardiac disorders are main causes of death and emerging as general health issue. Cardiac disorder is indicated with the stimulation of many signaling pathway linked with hypertrophy problems and maladaptation ventrical improvement. ACE2 gene polymorphisms are associated with several diseases, most probably in Asian countries populations [7]. In the human heart, Ace2 is positioned to cardio-myocytes, cardiac fibroblast, epicardial adipose tissues, and the coronary vascular endothelium [8].

Genetic ACE2 mutation results in uplift of Angiotensin II mediated cardio renal fibrosis and oppression in the cardiac system and kidney of stressed mouse while controlling of recombinant human ACE2 rhACE2 substantially recover the hypertension induced by angiotensin II, pathological hypertrophy, oxidant injuries, and heart dysfunctions [9]. Major regulatory role of the ACE2 of the renin angiotensin systems have been well defined in the spreading of diabetes related complexes, including heart diseases and kidney diseases [10].

Epithelial cells of lungs express high level of ACE2, which are relate with airways epithelial differentiations. Participation of ACE2 in acute respiratory distress syndrome, these are triggered by many disorders including SARS-CoV1 and COVID-19 or novel corona virus, have been established in many experimental model [11]. High level of ACE2 expressed to protect from hypertensive activity, while ACE2 low level provoke hypertensions. Nephric ACE2 expressions are correlates with blood pressure in animal model which are hypertensive active. In hypertensive rats and spontaneous hypertensive rat, nephric ACE2 mRNA level is decreased as compared to normotensive Wistar-Kyoto rat [12]. The modulatory effect on the Ang II AT1R and Angiotensin 1-7 made ACE2 credible target in prevention and treatment of chronic inflammations and inflammatory disorders, highlighted with the novel SARS-CoV2 pandemic [13]. Individuals with SARS-CoV2 developed

pneumonia with accelerations of injuries in affected individuals to multi-organs dysfunction by an inflammation causing cytokines and is a highlighted reason of deaths in individuals those are severely affected. Irregularity of Renin angiotensin systems (RAS) and the low level of ACE2 in individuals with SARS-CoV2 are more contributing factor to tissues and systematically induced inflammations [14].

Many researches have revealed modification in corona virus spike glycoprotein and host ACE2 receptors to anticipate and demonstrate capability of human to human transmissions of the viral infection [15]. Although, attention have not been given to explain the effects of natural genetics and expressions of human ACE2 variants for possible vulnerabilities and resistance to the corona virus infections. In vitro analysis have shown that ACE2 expressions are positively related with the corona virus infections [16]. Likewise, genetic variations in ACE2 has been come up with to influence the receptor interactions with the virus spike glycoprotein [17]. Contrary, new introductory studies show no such association among human variants of ACE2 and corona virus infections [18]. Several coding variants of ACE2 in human has been linked with cardio vascular diseases, hypertension, and diabetes [19]. Though many ACE2 variants, evaluated in the current investigations, but to date many functional effects have been expected, no observational data indicate their association with any of disorder or genetic disorders in human. However, no major changes in the general structure configuration of the ACE2 protein variant, only substituted amino acids present on one side of the peptidase sub-domains distant from the catalytic sites of ACE2 were observe compared to the wild variety [20]. It is therefore feasible to say that these ACE2 variants may be subject to neutral selection. Molecular protein modeling and related bioinformatics tools may provide valuable insights to predict the possible variants and complexities of the protein structure [21].

The frequencies of ACE2 distributed between female and male patients in population, as well as among different populations, indicating that the frequency of distribution of the ACE2 was not influence by the gender. In the summery, the study of the frequency distribution of coding variants of ACE2 in the Italian population with respect to global populations indicating a decreased rate of coding

variants in the Italian cohort study of the ACE2 gene, suggested that the susceptibility to infection with Covid-19 which rely on other genetic variants other than ACE2 or other genes [18].The potential contribution of noncoding variants found in the regulatory region such as promoter and enhancer of ACE2 to the risk of Covid-19 infections would be important to examine in this regard. Furthermore, research studies should be carried out to determine potential population specific effects that may explain variable vulnerability to Covid-19 infections in both Italian and foreign populations [13]. A related research on the Chinese population, consistent with our results, examined the genetic diversity of ACE2 in their population, find out a distinct frequency distribution of ACE2 variants in comparison to other populations.In addition, a higher allelic frequency of expression quantitative trait loci (eQTL) variants linked with higher expressions of ACE2 in tissues was found to indicate a different sensitivity or response to Covid-19 infection in comparison with other populations under same conditions [18].

SARS-CoV2 is a positive single stranded RNA virus (+ssRNA) that causes acute respiratory syndromes in humans. COVID-19 binds to their cellular target via ACE2, and its receptor recognition and fusion of cellular membrane are being mediated by Corona Virus spike glycoprotein. COVID-19 s glycoproteins bind to the RBD of ACE2 [22]. The similarity between SARS-CoV2 and SARS-COV1, and the similarity between SARS-CoV2 spike glycoprotein and the RBD of ACE2, indicates a strong binding affinity to human ACE2 in both cases [23]. Furthermore, SARS-CoV2 has a stronger affinity to ACE2 with respect to SARS-CoV1, a factor that likely caused increased individual to individual transmission of SARS-CoV2 [24]. The binding of +ssRNA virus to the RBD of ACE2 determines virus entrance in to the body and the rate of cell injury, which is directly proportional to ACE2 expression [25]. In addition, also ACE2 is expressed in the gastro intestinal tract (GIT), specifically the luminal surface of epithelial tissue, and acts as receptor for amino acid and nutrient up take [26]. This finding suggests that we should consider the feco oral transmission route and COVID-19 related GIT symptoms. Indeed, the widespread distribution of ACE2 across more than one organ may explain the observed multi-organ dysfunction in COVID-19 patients [27].

ACE2 enzyme is a main counter regulator that can degrade Ang II to Angiotensin 1-7, there by attenuating its effect on vaso-constriction, retention of sodium, and fibrosis. Though Ang II is the initial substrate for ACE2, that enzyme can also degrades Ang I to angiotensin (1-9) and take part in the chemical reaction such as hydrolysis of other peptide [28]. Many human studies, tissue samples from different organs, have found that ACE2 is broadly appeared in the lung alveolar epithelial cells, including the cardiac and kidney, as well as the main target cells for site of dominant injury [29]. Interestingly, the circulating level of soluble ACE2 are low and the functional role of ACE2 in the lung appears to be relatively lack under normal states but can be up regulated in some clinical conditions [30].

So, inhibitors of ACE and angiotensin receptor blockers have various effect on Ang (II), initial substrates of ACE2, the effect of following agents on ACE2 levels and activities might be predicted differently. In spite of substantial structural homology among ACE2 and ACE, their enzymes active site is well defined. As a result, ACE receptor blockers in medication are not directly affecting ACE2 performance. During experimental model has revealed mixed finding with respect to the effects of ACE blockers on ACE2 levels or activities in the tissue [30]. Although, available experimental model, there are few studies in human being concerning the effect of RAS inhibition on ACE2 expressions. In a study, the intervention of managing of ACE blockers in individuals with coronary arteries diseases do not influence Angiotensin (1-7) production, observation that call in to questions whether ACE blockers have directly effects on ACE2 directed Ang II metabolic activity [31]. Likewise, in other studies, out of individuals with hypertensive, Angiotensin 1-7 level appears to be un-affected after primary treatment with the ACE receptors blocker. Although, with exposure to captopril one therapy over a duration of 5 months, Angiotensin 1-7 level increases [32]. Moreover, limited studies have been found plasma ACE2 activities or urine ACE2 level in individuals who have received long term treatments with RAS inhibitor. In cross-sectional study involves individuals with heart failure, atrial fibrillation, aortic stenosis, and coronary artery disorder, plasma ACE2 activities were not higher among peoples who were intake angiotensin receptor blockers than among un-treated individuals [33]. During

cohort studies involved Japanese peoples with hyper-tension, urine ACE2 level was high among individuals who received long term treatments with the receptor blockers olmesartan than among untreated control individuals, but that associations were not found with the ACE inhibitors enalapril or with other angiotensin receptor blockers. These seemingly conflict data indicated the complexity underlying RAAS response to pathways modulator and reinforce the concept that findings from pre-clinical model might not readily translate to physiology of human. Such data do suggesting that effect on ACE2 should not be assuming to be uniform across RAS inhibitor or in response to therapy within a given drug substances [34]. It is significant to note down that the plasma ACE2 levels might not be reliable indicators of the function of the full length membrane bounded forms, so ACE2 is shedding from the membranes, a process that appeared as regulating by an endogenous inhibitors [35].

Inhibitors of ACE2, Ang II type 1 receptor blockers, and ibuprofen led to ACE2 up regulation which explains the urgent need to use and identify alternate ACE2 inhibitors. Thus, products derived from medicinal plants or natural products capable of selectively inhibiting the ACE2 recepter without inhibiting the function of the enzyme may be useful for preventing and treating the spread of COVID-19 in the humans without increasing the expression of ACE2 in infected individuals and thus increasing risk for SARS-CoV-2 [36].

Significant similarities existed among the ACE and ACE2 sequences, molecules with inhibitory effects on ACE may have the same effects on ACE2 and thus minimize the viral entry to the body [37]. The medicinally important plant for their inhibition on ACE2 protein. Researchers described about 140 medicinal species belonging to 73 families and 49 natural compounds that was purified with documentary ACE inhibition potentials. However, almost 15 medicinal species were found to be able to inhibit the angiotensin type 1 receptor (Ang T1R) in vitro [38]. More than 24 Chinese plants families were found to significantly inhibit the association of SARS corona virus and ACE2. Among them, species belonging to Polygonaceae, Labiatae, Oleaceae, Magnoliaceae, Lauraceae, and Nelumbonaceae exhibited the most sinificant inhibitory effects. These inhibitory effects

were attributed to emodin (1,3,8-trihydroxy-6-methylanthraquinone) produced in high levels in genus Rheum and Polygonum. Emodin inhibited the interactions of SARS corona virus spike glycoprotein and ACE2 in a dose dependent manner [39].

Turmeric is an everlasting herb that is belong to the Zingiberaceae (ginger) family. It is mostly cultivated highly in many countries mostly in India, Pakistan and China. The rhizome, the parcel of the plant utilized medicinally, yields a yellow powder. Dried turmeric rhizome is major source of curcumin, the fixing that gives curry powder its characteristic yellow color. It has numerous names such as curcum in the Middle Easterner's locale, Indian saffron, Haridra (Sanskrit, Ayurvedic), Jianghuang (yellow ginger in Chinese), Kyoo or Ukon (Japanese) [40]. Curcuma longa has been used in Asian countries for both its flavor and color. In Chinese and Ayurvedic pharmaceuticals turmeric especially utilized as an anti-inflammatory and for the treatment of jaundice, hematuria, menstrual challenges, hemorrhage, and colic. It is official within the Pharmacopoeia of China as well as in other Asians nations such as Japan and Korea and its utilization covers a wide run of wellbeing signs. In China it is taken orally and connected topically for urticarial and skin allergies, viral hepatitis and inflammatory condition of joints, sore throat and wound [41]. Oral administrations are the major route of taking for turmeric, it can also be used topically and via inhalation, Ayurvedic tradition or can be used for the treatments of acne, wound, boils, bruise, blistering, ulcer, eczema, insect bite, parasitic infection, hemorrhage and skin problems like herpes zoster etc [42].

Turmeric ingested orally in case of severe inflammations were found to be as effective as cortisone or phenyl butazone. Turmeric administered orally decreased swelling caused by inflammatory agents. Anti-inflammatory properties of turmeric may be attributed to its ability to stop both bio-synthesis of inflammatory prosta glandin from arachidonic acid, and neutrophil functions during inflammatory state. The volatile oil and also the petroleum ether, alcohol and water extracts of turmeric show anti-inflammatory effects [43]. Turmeric has been considered its influence on anti-inflammatory agents are useful for inflammation disorders. Investigation of

anti-inflammatory effects of turmeric showed a role for inactivation of NF-jB mediated inflammation [44].

Turmeric compounds such as curcumin can inhibit interferon, inducible proteins, Lipooxyenase, Cyclooxygenase, collagenase, phosphor lipases, thromboxane, leukotrienes, prostaglandin, hyaluronidase, monocyte chemo attractant protein 1, tumor necrosis and IL-12. They also decreased prostaglandins formation and stop leukotriene bio-synthesis by the LOX pathways [45].

The anti-viral consequences of turmeric have been reported for the various virus types such as Human immunodeficiency virus (HIV), Hepatitis C virus (HCV), Humans cytomegalovirus (HCMV), Epstein Barr virus (EBV), Bovine herpes virus 1 (BHV 1), Chikungunya virus, vesicular stomatitis virus (VSV), Ebola virus, Entero virus 71(EV71), Rift Valley fever virus (RVFV), Humans Noro virus (HuNoV), Fish viral hemorrhagic septicemia virus (VHSV), Influenza A virus (IAV), Parainfluenza virus type 3 (PIV-3), herpes simplex virus (HSV), flock house virus (FHV) and respiratory syncytial virus (RSV) [46]. Studies revealed that turmeric can prevent infections of virus by interfering with main step in replication cycle such as mode of viral attachment and genome replication as well as modulating cellular event [47]. According to the recent knowledge of literature, there is no report about the derivatives of turmeric to cause any health issue, and its relatively standard doses are beneficial for human health [48]. Studies revealed that patients with high risk or premalignant lesion shows that turmeric does not appear to have harmful effects even at high dosage. Several clinical trials were also performed to check the toxicity in humans which revealed little bit toxicity in humans [49]. The role of turmeric compounds have been broadly studied for in the regulatory process of renin angiotensin aldosterone system (RAAS). RAAS components well known to show its biological activities as antioxidant, anti-inflammatory and antimicrobial actions. Previous studies revealed the role of turmeric in the down regulation of ACE and angiotensin receptor expression in tissues of brain and vascular smooth muscle cells, respectively resulted to inhibiting Ang II AT1 receptors mediates effects of hypertensive and oxidative stress in experimental models [50]. Researchers revealed that the increased levels of AT2R and ACE2 expressions in

cardiac cell can be medicated with turmeric thus exhibiting the protective mechanism of turmeric with modulation of effects mediated by Angiotensin II receptors AT1R and AT2R. Up regulation of AT2R induce repression of AT1R expressions lead to Ang II AT2R mediated anti-inflammatory effect including to inhibit the activity of NF-κB and hypertension. In consequence, medication with turmeric compounds attenuated the pro-inflammatory effects induced by Angiotensin II angiotensin type 1 receptors axis leads lower down the level of pro-inflammatory cytokine TNF, interleukin-6 and Reactive Oxygen Species (ROS) [51].

1.2 Problem Statement

To evaluate anti-inflammatory property of turmeric against ACE2 recepter protein by using in-silico approach.

1.3 Aims and Objectives

We endeavored to undertake an in silico assessment of turmeric to determine its effect on the ACE2 due to its medicinal significance as anti-inflammatory agent, immune-stimulant, antiviral and anti-oxidant that had been proved by numerous scientific studies.

1. To investigate the interaction of turmeric against ACE2 receptor protein targets playing role as anti-inflammatory candidate by using CADD approach.
2. To find out the credibility of the selected compounds for oral bioavailable drugs by predicting the pharmacokinetics.

1.4 Significance of the Solution

In current situation, natural compounds target specific proteins in human cells need to be discovered by in-vitro as well as by in silico study are the basic needs of the era. This will be an effort to improve the natural compounds selectivity like properties of proposed natural compounds as drugs with no or possibly minimum side effects by using computational approaches.

