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IN-SILICO ANALYSIS OF TURMERIC AS AN ANTI-INFLAMMATORY AGENT AGAINST ACE2 RECEPTOR

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Abstract

Turmeric (*Curcuma longa*) is a rhizome containing the perennial plant of the ginger family. Turmeric is used for its medicinal properties in almost all diseases. With the increasing drug resistance problem, the interest to explore natural products with medicinal properties is increasing. The purpose of this research work is to find out the compounds from turmeric that can be used as anti-inflammatory agents. These compounds of turmeric were found in 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. A protein three-dimensional structure was prepared for molecular docking. Molecular docking was performed for this purpose and after that selected compounds of turmeric for Angiotensin Converting Enzyme 2 (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 validated on animal models to provide new treatment for inflammation in the body.

Key words: ACE2 protein; Anti-filamentary; Drug Design; *In-silico*; Molecular Docking; Turmeric.

Introduction

Angiotensin Converting Enzyme 2 (ACE2) is a glycoprotein that is from the family of zinc metallopeptidases (Douglas *et al*., 2004). ACE2 is predominantly present in major parts of the body like in heart tissues, kidneys, and lungs and a lot of tissues at lower levels of the colon (Tipnis *et al*., 2000). ACE2 is present on chromosome Xp22 and it is a 40 kb gene, apart from ACE gene that is found on chromosome 17. The human ACE2 protein is basically composed of 805 amino acids (Burrell *et al*., 2005). 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 to the 18 exons of ACE2. The genetics of ACE2 shows several new polymorphisms of ACE2, with specified geographical distributions. It has been described and linked with hypertension and heart diseases (Burrell *et al*., 2005). ACE2 is about 40% similar to the sequence of ACE, while it contains only a single catalytic zone. Residues of active sites, including Glu-Tyr-Met-Gly-His-Try and (Zn) binding motifs, are highly sustained (Zhang *et al*., 2001). The genetics of ACE2 shows several new polymorphisms of ACE2, with specified geographical distributions. It has been described and linked with hypertension and heart diseases (Burrell *et al*., 2005).

Worldwide Cardiac disorders are the main cause of death and emerging as a general health issue. The cardiac disorder is indicated by the stimulation of many signaling pathways linked with hypertrophy problems and maladaptation ventricle improvement. ACE2 gene polymorphisms are associated with several diseases, most probably in Asian countries' populations (Patel *et al*., 2014). In the human heart, ACE2 is positioned to cardio-myocytes, cardiac fibroblast, epicardial adipose tissues, and the coronary vascular endothelium (Patel *et al*., 2016).

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 (Zhong *et al*., 2010). 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 (Oudit *et al*., 2010).

Epithelial cells of lungs express high levels of ACE2, which are related 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 models (Yilin *et al*., 2015). High level of ACE2 expressed to protect from hypertensive activity, while ACE2 low level provokes hypertension. Nephric ACE2 expressions are correlated 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 (Crackower *et al*., 2002). Likewise, genetic variations in ACE2 have been developed to influence the receptor interactions with the virus spike glycoprotein (Li *et al*., 2005). Contrary, new introductory studies show no such association among human variants of ACE2 and corona virus infections (Cao *et al*., 2020). Several coding variants of ACE2 in humans have been linked with cardio vascular diseases, hypertension, and diabetes (Luo *et al*., 2019). 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 (Dong *et al*., 2020).

Medicinal plants are safely used for the treatment of many lethal diseases (Aromatic plants are both woody and herbaceous plants of several families that are widely used in medical care, the food industry, eco-tourism, and fruit (Shinwari *et al*., 2018; Anjum *et al*., 2019; Khan *et al*., 2019; Ovais *et al*., 2019; Najeebullah *et al*., 2020). Turmeric ingested orally in case of severe inflammations was 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 prostaglandin from arachidonic acid, and neutrophil functions during inflammatory state. The volatile oil, petroleum ether, alcohol and water extract of turmeric shows anti-inflammatory effects (Cronin *et al*., 2003). Turmeric has been considered as anti-inflammatory agents and useful for inflammation disorders. Investigation of anti-inflammatory effects of turmeric showed a role for inactivation of NF-jB mediated inflammation (Bronte *et al*., 2013).

In the current situation, natural compounds that 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. 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. In addition the current study was designed to check the interaction of turmeric against ACE2 receptor protein targets playing role as anti-inflammatory candidate by using CADD approach and find out the credibility of the selected compounds for oral bioavailable drugs by predicting the pharmacokinetics.

Material and Methods

Proposed diagram: The detail outline of the methodology is given in Fig. 1.

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 (Table 1). Human ACE2 was selected that is found to be majorly associated with the lung inflammation. Selected human ACE2 genes were choose that were involved in the direct occurrence of inflammation or in the pathway that lead to the inflammation.

Chemical compounds identification: The turmeric compounds were identified through the literature review. 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 inflammation in recent studies. These 30 chemical compounds that are found in the turmeric were taken and devised for the docking. Table 1 show the compounds which were used in this study.

Molecular docking: PatchDock server was used for molecular docking.

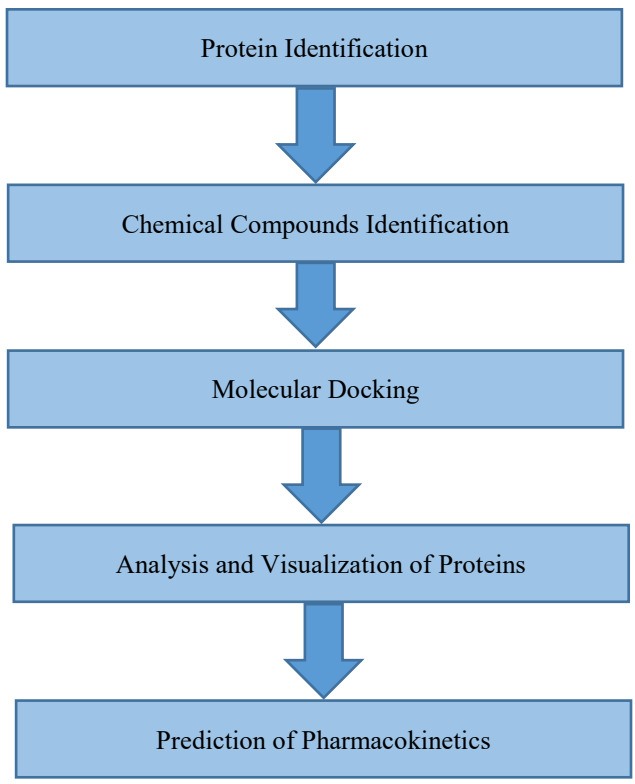


Fig. 1. Methodology overview.

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.

Ligand preparation: After identification of 30 natural compounds through literature review, the 3D structures of all the ligands were retrieved from PubChem website (https://pubchem.ncbi.nlm.