Chapter 2

Literature Review

2.1 Angiotensin Converting Enzyme 2 (ACE2)

Angiotensin Converting Enzyme 2 (ACE2) is integral trans-membrane glycoprotein that is member of M2 family of zinc metallo-peptidases [3]. Tissues of ACE2 are found predominately in the heart, kidneys and testes, and tissues wide variety include colon and lungs [4]. Previous studies reveals that ACE2 is restricted to the endothelium, but later studies showed ACE2 expression in cardiomyocytes, in the kidney on the luminal surface of tubular epithelial cells, and in testes on adult Leydig cells [3]. Similar to ACE, ACE2 has 2 domains: the amino-terminal catalytic domain and the carboxy-terminal domain. The catalytic domain has one active site (the zinc metallopeptidase domain) and shows about 40% sequence similarity with the amino domain of ACE [3, 4]. Despite the sequence similarity of their catalytic domains, ACE and ACE2 appear to act on different peptide substrates. Whereas ACE cleaves Ang I into Ang II, ACE2 cleaves a single residue from Ang II to generate Ang1–7, which has an opposing role to ACE by counterbalancing AT1R-mediated actions. Because ACE2 is a major Ang1–7-forming enzyme, [52] its identification has added further support to the biological significance of Ang-1–7 [4] Of note, gene targeting of ACE results in spontaneous hypotension, reduced sperm function, and kidney malformation [53]. When first ACE2 mutant mouse is

created by using homologous recombination, murine Ace2 gene disruption results in high levels of Ang II, and progressive worsening of the ability of cardiac with age [12]. On the other side, Collectrin Shows about 48% similarity with ACE2 carboxy-terminal zone. Recently collectrin shown to be a non-catalytic protein that has a critical role in absorption of amino acids in the kidney [54].

2.2 ACE2 Genetics

ACE2 is present on chromosome Xp22 and it is a 40 kb gene, apart from ACE gene that is found on the chromosome 17. Remarkably exons of ACE are very closely related with the 18 exons of ACE2. The genetics of ACE2 shows several new polymorphism of ACE2, with specified geographical distributions. It has been described and linked with hypertension and heart diseases [4]. Two alternative mice ACE2 gene transcripts have been found that are likely to occur from alternative splicing [55]. An alternative 5' untranslated human ACE2 exons have recently identified [56].

ACE2 protein in human is basically composed of 805 amino acids. ACE2 is about 40% similar to the sequence of ACE, while it contains only a single catalytic zone. Residues of active site, include the His, Glu, Trp, Tyr, Met, Gly, His and (Zn) binding motifs, are highly sustained. ACE2 protein is orientated with the catalytic site facing the extracellular space and N-terminus, circulating peptides can metabolized by catalytic site. There are number of possible regulatory sites in the small C-terminal cytoplasmic domain. Cytoplasmic domains of ACE2 share similarities with the renal and trans-membrane protein collectrin [5].

2.3 Role of ACE2

Renal juxtaglomerular cells secrete protease renin, which is acting on the angiotensinogen circulating precursor to produce angiotensin I. Angiotensin (I) converted in to Ang II, major effector material of RAAS, by the dipeptidyl carboxyl

peptidase angiotensin converting enzymes, with powerful vaso-constrictive, pro-inflammatory and pro-fibrotic properties. ACE inhibitor and angiotensin II receptor blocker (ARB) are therefore shown effectiveness in the development of stress, cardiac arrest and kidney injuries [57].

Angiotensin fragment other than angiotensin (II), in particular Ang (1-7), which mediated vaso-dilation, anti-proliferation and natural cell death, have also currently suggested to be important, thereby countering the effects of Ang (II) [58].

More complexities were introduced by the discovering of an ACE homologue, ACE2. This enzyme cleaves angiotensin I into Angiotensin (1-9), which can be converted to Angiotensin (1-7) by ACE.

The discovery of an ACE homologue, ACE2 added further complexities. This enzyme cleaved angiotensin I in to angiotensin 1-9, which can be transferred by ACE in to angiotensin 1-7 [59].

2.4 Role of ACE2 in Diseases

2.4.1 ACE2 and Cardiovascular Disease

Globally Cardio vascular disorders are main leading causes of death and lead to cause health related issues in public. The activation of many signaling pathways linked with pathologically induced hypertrophy and mal-adaptation ventricular remodeling decides cardio vascular dysfunction.ACE2 protein is localized to cardio-myocytes, heart fibroblast, cardial adipose tissues and the coronary vascular endothelial [8]. Angiotensin 1-7 is also found in cardio-myocytes, heart fibroblast and cellular endothelial and vascular smooth muscle [60].

ACE2 genetic mutation outcome is in enhancement of angiotensin II mediated cardio-renal fibrosis and hypertensive effects in cardiac tissues and renal system of hypertensively active mouse so the intake of recombinant human ACE2 exceptionally rescue the angiotensin (II) induced stress and cardiac dysfunctions [61].

Many ACE2 polymorphisms are related to cardiovascular disease. Post Myocardial infraction remodeling and coronary arteries diseases are the cause of heart failure [62]. Remarkably, myocardial infraction increases ACE2 mRNA action in hearts of mice, rat and human, although genetically ACE2 deletions result in exacerbating of myocardial infraction caused heart damage, infarcts area and matrix metallo-proteinase activations. Low level of ACE2 lead to upraise neutron-philic penetrations in the infarcts region, results in up regulation of cytokine that cause inflammation, interferon, IL 6, and the chemokines, Monocyte chemo attractant protein I, in addition to enhanced phosphorylation of extracellular kinases signaling pathway, changes that have been blocked with an angiotensin receptor blockers eventually results in maintaining in myocardial functions [63]. Over-expression of ACE2 and the action of angiotensin 1-7 make better myocardial infraction induced cardiac remodeling. Significantly, dropping of ACE2, as seen in explant humans heart patients from with dilated cardio myopathy, was enough to raise chance of cardiac disease [64].

Epicardial adipose tissues are main sources of inflammation causing cytokine that can have harmful impact on cardiac tissues. Low level of ACE2 increase the chance of macrophages polarization to pro-inflammatory phenotype in epicardial adipose tissues from individuals with heart failure by preserve removal fractions, with decrease in polarization to anti-inflammatory, phenotype macrophages, and exacerbates of heart failure with preserved ejected fraction in response to diet induced fatness [65]. Importantly, Ang 1-7 reduced macrophage polarizations in epicardial adipose tissues and preserve the heart functions of heavy ACE2 knockout mouse. Angiotensin 1-7 has potent anti-inflammatory effect on adipose tissues of obesed type 2 diabetes mouse and protects against diabetic heart complexities and nephropathy [66]. The ACE2 axis also provokes browning of adipose tissues lead toward improvement of metabolic effect and reduction of weight, which can deliberate more beneficial to the cardio vascular systems [67].

2.4.2 ACE2 and Vascular Disease

Deleterious arms blockage of the renin-angiotensin system have been the maintainer of the therapeutic managements of hypertensive patients. High level of ACE2 and the vaso-protective side of the RAS by ACE inhibitor and angiotensin receptors blocker clearly reinforce this. In addition, increased ACE2 expression protects against hypertensive activity in body, although decrease in ACE2 function worsen hypertension. Renal ACE2 expression is inversely related to blood pressure in experimental models of hypertension. In the spontaneous hypertensive mice and stroke prone to spontaneous hypertensive mice, renal ACE2 messenger RNA level is decreased compared with normotensive Wistar Kyoto mice [12]. Related study supports the important role of ACE2 in maintenance of normal blood pressure. Lentiviral over-expression of ACE2 result in increased expressions of anti-hypertensive component of the RAS and attenuate raise blood pressure level. Pretreatment with recombinant ACE2 prevented high blood pressure induced by Angiotensin (II) and reduced plasma Ang (II) while increase plasma angiotensin 1-7 level [68].

ACE2 and ADAM-17 were selectively knock out from all neuron, that disclose a reduction of inhibitory input to pre-sympathetic neuron related to regulation of blood pressure normally. Rats with ACE2 selected knock down from Sim 1 neurons in rat exhibited a blunt blood pressure elevations and preserved ACE2 activities during the development of salts sensitive hypertensive rats. The metallo-proteinase ADAM-17 is important for mediating ACE2 shed from the membranes bound zone that can be promoted by Angiotensin II, and releasing as soluble form of ACE2 in plasma [69]. Deficient in genetic ACE2 is linked with the up regulation of accepted mediators of atherogenesis and increases accountably to pro-inflammatory stimulus suggested an important role of ACE2 in suppression of vascular inflammation and Atherosclerotics disorder [70]. Moreover, ACE2 inhibitors blocked neuropeptides catestatinmediated protective effect in the development of atherosclerosis in a rat feed high fats containing food [71].

2.4.3 ACE2 and Diabetes Complications

The counter regulatory role of the ACE2 protein of the RAAS have been well distinguish in the progress of diabetic complexes, includes cardio vascular and kidney diseases [72]. Significance of ACE2 in diabetic patients come from its influence on diabetics complication where in diabetic induced vascular disorders are strong inter linked with move in the RAAS axis toward the pro-fibrotic, pro-inflammatory arms of RAAS decrease in the protective arms. Lack of the protective effect of the RAAS is associated to the regulations of tissues and circulating level of angiotensin II and its sequel in diabetic complications [73], Alteration with in the RAAS are considering as vital for the development of diabetes micro and macro vascular complexes [74].

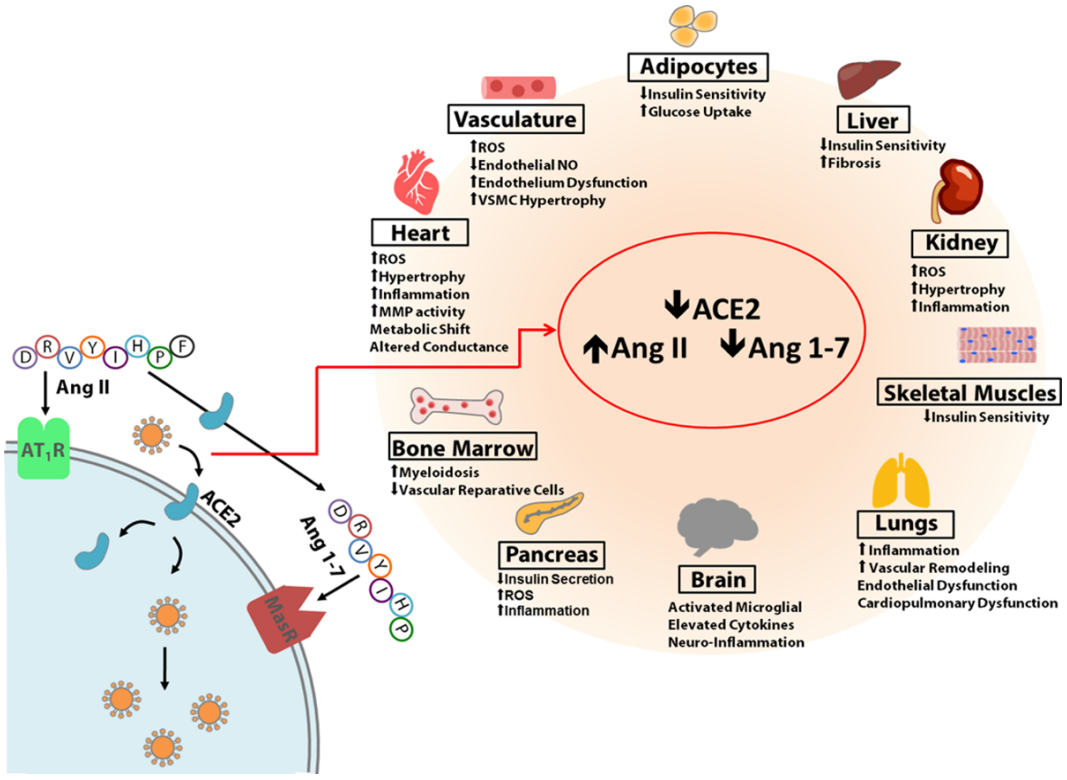


FIGURE 2.1: Low level of ACE2 (Angiotensin Converting Enzyme 2) worsening diabetic cardio vascular complication by the mechanisms of disorders [1]

The low level of ACE2 actions in diabetes complications lift up Ang II and lower down the angiotensin 1-7 level in tissue systemically. Increased Angiotensin II

Type 1 Receptor (AT1R) signaling derived many pathological activities in several end organs raise reactive oxygen species and promotes fibrosis, hypertrophy, and inflammation irritated by the lack of the protective effect of Angiotensin 1-7. Angiotensin II stimulations also systemically altered metabolic profiling and modulate sensitive to insulin influenced areas [1].

The blockage of the pro-inflammatory and pro-fibrotic arm of the RAS provided important kidney protection in both experimental model of diabetic complications and in individuals those have disease. So the low level of ACE2 increases diabetic renal damage, recombinant ACE2 is therapeutics in an experimental model of diabetic nephropathy and experimental Alport syndrome [75]. ACE inhibitors in type 1 diabetes and angiotensin receptors blockage with Losartan and Irbesartan in type 2 diabetes hinder the progression of nephropathy [76]. In diabetic nephric tubules, ACE2 gene expressions are decrease by 50%, which can reduced Angiotensin 1-7 formations and allowed Angiotensin II accumulations hence direct increase the expressions of transforming growth factor beta and growth factors of connective tissues, lead toward tubule interstitial fibrosis [77]. RAS blockage hinders kidney damage and ACE inhibitor therapies, result in a balance increase in ACE2, leading to kidney protections. So, reinforce for the low level of ACE2 contributes to vascular complexes in diabetes mellitus come from strong clinically and experiences of experimental models [78].

Damage to the retina of eye, the very important complications of diabetes mellitus and one of the raising cause of retinal dysfunction in working old individuals is associated with activation of trauma, pro-fibrotic, and pro-inflammatory arms of the RAS that could be effective to reduce by the ACE2 axis in experimental models [79]. Increased secretions of pro-inflammatory cytokines by bone marrow mesenchymal stem cell twist hematopoietic towards the generation of an increased in number of myelo monocytic cell [80]. Targeted tissues of diabetes complication secrete chemokine ligand 2 in response to high level of stress induced by glucose [81] facilitates the homing of ($CCR2^{+}$) cell to these regions and promoted the development of vascular complication [72]. Furthermore to an addition in myeloidosis, diabetes with complication have lowered bone marrow derived vascular reparative

cells and circulating angiogenic cell [82]. Increase in ACE2 mRNA was also important prediction for presence of micro vascular diseases in diabetic individuals. Diabetic patients who remained free of retinal damage despite more than forty years of minor glycemic control had increased mRNA level for gene of the vaso-protective axis of ACE2 compares with sex, age and glycaemia matched diabetic with retino-pathy. Improper function of $CD34^{+}$ cell from diabetes patients, activation of the protective arms of RAS, by showing the cell to Angiotensin 1-7 correct their abnormal function by restoring bio available NO and decrease reactive oxygen species. Angiotensin 1-7 genetic modifications of CD34+ cells restoring the in vivo vaso reparative functions of these cells in a mice retina ischemia perfusion injury models [83]. Moreover, intra ocular administrations of AAV ACE2 or Ang1-7 reduce diabetic induced retinal vascular leakage and inflammatory action, so prevented from retinal damage [84].

Diabetic individuals have unregulated renin angiotensin system, which may have influence their susceptibility to Covid-19. About 2000 individuals were confirmed SARS-CoV-2. Of these individuals, 173 had lethal disease, and of this, 16.3% were diabetic patients [85]. Several studies show that 141 patients those were admitted in the hospital due to severe infection of their SARS-CoV-2 infection, of these patients, 13% have effected with diabetic complications. It is interesting to know why diabetic individuals may be more susceptible for Covid-19 infections than the rest of population, and that was might be due to the decreased ACE2 level that were typically found in the vasculature of diabetic patients and diabetic animals model [86]. Uncertainly, reduced of ACE2 was linked with marked gut-dysbiosis, which was further add to in experimental model with diabetic Type 1 patients [82].

2.4.4 ACE2 and Lung Diseases

Epithelial cells of lungs express increased level of ACE2, which positively lies with the airways epithelial differentiation [87]. Involvement of ACE2 in acute respiratory distress syndrome that is activated by many diseases including SARS

corona virus and COVID-19 have been accepted in many experimental model [11]. ACE2 knockout mice show serious pathology for respiratory distress syndrome [88]. Further deficient in ACE, or treating by AR1 inhibitors of ACE2 knockout rat save them from respiratory distress syndrome incriminate the benefits of ACE2 and the analytic balances of the protected versus pro-inflammatory and fibrotic axis of renin system [89]. These kind of discoveries are compatible with evidences of beneficent effects of recombinant human ACE2 on lungs blood pressure and oxygenated process in an animal model of lipopolysaccharides inducing lung injury [90]. These kind of discoveries are compatible with evidences of beneficent effects of recombinant human ACE2 on lungs blood pressure and oxygenated process in an animal model of lipopolysaccharides inducing lung injury. Age related low level of ACE2 in the lungs co-relates with increasing death rate and damaged phenotype in old age individuals infected with SARS-CoV-2 [45]. ACE2 has been involve in severe pulmonary injury by getting an unbalance state in the RAS. Deposition influence in severe pulmonary injury.

1. Lungs ACE2 decrease and angiotensin II increase
2. Supply of ACE2 or inhibit in action of angiotensin II can enhance possible results
3. A lack of lungs ACE2 put out viral induced severe lungs injury

ACE2 plays role in maintaining of PH and fibrosis. Enhancing the activity of ACE2 lowered bleo-mycin inducing inflammation and fibrosis, results in improved lungs functionality and exercise range, and the ACE2 activator, protected animals from PH and fibrosis [88]. In addition, orally administrated a bio encapsulated forms of ACE2 protected and arrested the development of pH [91]. In addition, orally administrated a bio encapsulated forms of ACE2 protected and arrested the development of pH. Acceptance of its protecting effects come from researchers studies that is showing that pH is specified by decreased ACE2 activities and supplement of these individual with recombinant human ACE2 improves human lungs hemodynamics and decreases oxidative and inflammatory marker [92].

2.4.5 ACE2 and Hypertension

Activations of RAS activity is considered to be a primary hypertension modulator, and the most widely used of all blood pressure decreasing agents are therapies to block RAS activations. The anti-hypertensive efficacy of these agents is partially mediated by their capacity or signaling of these agents to minimize angiotensin II. Anyway, the anti-hypertensive effects of traditional RAS blocking are also partially determined by the ability of angiotensin receptor blocker and ACE inhibitors to increase circulating angiotensin 1-7 levels. In addition, inhibiting Ang 1-7 vascular activity in spontaneously hypertensive mice received RAS blockage attenuation the anti-hypertensive response to these medications [93].

Surely, ACE2 expressions are abnormal in spontaneous hypertensive rats, in which one genetic component of this phenotype tracks to the locus of ACE2. In addition, ACE2 deficiency is co-related with modest systolic hypertension, though the mice genetic background significance alteration of the cardio vascular phenotype. ACE2 knout out mouse also having a heightened hypertenssion response to angiotensin (II) mixture linked with magnified aggregation of Angiotensin (II) in the kidneys. ACE2 knout out mouse also having a heightened hypertenssion response to angiotensin (II) mixture linked with magnified aggregation of Angiotensin (II) in the kidneys [94].

ACE2 and RAS are also involved in pathology of main hypertension. Specially, the rostral ventro-lateral medulla is a relay point that provides supra-spinal excitatory input to sympathetic pre-ganglionic neuron in the regulations of healthy blood pressure. In the spontaneous hypertensive rats, ACE2 expressions are decreased in the rostral ventricular medulla [95] and persistent over expression of ACE2 in the rostral ventricular medulla results in an important attenuation of high blood pressure in these models [96]. Additionally, the injection into the nucleus tractus solitari of the ACE2 inhibitor MLN4760 decreases reflex bradycardia in response to baroreceptor stimulation in mice, indicating an additional role for the main ACE2 in the regulations of baroreceptor responsiveness [97].

2.4.6 ACE2 and COVID-19

COVID-19 Pandemic

On 11^{th} March, 2020, the WHO talk about the outbreak of COVID-19 a worldwide pandemic, reported individual to individual transmissions occurring in all over the world. After, the pandemic has increased rapidly to well over millions cases and became cause of millions of deaths worldwide by the end of March and start of April 2020. Though, prior to appearances of Corona Virus in 2002, coronaviruses were habitually assume as unimportant pathological agent that is circulating naturally in several hosts and many species that can transfer infection to humans which is only causing mild upper respiratory tract infection and symptom of the common flu [98]. Additionally, for understanding of the seriousness of worldly health issues produce by Covid-19 and improve treatments for infected individuals. We should know the function of ACE2 in Covid-19 infection. Furthermore to respiratory involvement and multi organ malfunctioning occurs in response to Covid-19 infections [99]. Although respiratory symptoms are pre-dominant, severe heart and kidney injury, gut, and hepatic abnormalities have also noted in infected individuals, suggests myocardial, renal, enteric and liver impairment in SARS-CoV-2. Likewise, Corona virus infection also results in systemic expression with damage to cardiac tissues, gut, liver, kidney, and many organ tissue [100].

2.5 ACE2 as receptor for Covid-19

Covid-19 is eventually differentiated from the old SARS Corona Virus of 2000 by 380 amino acids substitution, which interpret to variations in five of the six important amino acid in the receptors binding domain among the virus spike glycoprotein with surface revealed hACE2 [101]. Virus spike proteins are well established as an important influence of host tropism and represents a key target for therapeutics and drug preparation. In addition, protease of host cells are significance for Corona Virus entry and infected cells as both spike glycoprotein

and ACE2 are proteolytically modified during process. Covid-19 binding affinities with ACE2 appears to be highly strong than old Corona Virus, with changes in various amino acids residue allow for increased hydrophobic interaction and salt bridge emergence, which may show the considerable worldwide effect of severe respiratory syndrome than the starting SARS-CoV [102]. Additionally, Covid-19 has developed to make use of a broad disposition of host protease includes Cathepsin L, Cathepsin B, trypsin, factor X, elastase, furin, and trans-membrane proteases serine 2 for spike protein prepare and ease the cell entry follow the receptor bindings [103]. Until now, trans-membrane proteases serine 2 and cathepsin B facilitates spike glycoproteins prime of Covid-19, and a serine proteases inhibitors combined with Cathepsin B inhibitor blocked Covid-19 infection [104].

The entrance of initial SARS-CoV and Covid-19 in to cells is ease with the interactions among virus spike proteins with extra-cellular domain of the trans-membrane ACE2 protein, observed by successive down regulation of epithelial ACE2 expressions (Figure 2.2) [105]. In cohort study of 13 SARS-CoV-2 infected individuals, circulating Angiotensin II level was noticeably raise compare with health control linearly related with virus loads and provid directly linked between tissues ACE2 down regulation with systemic RAS imbalances, and facilites the development of multi organ damage from Covid-19 infection [106]. Possible therapeutics strategy may include stopping of the binding of humans ACE2 and Covid-19 by preventing the receptors binding domain (RBD) of the virus spikes glycoprotein. Additionally, this receptors binding domain preventing strategies, other possible treating options might include confine uses of ACE2 driven peptide, small molecules inhibitor, ACE2 antibodies and one chain antibodies fragments against ACE2 [1].

ACE2 mediated cardio vascular protections are lost following endocytosis of the enzymes along with SARS-CoV-2 severe and acute respiratory syndrome viral agents. Angiotensin II level raise with increased activities of Angiotensin type 1 receptor (AT1R) at the cost of ACE2 or Ang 1-7 driven pathway leading to adverse fibrosis, hypertrophy, increased reactive oxygen species (ROS), vaso-constriction, and gut-dysbiosis.

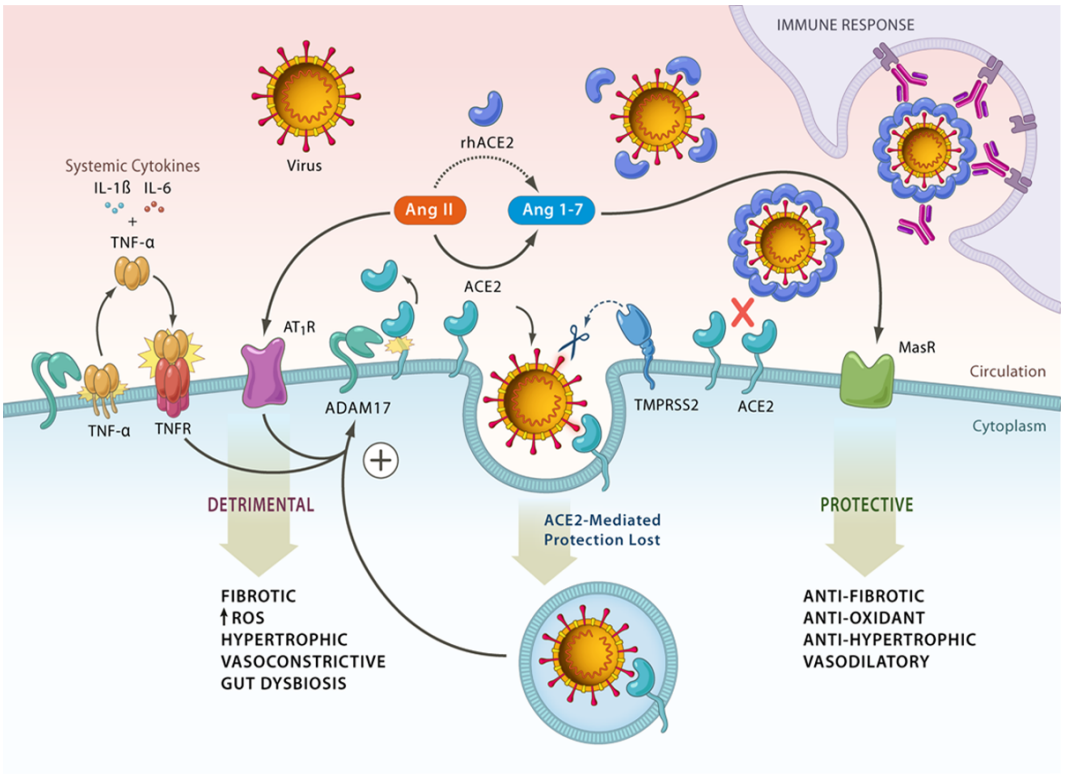


FIGURE 2.2: Significant role of ACE2 in COVID-19 [1]

ACE2 mediated cardio vascular protections are lost following endocytosis of the enzymes along with SARS-CoV-2 severe and acute respiratory syndrome viral agents. Angiotensin II level raise with increased activities of Angiotensin type 1 receptor (AT1R) at the cost of ACE2 or Ang 1-7 driven pathway leading to adverse fibrosis, hypertrophy, increased reactive oxygen species (ROS), vaso-constriction, and gut-dysbiosis. A Disintegrin and Metallo-proteinase-17 (ADAM-17) mediated proteolytic cleavage of ACE2 is up regulated by endocytosed SARS-CoV-2 spike proteins. Activation of the AT1R by elevated Ang II levels also further increases ADAM-17 activity. ADAM-17 correspondingly also cleaves its primary substrate releasing soluble TNF-α (tumor necrosis factor-α) into the extracellular region where it has auto- and paracrine functionality. TNF-α activation of its tumor necrosis factor receptor (TNFR) represents a 3^{rd} pathway raising ADAM-17 activity. TNF-α along with systemic cytokines released due to COVID-19 infections and in conjunction with comorbidities such as diabetes mellitus and hypertension may result to a cytokines storm. [1].

2.6 Association of ACE2, ADAM-17 and Inflammation

TNF-α (Tumor Necrosis Factor-α) is a type of cytokine which is involved in inflammations, and its extracellular domains let go and activation is derived from the membrane bound proteases coined TACE (TNF-α converting enzyme), also known as ADAM17 (A disintegrin and metalloproteinase-17) [106]. ADAM17 is a type I trans-membrane protein belongs to the adamalysin sub-family of zinc dependent metallo proteases [107]. From the discoveries that ADAM17 breakdown into TNF-α, the substrates specification of the enzymes have inflate to include many cytokines and receptor, a lot of which contributed to initiate and worsen the inflammation [107]. Significantly, ADAM17 was also begin to conciliate proteolysis and ecto-domain get rid of ACE2 [108].

Angiotensin II mediated activations of AT1 receptor activates a signaling cascade leading to the activations of p38 MAPK (mitogen activated protein kinase) and ADAM17 phosphorylation by NAPDH oxidase 2 induced reactive oxygen species formation [109].

Phosphorylation upgrade the stimulant activity of ADAM-17, while enhancing the disperse of ACE2, resulting in low level of ACE2 at the membrane and impaired conversions of Angiotensin II (Ang 1-7), leads towards RAS mediated harmful effect in a positive type of cycle feedback [110]. Most importantly consumption of ACE2 at the cell surface is a critical pathological infection outcome of Covid-19. Covid-19 is endocytosed by cell to make a complex with ACE2, while, the primary damaging effect of virus infections began with lack of tissues protection mediated by ACE2 [111]. ADAM17 activities are up regulated upon binding of corona virus with ACE2 and facilitate the virus entering in body, although knock down of ADAM17 by small interfering RNA with severe weakened SARC corona virus cellular entry. The molecular mechanism of SARS corona virus and another type of human corona virus that only cause acute respiratory symptoms, HNL63 corona virus, were compared. Though HNL63 corona virus also uses ACE2 as a receptor

during cellular entry, it was not induced ADAM17 activations and shedding of ACE2 ecto domain [112]. As a consequence, this type of study clarify the major role of ADAM17 mediates shed of ACE2 in corona virus infectivity and may inform the inconsistency in seriousness among corona virus sub-types. In addition, lack of membrane ACE2 promoted Angiotensin II accumulations, which also activated ADAM17 activities, so keep in existence membrane shedding of ACE2, RAS over activation, and inflammatory effects of lungs [108].

2.7 Association of ACE2, COVID-19 and Inflammation

The regulatory effect on the angiotensin II or AT1R and Ang 1-7 MasR axis make ACE2 a feasible targets in prevention and treatment of severe inflammation and inflammatory disorders, as highlighting with novel SARS-CoV-2 pandemics [113]. Patients with SARS-CoV-2 developed lungs inflammation with increasing of injuries in easy targeted patient to multi-organs damage derived in part by inflammatory cytokines storm and is a remarkable cause of deaths in individuals those are critically affected. When the immunity is activated due to factor such as COVID-19 infections, there will be an unbalance of Th17 or Treg cells functionality and over activation of immune system, which secretes huge number of pro-inflammatory cytokine. Unbalancing in the RAS system and the decrease in ACE2 in individuals with SARS-CoV-2 are more contribution of factor to tissues and systemic inflammation [114]. Lipopolysaccharide induced severe lungs injury reduced the expressions of ACE2, precipitated inflammatory injuries, and up regulated expressions of (RAS), Angiotensin II, ACE, and angiotensin type 1 receptor. After administration of recombinant human ACE2, lung functions and pathological injuries improving with attenuation of inflammation. In addition, rhACE2 is very useful and can overcome the severe lungs injury induced by Covid-19, acids inhalation, and sepsis [115].

ACE2 knock out rat explored severely acute respiratory distress syndromes (ARDS) or acute lungs injury disorder, increasing vascular permeability, increasing pulmonary edema, neutrophil accumulations, and deteriorations of lung functions compared with normal and controlled rat [116]. Deficient in ACE particularly treated the severe phenotypes of rat with one mutation of ACE2 in acute lung injury by more deletions of the ACE gene, suggested that the balances of ACE2 or level of ACE play an important role in lung injury or lungs protection during an inflammation storm. Angiotensin receptor blocker induced ACE2, Angiotensin 1-7, and Mas expressions in line with the reduction of pro-inflammatory cytokine and induction of Interleukin-10, an anti-inflammatory cytokines. We revealed that ACE2 knock out hypertensive rat showed increase of pro-inflammatory cytokine, IL-1β, Interleukin 6, and TNF-α, and chemokine ligand 5 while taking of recombinant human ACE2 rescuing Angiotensin II inducing T-lymphocyte mediated inflammations. Blockage of Mas receptor by D-Ala7-Ang 1-7 completely obstruct the Ang 1-7 mediate anti-inflammatory effect while AVE0991, the boost of Angiotensin 1-7 receptor, imitate the action of Ang (1-7) [117].

2.8 ACE2 as Therapeutics Target

2.8.1 Pharmacologically Antagonist of the RAAS and ACE2 Expressions

Recent pharmaco-therapies focus to attain several level RAAS blocked by distinct mode of actions. Even though ACE2 proteins are not the direct cellular targets of these therapeutics, ACE2 genes transcription, translation, and ultimately catalytic activity is modified due to the complex nature of the RAAS [118]. Pathologic neuro hormonal activations of the RAAS encourages the growth and development of cardiovascular disorders. Inhibiting the ACE, angiotensin II and AT_1 receptors through limiting the formation and actions of Angiotensin II potentiate the effect of ACE2 as the endogenous RAAS counter regulator. The angiotensin receptor

blockers frequently increasing ACE2 mRNA expressions, proteins level, and catalytic activity in the heart, kidney, and thoracic aorta, but the translation to the proteins level and activities differ among animal model and tissue for ACE blockers [119]. Combinations of lisinopril and losartan treatment in the mice that have normal blood pressure abolish the rise in level of ACE2 mRNA level seen individually but remained losartan induced increase in ACE2 activities in the heart. Furthermore, lisinopril in mice those have normal blood pressure raise ACE2 mRNA without affecting ACE2 activities in the heart, but the incompatibility was found in the kidney [120]. These observations can be referred to tissue-specific regulations of ACE2, as increase ACE2 proteins level was observed in the heart, but ACE2 activities were increased in kidney of experimenter mice, in addition to the complexity of the tissues RAS [121]. Dual RAS blockage with perindopril and losartan normalizes diseases in type 1 diabetes Akita angiotensinogen transgenic rats mediates a decrease in the appearance of ACE2 mRNA and protein levels in the kidney [122].

Ang II can regulated ACE2 expressions through the AT_1 receptor. Human healthy body parts are characterized by having ACE2 mRNA and protein expressed in high level these healthy organs are heart and kidney, with average ACE appearance. RAAS over activation in CVD increases angiotensin receptors stimulation by Ang II, promoting ERK1/2 and p38 MAP Kinase signaling pathways to down regulate ACE2 while up regulating ACE expression [123]. Activations of p38 MAP Kinases up regulate ADAM17 activities are though post-translational phosphorylation of the cytoplasmic domain resulted in shedding of surface ACE2 in a positive feed-back loop and can explain the effect of angiotensin receptor blocker in increasing ACE2 protein levels and activities [124]. Enhancement mechanism of ACE2 mRNA level by ACE inhibitor and angiotensin receptor blocker required more characterization. Furthermore, mineralocorticoid receptors antagonist increase ACE2 mRNA expressions and functions in sample form patient with persistent heart failure, wild type rat, and rat to varying degree between tissues but not in the cardiac system of a mice high blood pressure diseased models [114]. Spironolactone, a non-selective mineralocorticoid receptors antagonist, avoided the increase

in both ACE and AT_1R mRNA level, and the linked increasing in AT_1R density from aldosterones signaling in cardio-myocytes [125]. Activations of mineralocorticoid receptor also stimulated overlapping down streams signaling pathway with AT1R, includes the ERK2 and p38 MAP Kinases pathway [126].

TABLE 2.1: Pharmacological agents and their effects on RAS system and Genetics of ACE2

Pharmacological and their effects on RAS compounds as well, Genetic ACE2 Expressions, Proteins Level, and Cellular Activities				
Pharmacological Agent	**Experimental Model or Subject**	**Tissues**	**Observations**	**Ref**
ACE Inhibitors				
Lisinopril	Lewis rats	Heart	Decrease in Plasma Ang II, increase in plasma Ang 1-7 and ACE2 mRNA,But do not have effect on cardiac ACE2 activity	[127]
Enalapril	Coronary artery ligation in sprague Dawley rat	Heart	Increased in plasma and cardiac ACE2 activity	[128]
Lisinopril	Transgenic Ren2 rats	Heart or kidneys	Decrease in plasma Ang II, increase in Renal ACE2 mRNA and activity	[129]
Lisinopril	Lewis rat	Kidney	No change in kidney ACE2 mRNA but increase ACE2 activity	[120]
Angiotensin receptor blocker				

Losartan	Coronary artery ligation in Lewis rat	Heart	Increase in plasma Ang II and ACE2 mRNA 28 days post-surgery	[118]
Irbesartan	C57BL/6 mice	Heart	Increase in cardiac ACE2 mRNA, Irbesartan prevented Ang II induced decrease in ACE2 protein level	[108]
Losartan	Transgenic Ren2 rats	Heart or kidney	Increase in plasma Ang II, Ang 1-7 and renal ACE2 mRNA and activity	[129]
Telmisartan	C57BLKS/J mice	Kidney	Following 2 weeks administration, increased ACE2 protein level	[130]
Irbesartan	C57BL/6 mice	Aorta	Treatment with irbesartan significantly augmented ACE2 protein level	[119]
Mineralocorticoid receptor blockers				
Spironolactone	Patients with heart failure	Monocyte derived macrophage	Increase in ACE2 activity and ACE2 mRNA expression	[131]
Eplerenone	Balb/C mice	Heart or Kidneys	Increase in cardiac ACE2 activity	[131]

Eplerenone	Wister rat	Heart	Prevent aldosterone induced reduction in cardiac ACE2 mRNA expression	[114]

2.9 Effecting ACE2 activity by small Molecules Drugs

Promoting the ACE2 or Angiotensin 1-7 by recombinant human ACE2 or the Angiotensin 1-7 receptor agonists AVE0991 could have beneficial therapeutics effect in heart disorder and lungs disorder from adverse etiology. The Angiotensin (1-7) receptors agonist AVE0991 have been shown to exert cardio-renal and lung protective effect, and treated with recombinant human ACE2 improved the symptom of acute lung injury, heart, and kidney injury in many pre-clinical models. Maintaining ACE2 level in individuals with or predisposed to common cardiovascular disease states such as diabetes mellitus, high blood pressure, and obesity wards off the advancement of these co-morbidities to point where the patient deal Covid-19 by maintaining negative counter regulation level of ACE2 or Angiotensin 1-7 [132].

In order to prevent spike glycoprotein associations with endogenous ACE2 while spontaneously controlling RAAS system, recombinant human ACE2 functionally suitable circulating virus particles may provide therapeutic benefits in SARS-CoV-2 and is entering phase II clinical trials in European countries [133].The possible drawback of recombinant ACE2 protein is the limited penetration and function of its large molecular size against tissue RAS. In particular, pharmacological RAAS blockade agent, receptor blockers have ability to modulate both systematic and tissue RAAS and high level of ACE2 expressions and activities experience in experimental model at the same time. In SARS-CoV-2 individuals with hypertension, the direct effects of RAS inhibition remained unclear, clinical evidences are miserably required to find out the related pros and cons of linked with the use of

drugs [134]. The introduction of angiotensin receptors blocker in individuals already infected with SARS-CoV-2 can, however, be an effective therapeutic choice to resolve the viral mediated RAS imbalance and is currently being investigated in many clinical trials [1].

Possible use of ACE2 as a treatment is also mediated by the use of *Lactobacillus paracasei*, a probiotic species which may be modified to show recombinant protein. Mouse treatment with recombinant Lactobacillus paracasei showing secreted ACE2 by fusion with nontoxic cholera toxin subunit B acting as a trans-mucosal transport facilitator expresseded increased in ACE2 level in organs tissue to decreased diabetes retino-pathy [135].

2.10 Turmeric (*Curcuma longa*) Compounds targeting ACE2

Scientific name of turmeric is "*Curcuma longa*". This flowering plant is belonging to ginger family, Zingiberaceae. It is a plant which is rhizomatous and perennial plant. The height of turmeric herb is 1 m long. The leaves are arranged in alternately and divided into two rows. They consists of sheath of leaves, a petiole and a blade of leaves. From the leaf sheath, a false stem is produced. The leaf blades length are normally 76-115 cm and width of 38-45 cm. Its shape is oblong to elliptical and narrowing at the tips. The flowers are bisexual and zygomorphic. Through the three compartments, the fruit capsule opens. From approximately 110 species of the genus Curcuma L., about 20 species phyto-chemically have been studied [136]. The most chemically studied genus of Curcuma is turmeric. At least 235 compounds, primarily phenolic compounds and terpenoid, along with diarylheptanoid, diarylpentanoid, monoterpene, sesquiterpene, diterpene, triterpenoid, alkaloids and sterols have been identified to date. At least 235 compounds, primarily phenolic compounds and terpenoid, along with diarylheptanoid, diarylpentanoid, monoterpene, sesquiterpene, diterpene, triterpenoid, alkaloids and sterols have been identified to date [137].

2.11 Medicinal significance of Turmeric in Multiple Diseases

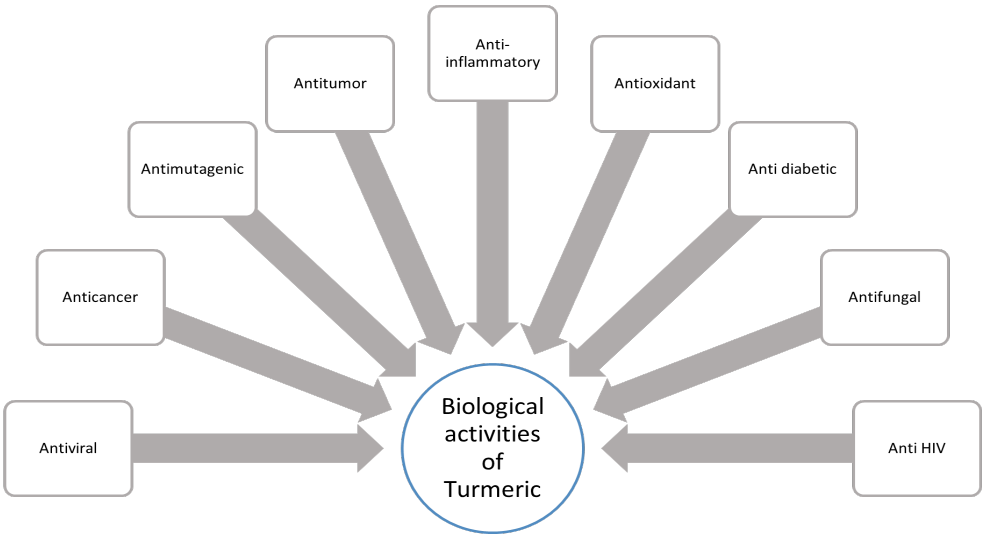


FIGURE 2.3: Biological activities of Turmeric [2]

2.11.1 History of Turmeric

It is unknown the precise origin of turmeric. Ayurveda is an old Indian, natural healing method that is still experienced. Ayurveda refers to "science of life"– ayur means "life" and Veda means "science or knowledge". Inhaling gases from burning of turmeric has been utilized to alleviate congestion since old times. For the healing of wounds, turmeric juice was utilized. All kind of skin disorders (smallpox, chicken pox, blemishes and shingles) were also treated with turmeric extract. There are about hundred different terms for turmeric used in Ayurvedic literatures such as Jayanti meaning "one who is victorious over diseases" and Matrimanika meaning "moon light". For the healing of wounds, turmeric juice was used. In the Asian countries, it is most favorable material, both among Aryan and the Dravidian cultures. Its impotence can stretch far to the beliefs of old native peoples in the history. Usually in the north turmeric is known as haldi that is derived from the Sanskrit word haridra, and in south as manjal a word derived from Tamil literature.In Asia turmeric has revealed long history of

medicinally importance especially in southern areas and commonly used in the system of Ayurvedic and Unani. Ayurvedic collection, dated to 250 B.C., suggests a turmeric-containing ointment to alleviate the symptoms of food-poisoning.In Hindu culture and worship, turmeric has a significant value. It is used to worship the God of the Light. Turmeric also used during India's solar era to worship the sun. In the Ayurveda of India, it was reported. It is also worn by the peoples as method of purification. The use of turmeric is recorded in different forms in India. Buddhist also has been used turmeric. Buddhist monks also traveled to different places of the globe to dye their robes. There is also evidence that turmeric was used about thousand years ago as part of herbal medicine in China.It was referred to as Pent Sao of the seventh century in China. Turmeric was not part of European countries till recent study. There has been only few indications showing its use significance in Western world. Although turmeric has always been a significant component of the Ayurvedic Systems.Until the late twentieth century, herbalists of western countries did not acknowledge the turmeric advantages. However in the middle of twentieth century, turmeric began to gain prominence globally too. To find its advantages, there are several research studies and experiment done today. In 1280 AD, as it was used for dying the clothes, Marco polo referred to turmeric as Indian Saffron. He claimed that he discovered a kind of herb with all saffron characteristics, but it's a rhizome. By the 700 AD, turmeric used by the Chinese peoples, by 800 AD to eastern Africa and by 1200 AD to western Africa and started to become famous all over the world. In China, turmeric is used as a medicine, especially medicines for spleen, liver and stomach. They use it as an antibiotic, antiviral and analgesic for relaxation and purification. The most famous producers and consumers of turmeric is subcontinent Asian. Bangladesh, Pakistan, Sri Lanka, Taiwan, China, Burma and Indonesia are among the other producers in Asian countries. In the Caribbean and Latin America, turmeric is also grown in Jamaica, Costa Rica, Peru and Brazil also. It is identified all over the South and South East Asia with a few species spreading to China, Australia and South Pacific. Around 15th century Vasco-de-Gama, a Portuguese sailor, after visiting to Indian cultures, brought this spice to the Europe [138]. Turmeric

is considering as a significant spice all over the globe especially in the Eastern peoples [139]. Apart from its use as a spice, Turmeric is also used as folk medicine in Asian countries like India, Bangladesh and Pakistan because of its medicinal properties [140]. Turmeric is commonly used as domestic cure in different diseases in Ayurveda, Unani and Siddha medicines [141].

2.11.2 Use of Turmeric as Folk medicines

Folk medicines, turmeric extracts have been used in curative preparations over the hundreds of years in several parts of the globe. In Ayurvedic practice, turmeric is used in different therapeutics properties includes, such as strengthen the energy of body, relieving gas, eliminate worm, improvement of digestive system, regulation of menstrual cycle, dissolving gall stones, and relieves joints pain. India, Bangladesh, Iran use turmeric as an anti-septic for healing of wound, burns, and bruises, and as an anti-bacterial agent. In Pakistan, it is used as an anti-inflammation substance, and as a remedy for gastro-intestinal discomfort linked with irritable bowel syndromes and many digestion problems. In Pakistan and Afghanistan, turmeric is thought to clean cuts and initiate their recovery by applying it on a piece of burn clothes that are placed over a cut. Indian people use turmeric, in addition to its Ayurvedic application, to purify blood and for treatment of skin allergies. Mixture of turmeric is used by female in different parts of Asian countries to remove superfluous hair and also applied to body of the brides and grooms prior marriage in some parts of India, Bangladesh, and Pakistan, where it has an idea to make to glowing skin and keep disease causing bacteria away from the body. Recently turmeric is utilized in the formulations of many sun-screens. Many multinational business are interested in producing turmeric containing face cream [142].

In Ayurvedic medicines, turmeric has been well documented for treatments of many respiratory disorders such as pleurisies, bronchitis hyper activity, and skin allergies, as well as for liver disorders, auto immune disease, wounds due to diabetes, flu, cough, and sinuses [143]. In traditional Chinese medicines, it is used for treatment of disorders linked with abdomen pain [144]. From old times, as

prescribed by Ayurvedic medicines, turmeric has been used to treat sprain and inflammation [143]. In both Ayurvedic and traditional Chinese medicines, turmeric is considered a bitter digestive and a carminative. Unani practitioner also used turmeric in order to remove phlegms or kapha, as well as to open blood vessel in order to increase blood circulation. It might be integrated in to diet, including rice and bean dishes, to boost regulation of digestive system and minimize gas and bloating. It is a cholagogue, which promote to the productions of bile in the liver and also facilitates bile excretions through the gallbladders, which can enhance the abilities of the body for digestion of food. Though, turmeric mixture with milk or water is administered to treat digestive disorders as well as cold and sore throat [142].

2.12 Use of Turmeric as Modern medicine

Turmeric has been linked to many medical characteristics. Rhizomatous part of turmeric is also known to have therapeutic properties and has been using as an anti-diabetic, hypolipidemic, anti-inflammatory, anti-diarrheal, hepatic protective, anti-asthmatic and anti-cancer drugs. Turmeric is widely used in treatment of skin [145].

2.12.1 Respiratory Disorder

The extracts from turmeric root is given in respiratory disorders. In coryza and cough boiled turmeric in milk and mixed with jugglery given internally. In continuous cough, and throat infections the decoction of rhizomes are used for gargling and also the pieces of rhizome is slowly burnt and given for chew the pieces [146]. The chemical constituent of turmeric like Tumerone, curcuminoid, Curcumin and tetrahydro-curcumin have anti-asthmatic actions. In asthma and congestion patients, fumes of turmeric dhumvarti is given [147].

2.12.2 Inflammatory Disorder

Turmeric has been revealed to inhibit a number of varying molecule involves in inflammation including phospholipases, LOX, COX 2, leukotriene, thromboxane, prostaglandin, nitric oxide, collagenase, elastase, hyaluronidase, MCP-1, interferon inducible proteins, tumor necrosis factors, and IL-12 [148]. Many researchers proved bis-demethyl curcumin (BDC) is more potent as an anti-inflammatory agents as shown by suppressions of tumor necrosis factor induces NF-κb activations, much powerful as an antiproliferative agents, and more strong in inducing reactive oxygen species (ROS). Hispolon analogues, which lacks one aromatic unit in relation to turmeric compounds, also exhibited enhanced anti-inflammatory and anti-proliferative activity [149]. The beneficial effects of turmeric (anti-inflammatory compounds) in sepsis appeared to be mediated by the up regulation of PPAR-γ, led to the suppressions of pro-inflammatory cytokines, tumor necrosis alpha expressions and released [150].

2.12.3 Diabetes Mellitus

In a scientific and systemic analysis shows the anti-diabetic, hypolipidemic and hepato-protective effects of turmeric freeze dried rhizome powder milk dissolved that can be useful as an effective and good safety anti-diabetic dietary supplements of high capacity [151]. Turmeric is known to have curcumin, glycoside, terpenoids, and flavonoid. Maximum inhibition of the enzyme human pancreatic amylases were obtaining with turmeric isopropanol and acetone extracts. These inhibitory actions on human pancreatic amylases induce reduction in starch hydrolysis lead to lower the glucose level [152].

2.12.4 Cardio-Vascular Disease

The anti-oxidants present in turmeric prevents cholesterol damage, thus help to protects against atherosclerosis. Indeed, the ability of anti-oxidant to reduce free

radicals in turmeric is close to that of vitamin C and E. So the anti-oxidant activity of turmeric is not degrade by heat (unlike most vitamins), even use as a spice in cooking that provides beneficial effects. Animal study shown that turmeric lower the cholesterols and tri-glycerides and other fats that circulate in the blood stream and is a risk factor for cardio vascular disorder. In a newly study of atherosclerosis, mouse was feeding a standard American food, high in refine carbohydrate and fats, but low in fibers. Some of the mouse, however, received this food plus turmeric mixed in with their food. Approximately after five months on this controlled foods, the mouse that consume the turmeric with their diet had about twenty percent less blockage of the artery system, than the mouse feed the diets without the turmeric [153]. During a recent research, rabbits were feed with turmeric containing food prepared to cause atherosclerosis. A lot of risk factor for the diseases were improved, including reduce in cholesterols, tri-glycerides, and free radicals damages [154].

2.12.5 Neuroprotective Activity

Turmeric significantly decreases the adverse effects of ischemia by attenuating nitrosative and oxidative stress. Ischemia causes by collapse of capacity of mitochondrial membranes, cytochrome C release, and alteration of the Bax-Bcl 2 ratio and subsequent capiases activations leading to induction of apoptosis in sequential fashion was reversed importantly by turmeric compounds. Therefore, there is evidence for the clear neuroprotective effectiveness of turmeric oil, with an outstanding therapeutics window for the prevention of ischemic brain injury [155].

2.12.6 Alzheimer Disease

Turmeric when feed to over age mouse with advanced plaque deposited equilent to those of Alzheimer disease, turmeric decrease the plaque depositions. It decreased oxidative damage and reversed the pathology of amyloids in transgenic mouse

with Alzheimer's disease. The potent anti-oxidant and anti-inflammatory effects of turmeric have also relieved Alzheimer's disease symptoms marked by inflammatory and oxidative effects [156].

2.12.7 Anti-cancer Activity

Turmeric has been found to have anti-cancer activity with its effects on a change of biological pathway involving in mutagenesis, oncogene expressions, cell cycle regulation, natural cell death, tumor genesis and metastasis. Turmeric has shown anti proliferative effects in many kinds of cancer, and is a blocker of the transcription factor NF-B and downstream genes product (including Bcl 2, COX2, Cyclin D1, TNF a, IL and NOS). Additionally, turmeric affects a variety of growth factors receptor and cell adhesion molecule involved in tumor genesis, angio-genesis and metastases [157]. Turmeric as a natural plant chemical can communicated with these new target and shown synergism to chemo therapy. In addition, turmeric is well tolerating in humans. Hence, EGFR miRNA autophagy and cancer stem cells based therapies in the presence of turmeric compounds may be encouraging mechanism and target in the therapeutics strategies of lungs cancer. Hence, EGFR miRNA autophagy and cancer stem cells based therapies in the presence of turmeric compounds may be encouraging mechanism and target in the therapeutics strategies of lungs cancer. [158].

2.12.8 Anti-allergic Activity

Turmeric derivatives suppress compound 48/80 induced Rat Peritoneal Mast Cell (RPMC) de-granulation and histamine released from rat peritoneal mast cells. In vitro systemic anaphylaxis caused by turmeric inhibits compounds 48/80 and against Ig E mediates in vivo passively cutaneous anaphylactic responses. Turmeric compounds have abilities to hinder non-specific and specific mast cells dependent allergy causing reaction in body [159].

2.12.9 Anti-dermatophytic Activity

Leaves of Turmeric plant used as anti-fungal substances that can be utilized as therapeutics for the treatment of human's disease causing fungi on account of its several in-vitro and in-vivo anti-fungal activities, anti-fungal actions, increased shelf life, its tolerability of heavy inoculum density, heat stability and the vast range of anti-dermatophytic activities and absence of any detrimental property. Curcumin compounds attained from the turmeric extracts which contain abilities to protected the skin from dangerous ultraviolet rays induce by showing anti-mutagen, anti-oxidant, and free radicals scavenging, anti-inflammatory and anti-carcinogenic properties [160].

2.12.10 Turmeric Prevent Resistance of Drug

Turmeric prevent against drug resistance. It presents new kind of abilities to stop the up regulation of glycol-protein and mRNA induce by Adriamycin. The avoidance are also structurally and functionally linked with the raised drug intracellular gathering and parallel to increase Adriamycin cell toxicity [161].

2.13 Turmeric and SARS-CoV-2

Numerous turmeric compounds showed to have activities against viruses. Studies using neuraminidase activations examined that 4-5 active turmeric compounds decreased H1N1 induced neuraminidase activations in H1N1 infected lungs epithelial cell. Tetramethyl curcumin and curcumin even down regulated nucleo-protein expressions [162]. Many studies have found that turmeric compounds derivatives are useful for executive influenza virus. Researchers have found that, by using various types of pathways, monoacetylcurcumin and curcumin can both avoid influenza virus infection. Important anti-viral activities of curcumin have also been investigated against the highly pathogenic avian influenza H5N1 virus in Madin Darby canine kidney (MDCK) cells in vitro by interfering with the function of

viral haem-agglutination. The results of anti-viral activities of turmeric derivatives extract were calculated by up regulation of mRNA expressions of the tumor necrosis factor alpha and interferon beta genes against viral entities in the tested kidney cells [163]. Turmeric has been observed to be effective in other virus induced disorders such as AIDS because of its HIV protease inhibitory activities and integration with its interdependent action on other medicinal drugs [164]. Other viruses, such as hepatitis B, hepatitis C, zika virus, chikungunya virus and dangue virus, have also been shown to intervene. The major cause of mortality with SARS-CoV-2 is respiratory distress syndromes with fulminant hypercytokinaemia and multi organs failure.

By blocking nuclear factors and suppressing the development of inflammation inducing cytokines, turmeric compounds have been observed to attenuate influenza virus induced lung tissues injuries. Turmeric is a natural peroxisome proliferator compound activated by gamma receptors that inhibit the inflammatory process by lowering the development of cytokines. It could also play the same role in protecting against SARS related lung damage [165].

From past centuries, turmeric is used as safe drug. It has been shown as efficacy inhibitory agent influenza (A) virus infection by boosting the immunity of body for prevention of injuries of lung tissues. To check the efficacy of compounds derived from turmeric against Covid-19 well organized studies should performed and finds its worth as a suitable treatment for this notorious virus [166].

Chapter 3

Material and Methodology

3.1 Proposed Diagram

Figure 3.1: shows the detail outlines of our research methodology.

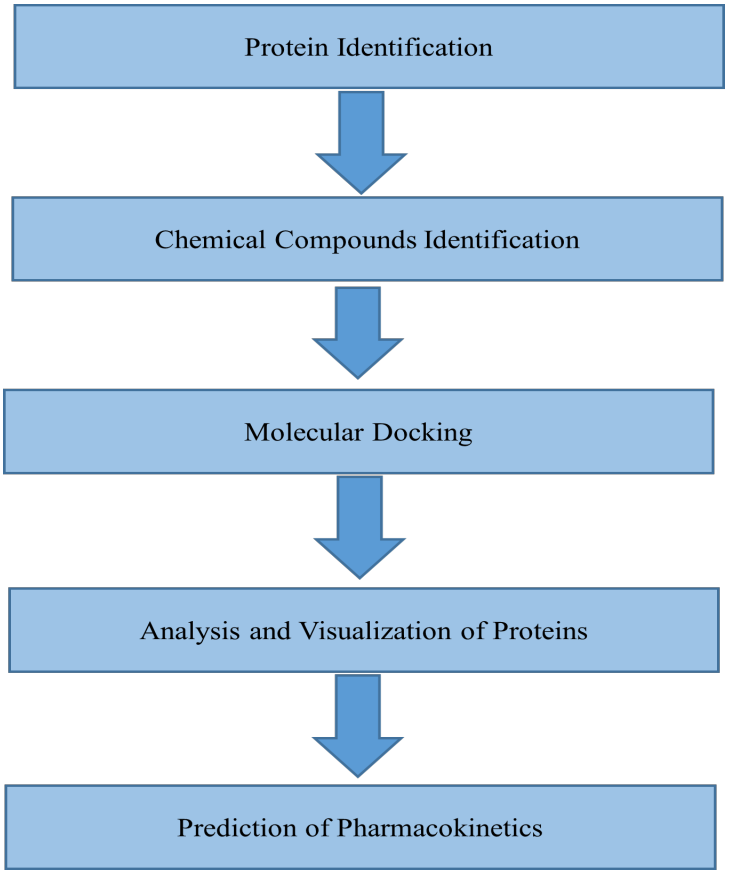


FIGURE 3.1: Methodology Overview

3.2 Protein Identification

Protein involved in inflammation was identified through the literature review. The PubMed IDs of papers that contain information regarding our studies were used for literature review. Human ACE2 was selected that is found to be majorly associated with the lung inflammation. Selected human ACE2 gene that was involved in the direct occurrence of inflammation or in the pathway that lead to the inflammation. The three dimensional structure of targeted protein was downloaded from Protein Data Bank.

FIGURE 3.2: 3D structure of Ace2 Protein

3.3 Chemical Compounds Identification

The turmeric compounds was identified through the literature review. Research articles were used for literature review which contain information related to our studies.

TABLE 3.1: Compounds name and their PubChem ID's

Sr.no	**Natural compounds**	**Pubchem ID**
1	Curcumin	969516
2	Demethoxycurcumin	5469424

3	Bisdemethoxycurcumin	5315472
4	Tetrahydroxycurcumin	129762283
5	1, 7 - Bis (4-Hydroxyphenyl) - 1 - Heptene-3, 5-Dione	9796708
6	Cyclocurcumin	69879809
7	1, 7-Bis (4-Hydroxy-3-Methoxyphenyl) - 1, 4, 6-Heptatrien-3-One	10904292
9	Calebin-A	637429
10	(E)-Ferulic Acid	445858
11	Vanillic Acid*	8468
12	Vanillin	1183
13	Demethyl Curcumin	5469426
14	1 - (3,4-Dihydroxyphenyl) - 7 - (4-Hydroxy-3-Methoxyphenyl) Hepta-1, 6-diene-3, 5-Dione	390474
15	1, 7- Bis- (4-Hydroxyphenyl)-1, 4,6-Heptatrien-3-One	71346280
16	1 - (4-Hydroxyphenyl) - 7 - (3 4-Dihydroxyphenyl)-1 6-Heptadiene-3 5-Dione	68738786
17	1, 7 - Bis(4-Hydroxyphenyl) - 1 - Heptene - 3, 5-Dione	9796708
18	1 - (4-Hydroxy-3-Methoxyphenyl) - 5 - (4-Hydroxyphenyl) - 1, 4-Pentadiene-3-One	10469828
19	4" - (4"'-Hydroxyphenyl) - 2" - Oxo - 3"- Butenyl - 3 - (4'-Hydroxyphenyl-3'-Methoxy) - Propenoate	17541200
20	E - 4 - (4-Hydroxy-3-Methoxyphenyl)- 3 - Buten-2-One	5354238
21	(Z)-Ferulic Acid	1548883
22	P-Cymene	7463
23	M-Cymene	10812
24	Terpinen-4-Ol	11230
25	4-Terpinol	24005967
26	Limonene	22311
27	Terpinolene	11463
28	Thymol	6989

29	Carvacrol*	10364
30	(E)-Carveol	94221

There were more than 235 turmeric compounds that were known but only 30 compounds were used in this study. These 30 compounds were found to be used in the treatment of inflammaion in recent studies. These 30 chemical compounds that are found in the turmeric were taken and devised for the docking. Table 3.1 shows the compounds which were used in this study.

3.4 Molecular Docking

Consequently docking contributed fundamental part in the rational drug designing. It helps in the detection of novel small molecular compounds, revealing the important properties, such as high binding interaction with target protein having reasonable absorption, distribution, metabolism and excretion (ADME) profile and drug likeness, which helps in selection of lead for the target [167]. PatchDock server was used for molecular docking.

3.5 Receptor Preparation

3D protein structure was downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) and Protein Data Bank https://www.rcsb.org/ in PDB format. Proteins were devised for refining in Discovery Studio Visualizer tool by removing.

- Water molecules
- Hetro-atoms
- Ligands

After removing water molecules, hetro-atoms and ligand, 3D structure was save in .PDB format for further proceedings.

3.5.1 Ligand Preparation

After identification of 30 natuarl compounds through literature review, the 3D structures of all the ligands were retrieved from PubChem website and PubChem ID's of selected natural compounds were collected from PubChem. All the Ligands were added in Discovery Studio Visualizer tool one by one by removing hydrogen atoms. After removing hydrogen atoms from ligands, saved in PDB format. Because PDB format is accepted by PatchDock.

3.6 Docking Simulation

PatchDock is freely available online server which is used for docking of receptor and ligand. The detected ligand-protein interaction was chosen that shows highest interaction of ligand with targeted protein.

3.7 Analysis and Visualization of Proteins

For the interpretation of docking results; interactions between ligand and targeted protein were calculated. After docking simulation, following compounds with the highest interaction with targeted protein were selected. Discovery Studio Visualizer, a desktop based visualization tool, was utilized to study these ligand-protein intration. The PDB format of complex protein was uploaded in Discovery Studio. Complex proteins were visualized by Discovery Studio by selecting their interaction residue with the ligand.

3.8 Calculation of Pharmacokinetic Parameters

The Molinspiration online toolkit was used to predict the drug likeness properties of the compounds. To prove the pharmaceutical fidelity of, the orally active drugs should have utilized drug likeness properties. In this project multiple parameters were calculated such as the number of hydrogen-bond donors, miLogP, the number of hydrogen-bond acceptors, TPSA, molecular mass of the compounds and the number of rotatable bonds. Violations Lipinski's rule of five [4] was also calculated.

3.8.1 Rule of Five Properties

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

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

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

Chapter 4

Results and Discussions

In this chapter the results obtained from the implementation of the methodology as mentioned in Chapter 3 are discussed in detail in-silico analysis of turmeric against ACE2.

4.1 Retrieval of Identified Protein and Turmeric Compounds

After identification of targeted proteins, the 3D structure of human ACE2 was downloaded from RCSB database in PDB format. Protein structure was also optimized by using Discovery studio. Through literature 30 Turmeric compounds were selected and their structures were visualized in Discovery studio and optimized.

4.2 Molecular Docking

Molecular docking simulations were performed by online PatchDock tool for the purpose of understanding the mechanisms of inflammation causing protein inhibition by turmeric compounds and to find out the binding interactions between

protein's amino acids and the ligands. All the selected ligands were docked against all the ACE2 protein that is reportedly found to be associated with the lungs inflammation. Ligands are shown in table 4.1 along with their PubChem ID and compounds name. Ligands that show best associations with proteins on the basis of amino acids residues were selected and went for further study.

TABLE 4.1: Compounds name that show best association with ACE2 protein

Sr.no	**PubChem ID**	**Compounds name**
1	969516	Curcumin
2	5469426	Demethylcurcumin
3	129762283	Tetrahydroxycurcumin
4	390474	1 - (3,4-Dihydroxyphenyl) - 7 - (4-Hydroxy-3-Methoxyphenyl) Hepta-1, 6-diene-3, 5-Dione
5	445858	(E)-Ferulic Acid
6	8468	Vanillic Acid
7	10364	Carvacrol
8	94221	(E)-Carveol
9	5354238	E - 4 - (4-Hydroxy-3-Methoxyphenyl) - 3 - Buten - 2 - One
10	11463	Terpinolene
11	1183	Vanillin
12	1548883	(Z)-Ferulic Acid
13	6989	Thymol
14	22311	Limonene
15	11230	Terpinen-4-Ol

These are top 15 ligands for protein that were selected on the basis of association with amino acids residue. The S score is considered as the drug score. Some of ligands show strong associations with more than one binding site of the protein.

TABLE 4.2: Selected turmeric compounds with their hydrogen bond and Amino acid residue

Compounds Name	**Ligand atoms interacting with amino acids**	**Amino acid interactions**
Curcumin	C, H	GLN81, GLU208
Demethylcurcumin	C,H	TRP208, SER511, LYS562, GLU564
Tetrahydroxycurcumin	C.H	TYR158, LEU162, ASP615
1 - (3,4-Dihydroxyphenyl) - 7 - (4-Hydroxy-3-Methoxyphenyl) Hepta-1, 6-diene-3, 5-Dione	C,O	GLN102, TYR202, TRP203, SER511
(E)-Ferulic Acid	C,H,O	GLN98, ALA99, GLU564, TRP566
Vanillic Acid	C,H,O	GLN98, GLY205, GLU208, LYS562, GLU564. TRP566
Carvacrol	C,H	LEU95, ASP206, VAL209, LYS262, TRP566
(E)-Carveol	C,H	LEU95, GLU208, VAL209, LYS562, GLU564, PRO565, TRP566
E - 4 - (4-Hydroxy-3-Methoxyphenyl) - 3 - Buten-2-One	C,H,O	LEU95, TYR196, VAL209, ALA396, LYS562
Terpinolene	C	LEU95, ALA99, LYS562, TRP566
Vanillin	C,H,O	GLN98, ASP206, LYS562, PRO565, TRP566

(Z)-Ferulic Acid	C,H,O	ASP206, GLU208, VAL209, ALA396, LYS562, GLU564, PRO565, TRP566
Thymol	C,H	GLN98, ALA99, LYS562, TRP566
Limonene	C	VAL209, LYS562, TRP566
Terpinen-4-Ol	C,O	ALA413, PHE438, LYS441

ACE2 gene encodes for ACE2 protein with 805 amino acids that is 40% similar in sequence to ACE protein. Critically active site residues, including the His-Tyr-Met-Gly-His zinc-binding motif, are highly conserved. ACE2 is glycoprotein orientated with the N terminus and the catalytic site facing the extracellular space, where it can metabolize circulating peptides. The small C-terminal, cytoplasmic domain has a number of potential regulatory sites. It localized on X chromosome no.7 [5]. Ace2 is found in lungs, kidneys, colon and heart as well [4].

ACE2v protein shows best interaction with turmeric compounds listed above. Selected turmeric compounds show hydrogen bonding with amino acid residues of ACE2 protein. Vanillic acid shows four hydrogen bond with LYS562, GLU564, TRP566. Curcumin and (Z)-Ferulic Acid show three hydrogen bonding with amino acid residues of human ACE2 protein. Demethylcurcumin, (E)-Ferulic Acid, Carvacrol, (E)-4-(4-Hydroxy-3-Methoxyphenyl) But-3-En-2-One and Thymol all these ligands show only 1 hydrogen bond with human ACE2. 1-(3,4-Dihydroxyphenyl)-7-(4-Hydroxy-3-Methoxyphenyl)Hepta-1,6-diene-3, 5-Dione.

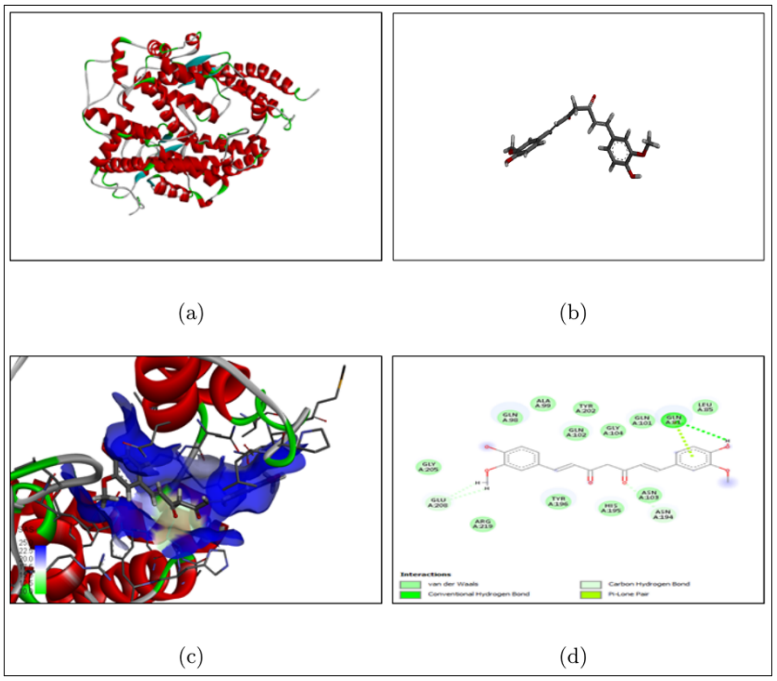


FIGURE 4.1: Analysis of Molecular Docking of Human ACE2 and Curcumin (a) 3D structure of Human ACE2 (b) 3D structure of Curcumin (c) SAS of Protein and ligand interaction (d) 2D structure of active site of Ligand and amino acids residues of protein

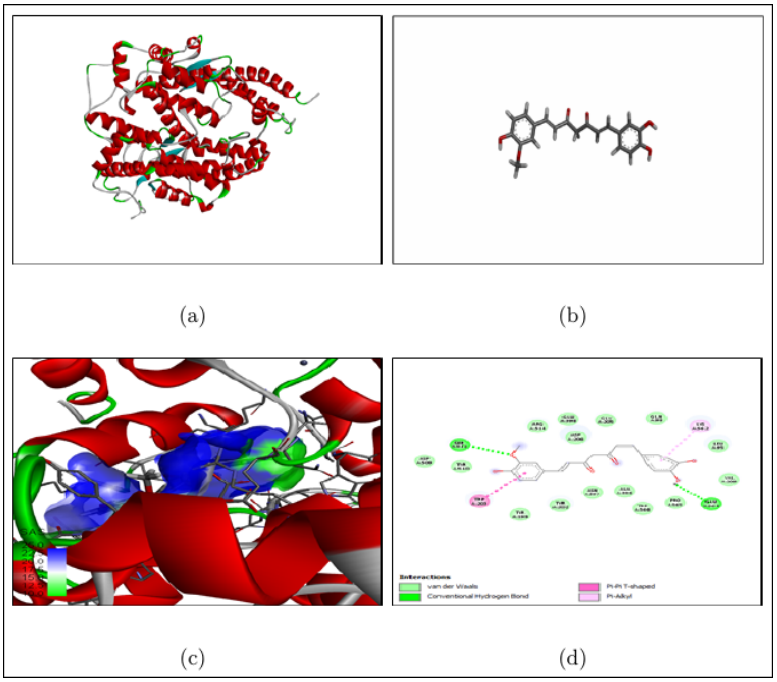


FIGURE 4.2: Analysis of Molecular Docking of Human ACE2 and Demethylcurcumin (a)) 3D structure of Human ACE2 (b) 3D structure of Demethylcurcumin (c) SAS of Protein and ligand interaction (d) 2D structure of active site of Ligand and amino acids residues of protein

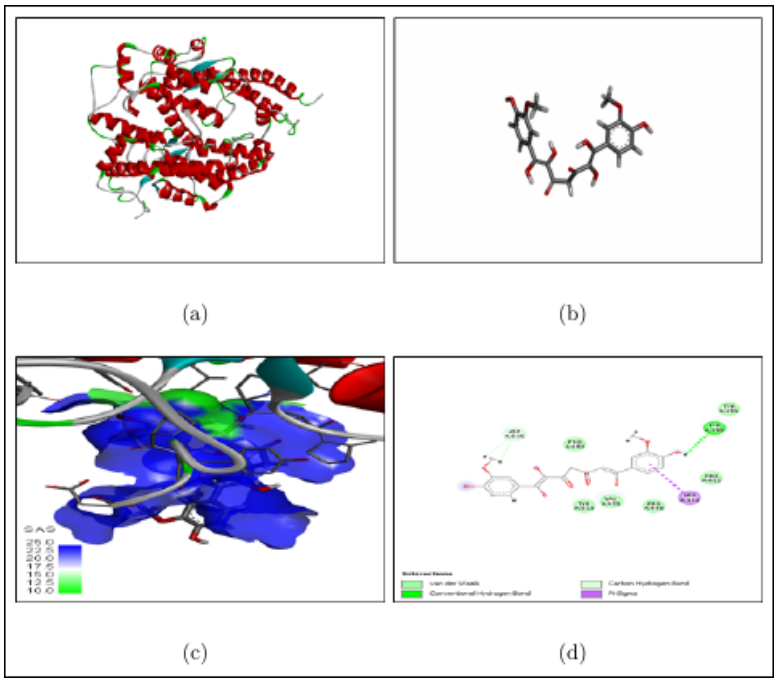


FIGURE 4.3: Analysis of Molecular Docking of Human ACE2 and Tetrahydroxycurcumin (a) 3D structure of Human ACE2 (b) 3D structure of Tetrahydroxycurcumin (c) SAS of Protein and ligand interaction (d) 2D structure of active site of Ligand and amino acids residues of protein

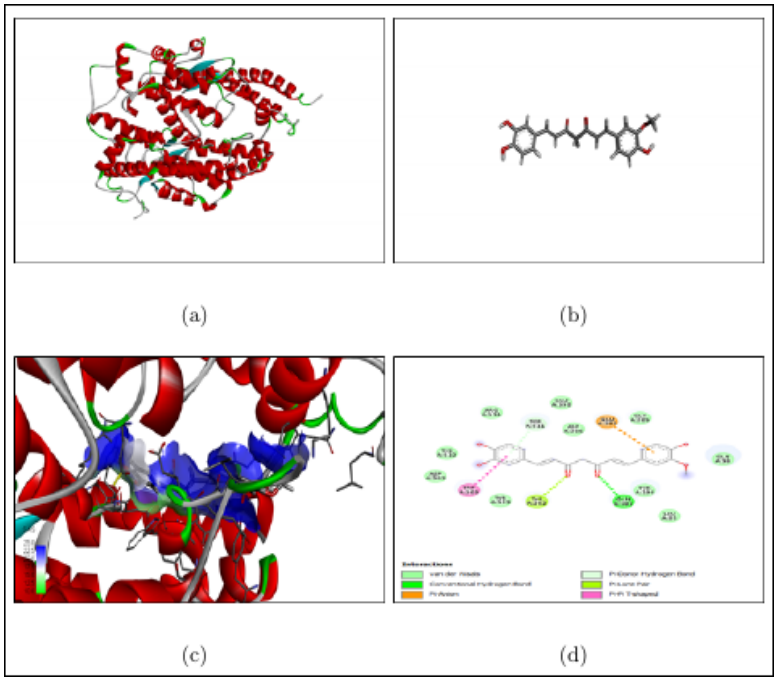


FIGURE 4.4: Analysis of Molecular Docking of Human ACE2 and1-(3,4-Dihydroxyphenyl)-7-(4-Hydroxy-3-Methoxyphenyl)Hepta-1,6-diene-3, 5-Dione (a) 3D structure of Human ACE2 (b) 3D structure of 1-(3,4-Dihydroxyphenyl)-7-(4-Hydroxy-3-Methoxyphenyl)Hepta-1,6-diene-3, 5-Dione (c) SAS of Protein and ligand interaction (d) 2D structure of active site of Ligand and amino acids residues of protein

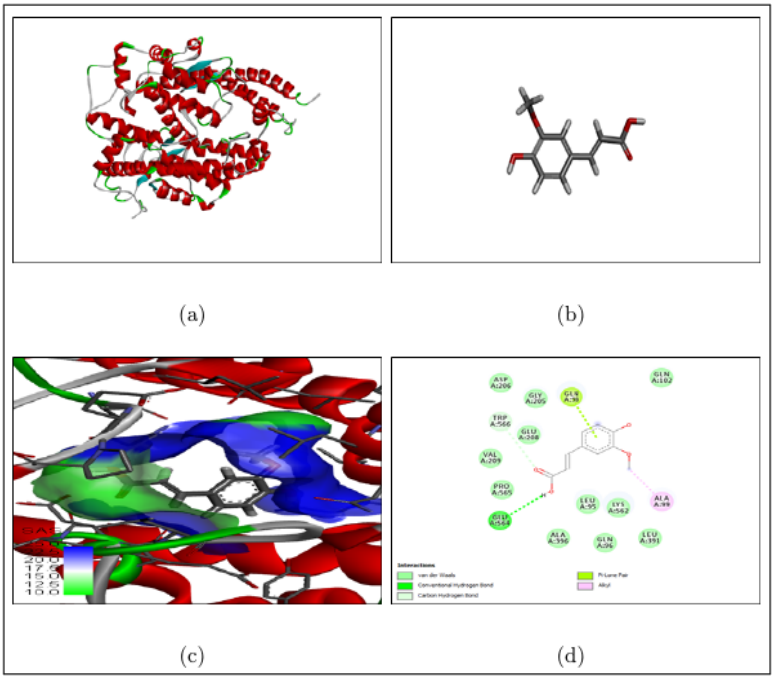


FIGURE 4.5: Analysis of Molecular Docking of Human ACE2 and (E)-Ferulic Acid (a) 3D structure of Human ACE2 (b) 3D structure of (E)-Ferulic Acid (c) SAS of Protein and ligand interaction (d) 2D structure of active site of Ligand and amino acids residues of protein

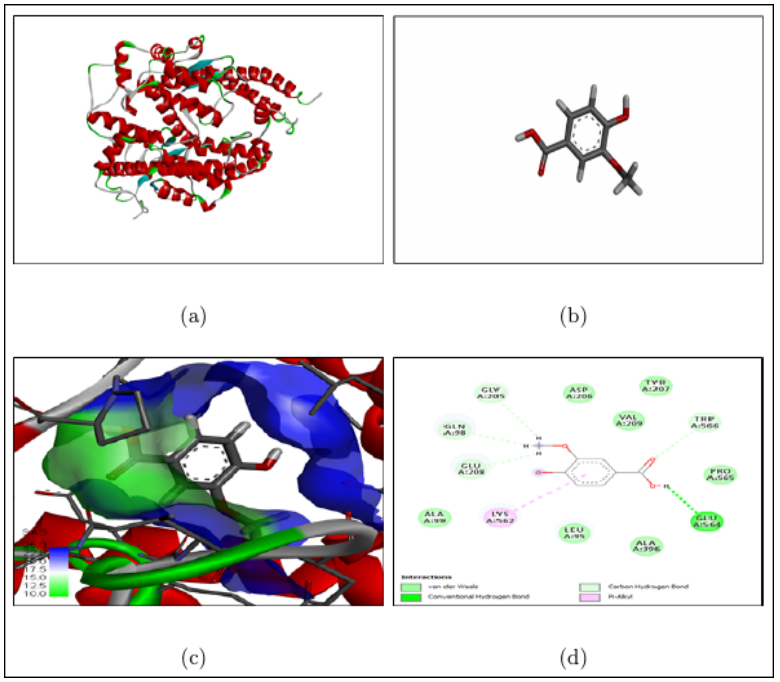


FIGURE 4.6: Analysis of Molecular Docking of Human ACE2 and Vanillic Acid (a) 3D structure of Human ACE2 (b) 3D structure of Vanillic Acid (c) SAS of Protein and ligand interaction (d) 2D structure of active site of Ligand and amino acids residues of protein

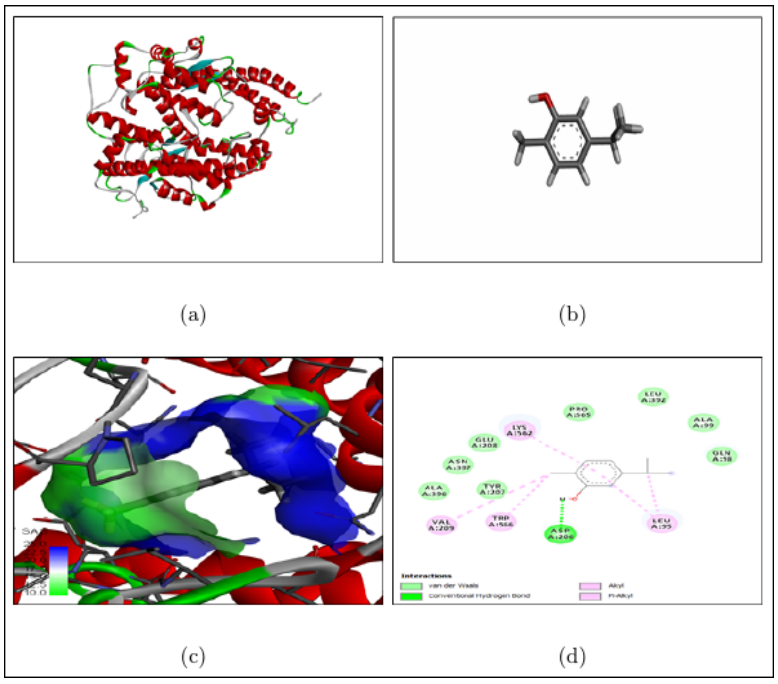


FIGURE 4.7: Analysis of Molecular Docking of Human ACE2 and Carvacrol (a) 3D structure of Human ACE2 (b) 3D structure of Carvacrol (c) SAS of Protein and ligand interaction (d) 2D structure of active site of Ligand and amino acids residues of protein

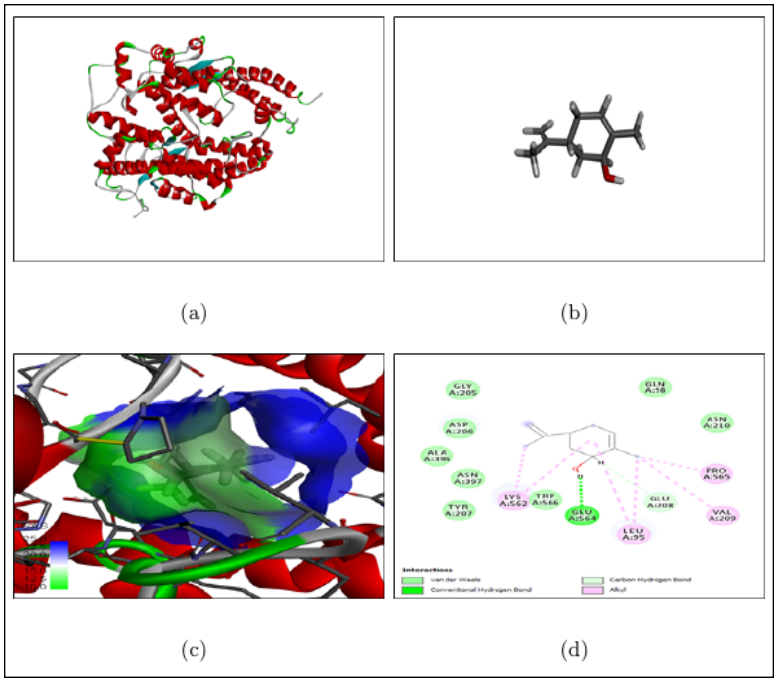


FIGURE 4.8: Analysis of Molecular Docking of Human ACE2 and (E)-Carveol (a) 3D structure of Human ACE2 (b) 3D structure of (E)-Carveol (c) SAS of Protein and ligand interaction (d) 2D structure of active site of Ligand and amino acids residues of protein

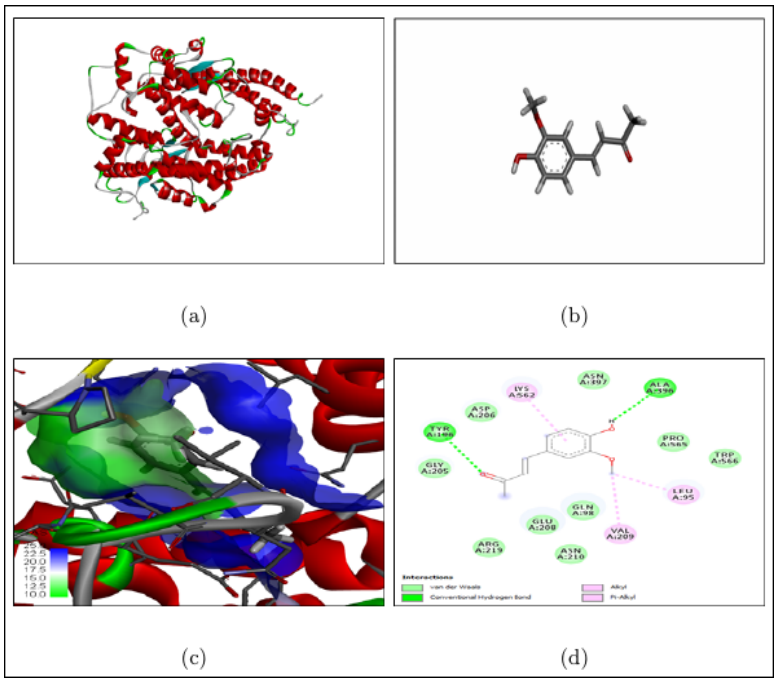


FIGURE 4.9: Analysis of Molecular Docking of Human ACE2 and (E)-4-(4-Hydroxy- 3Methoxyphenyl) But-3-En-2-One (a) 3D structure of Human ACE2 (b) 3D structure of (E)-4-(4-Hydroxy- 3Methoxyphenyl) But-3-En-2-One (c) SAS of Protein and ligand interaction (d) 2D structure of active site of Ligand and amino acids residues of protein

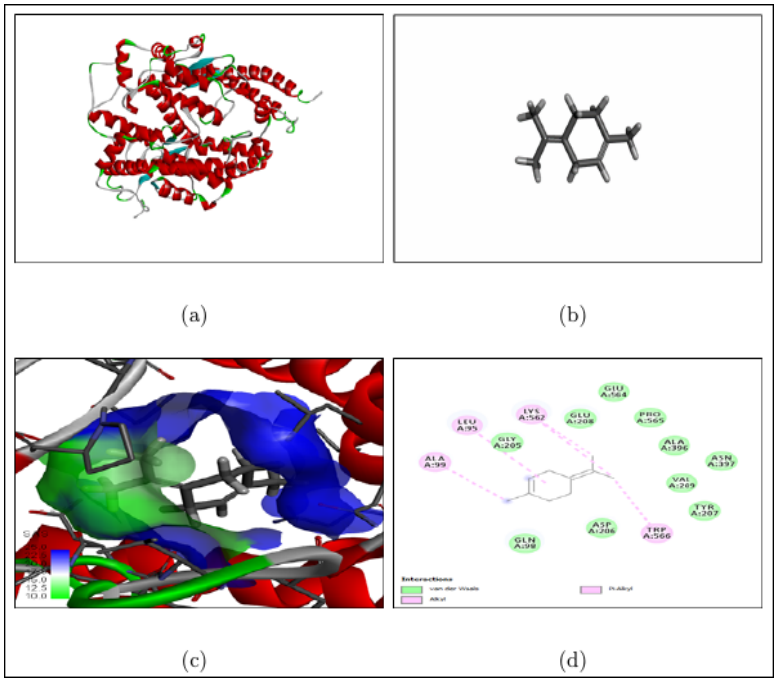


FIGURE 4.10: Analysis of Molecular Docking of Human ACE2 and Terpinolene (a) 3D structure of Human ACE2 (b) 3D structure of Terpinolene (c) SAS of Protein and ligand interaction (d) 2D structure of active site of Ligand and amino acids residues of protein

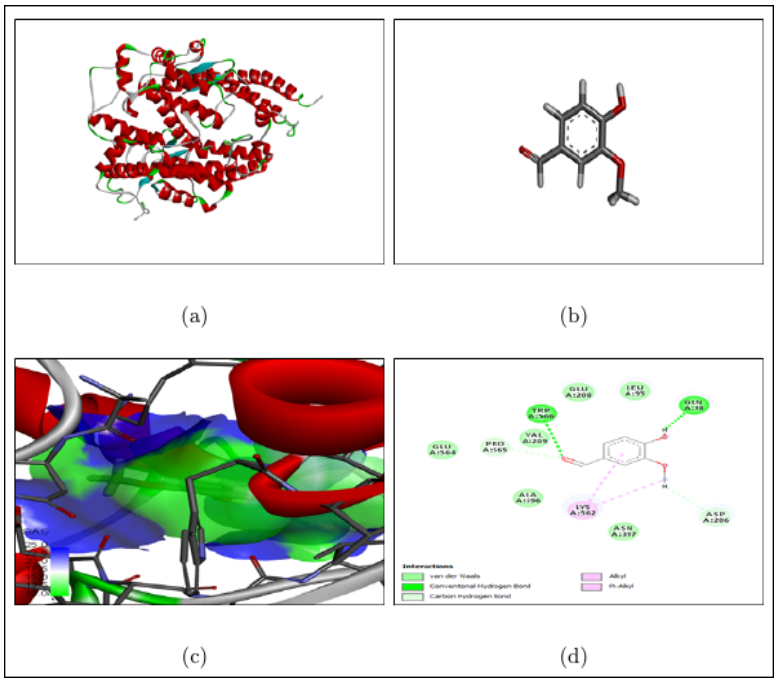


FIGURE 4.11: Analysis of Molecular Docking of Human ACE2 and Vanillin (a) 3D structure of Human ACE2 (b) 3D structure of Vanillin (c) SAS of Protein and ligand interaction (d) 2D structure of active site of Ligand and amino acids residues of protein

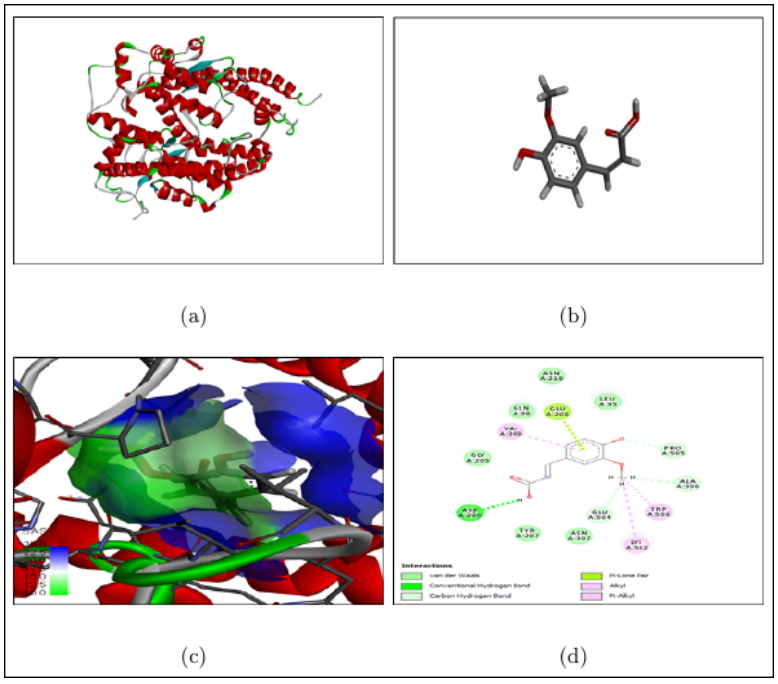


FIGURE 4.12: Analysis of Molecular Docking of Human ACE2 and (Z)-Ferulic Acid (a) 3D structure of Human ACE2 (b) 3D structure of (Z)-Ferulic Acid (c) SAS of Protein and ligand interaction (d) 2D structure of active site of Ligand and amino acids residues of protein

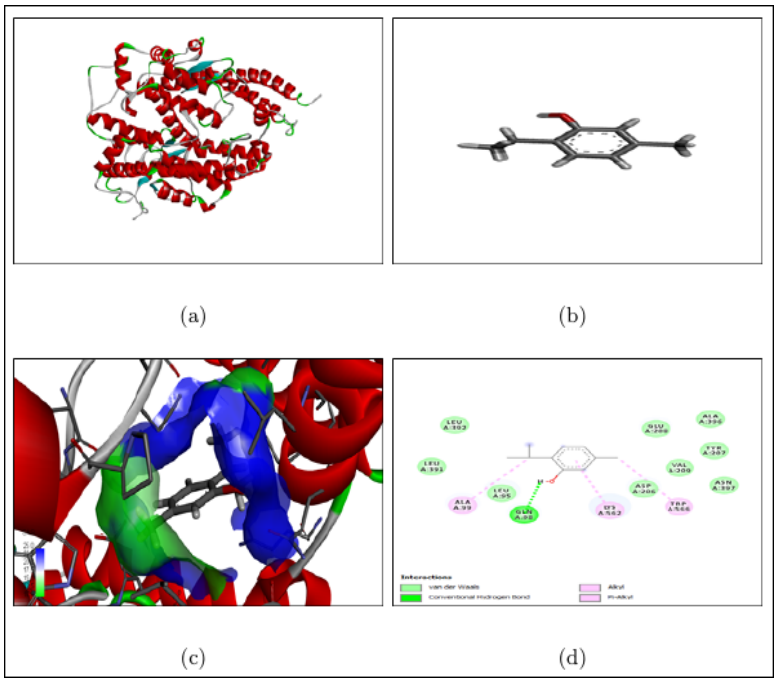


FIGURE 4.13: Analysis of Molecular Docking of Human ACE2 and Thymol (a) 3D structure of Human ACE2 (b) 3D structure of Thymol (c) SAS of Protein and ligand interaction (d) 2D structure of active site of Ligand and amino acids residues of protein

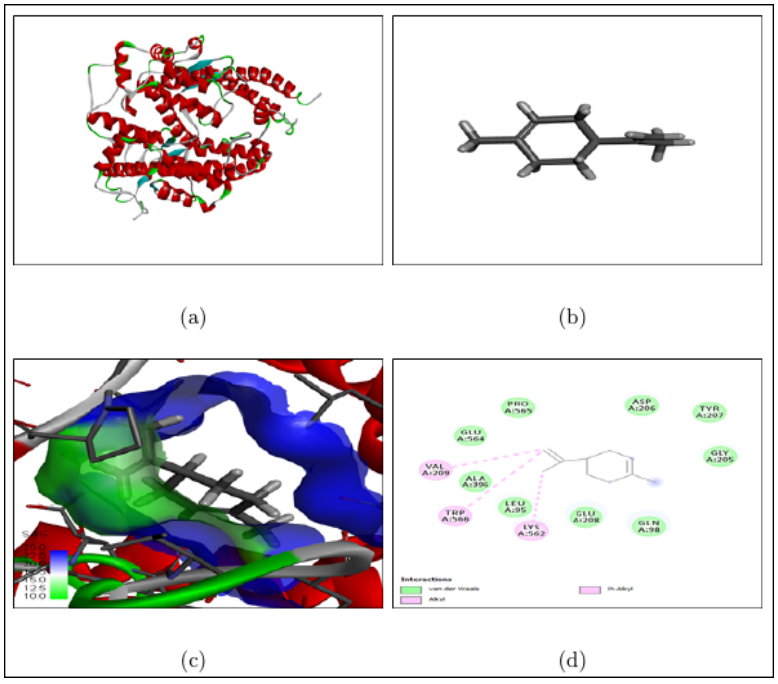


FIGURE 4.14: Analysis of Molecular Docking of Human ACE2 and Limonene (a) 3D structure of Human ACE2 (b) 3D structure of Limonene (c) SAS of Protein and ligand interaction (d) 2D structure of active site of Ligand and amino acids residues of protein

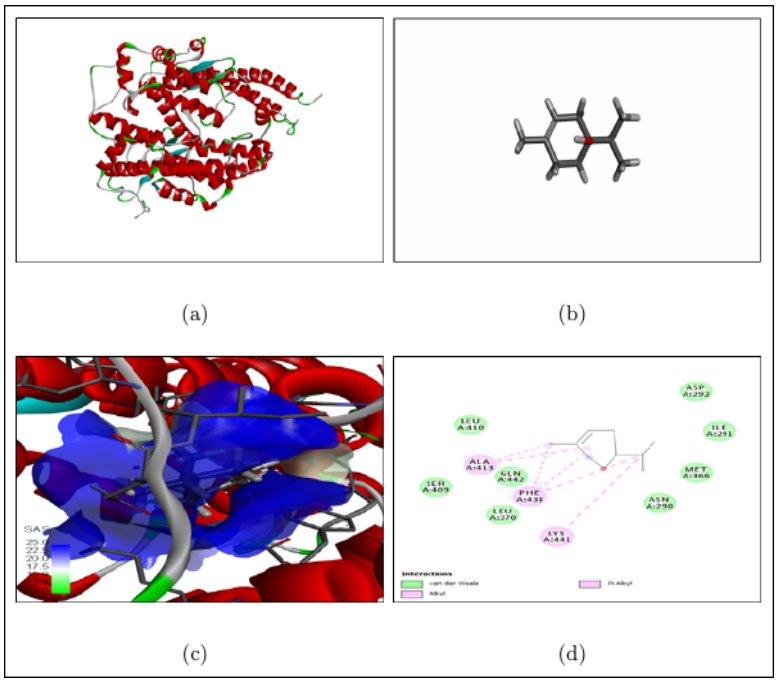
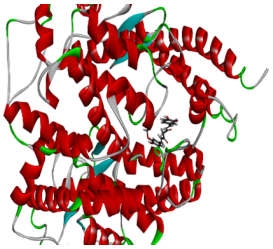
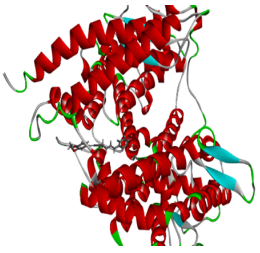
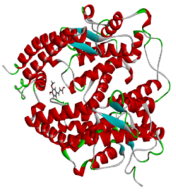
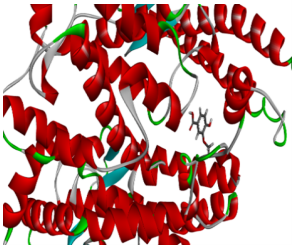
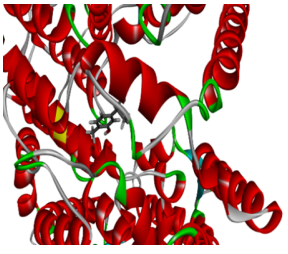
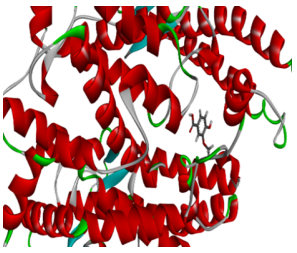
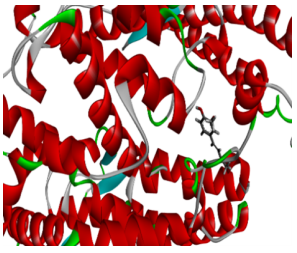
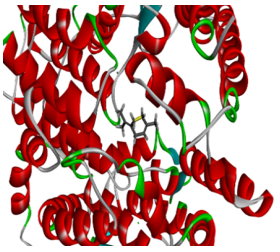
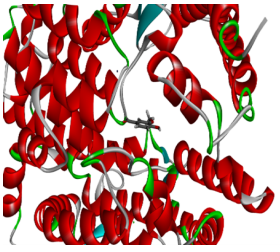
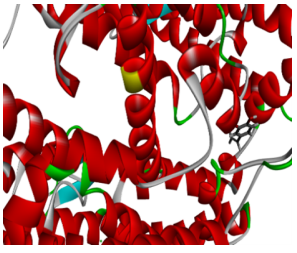


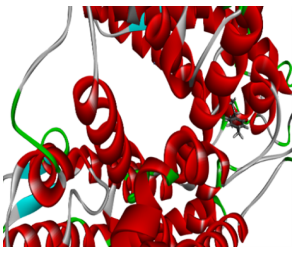
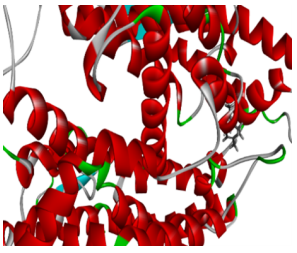
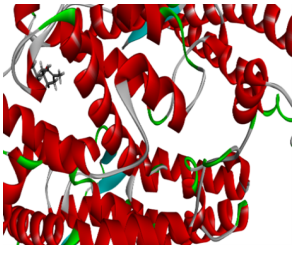
FIGURE 4.15: Analysis of Molecular Docking of Human ACE2 and Terpinen-4-Ol (a) 3D structure of Human ACE2 (b) 3D structure of Terpinen-4-Ol (c) SAS of Protein and ligand interaction (d) 2D structure of active site of Ligand and amino acids residues of protein

TABLE 4.3: Docking results with Patchdock scores, ACE value and structures of complex

Sr	**Compounds name**	**Pub Chem ID**	**Patch dock Scores**	**ACE Value**	**Structures of Complex**
1	Curcumin	969516	4970	-87.13	
2	Demethyl curcumin	5469426	4610	-123.80	

3	Tetra hydy-oxy curcumin	129762283	5050	-290.99	
4	1-(3, 4-Dihydroxy phenyl)-7-(4-Hydroxy -3-Methoxyphenyl) Hepta-1, 6-diene-3 , 5-Dione	390474	4684	-106.43	
5	(E)-Ferulic Acid	445858	3212	-96.14	
6	Vanillic Acid	8468	2818	-92.52	
7	Carvacrol	10364	3188	-95.88	

8	(E)-Carveol	94221	2862	-103.12	
9	E-4-(4-Hydroxy-3-Methoxyphenyl)-3-Buten-2-One	5354238	3326	-56.96	
10	Terpinolene	11463	3100	-90.87	
11	Vanillin	1183	2776	-77	
12	(Z)-Ferulic Acid	1548883	3252	-112.96	

13	Thymol	6989	2996	-63.36	
14	Limonene	22311	3056	-116.72	
15	Terpinen-4-Ol	11230	29.34	-60.34	

4.3 Pharmacokinetic Properties

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

There are multiple computational approaches that are being used to determine the absorption, metabolism, distribution, excretion, and toxicity of the new compounds that have the potential of becoming drugs. Pharmacokinetics properties

are determined by the Molinspiration online toolkit which is used for checking physiochemical properties of the selected 15 compounds after the docking simulation that lead towards further scrutiny. Pharmacokinetic properties are determined on the basis of the Lipinski's rule of five [168].

According to this rule, all the potential oral drug candidates must have molecular weight less than 500 amu, value of LogP is less than or equal to 5, hydrogen-bond donor sites must be five or less than five, and hydrogen-bond acceptor sites should be ten or less than ten [169].

Based on the Vebers rule, the number of rotatable bonds in the orally bioavailable drugs should be less than or equal to 10 and topological polar surface area (TPSA) value should be less and or equal to the 140 that is considered as a good descriptor for suitable drugs as it is involved in the passive molecular drug transport through membranes [170]. If any drug, is violating any of the above given rule than it will have problems regarding bioavailability. Table is presenting the results of physiochemical properties of the compounds.

TABLE 4.4: Physiochemical properties of Turmeric compound good for oral bioavailability

Compounds Name	**TPSA**	**MW**	**LogP**	**HBD**	**HBA**	**n-ROTB**
Results	**<=140**	**<500**	**<= 5**	**<5**	**<10**	**<=10**
Curcumin	93.07	368.38	2.3	2	6	8
Demethylcurcumin	104.06	354.36	2.00	3	6	7
Tetrahydroxycurcumin	173.98	432.38	2.15	6	10	8
1-(3,4-Dihydroxyphenyl)-7-(4-Hydroxy-3-Methoxyphenyl)Hepta-1,6-diene-3, 5-Dione	104.06	354.36	2.00	3	6	7
(E)-Ferulic Acid	66.76	194.19	1.25	2	4	3
Vanillic Acid	66.76	168.15	1.19	2	4	2
Carvacrol	20.23	150.22	3.81	1	1	1

(E)-Carveol	20.23	152.24	2.70	1	1	1
E-4-(4-Hydroxy-3-Methoxyphenyl)-3-Buten-2-One	46.53	192.21	1.55	1	3	3
Terpinolene	0.00	136.24	3.67	0	0	0
Vanillin	46.53	152.15	1.07	1	3	2
(Z)-Ferulic Acid	66.76	194.19	1.25	2	4	3
Thymol	20.23	150.22	3.34	1	1	1
Limonene	00.0	136.24	3.62	0	0	1
Terpinen-4-Ol	20.23	154.25	2.60	1	1	1

TABLE 4.5: Turmeric compounds passing Lipinski's rule of five

Compounds Name	**Passing Lipinski's Rule**
Curcumin	Yes
Demethylcurcumin	Yes
Tetrahydroxycurcumin	No
1-(3,4-Dihydroxyphenyl)-7-(4-Hydroxy-3-Methoxyphenyl)Hepta-1,6-diene-3, 5-Dione	Yes
(E)-Ferulic Acid	Yes
Vanillic Acid	Yes
Carvacrol	Yes
(E)-Carveol	Yes
E-4-(4-Hydroxy-3-Methoxyphenyl)-3-Buten-2-One	Yes
Terpinolene	No
Vanillin	Yes
(Z)-Ferulic Acid	Yes
Thymol	Yes
Limonene	No
Terpinen-4-Ol	Yes

4.4 Lead Identification

The docking score and binding interactions of all ligands have been analyzed. Out of 30 ligands; top 15 ligands which showed high binding interactions were selected for physiochemical properties prediction and effectiveness. The twelve ligands has been selected as lead compounds as it has been identified as the most active from all molecules shown interactions with the target receptors. These compounds fulfill all the requirements that are required for an oral bioavailable drug.

Chapter 5

Conclusion and Future Direction

The purpose of this project was to find out the competent drug targets for the inflammation in lungs that is caused by ACE2. Physiochemical properties of protein was checked. 30 Turmeric compounds mined from the literature were docked for protein and best turmeric compounds were selected for targeted protein.

Turmeric compounds shown strong interaction with protein were selected. After the docking results, on the selected compounds, physiochemical properties analysis was performed that is based on the Lipinskis rule of five. After this, ligands that passed the Lipinskis rule of five were selected remaining ligands were removed from the candidates of the drugs for the lungs inflammation that shows the presence of toxicity. All these drug targets were tested against the pharmacokinetics properties.

The remaining compounds that include Curcumin, Demethylcurcumin, 1 - (3, 4-Dihydroxyphenyl) - 7 - (4-Hydroxy-3-Methoxyphenyl) Hepta-1,6-diene-3, 5-Dione, (E)-Ferulic Acid, Vanillic Acid, Carvacrol, (E) - Carveol, E - 4 - (4 - Hydroxy - 3 - Methoxyphenyl) - 3 - Buten - 2 - One, Vanillin, (Z) - Ferulic Acid, Thymol and Terpinen-4-Ol are considered as the competent for the oral bioavailable drugs for anti-inflammatory agents. These are the compounds that pass rule that is important for the formation of any drug such as absorption, toxicity, metabolism and excretion.

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

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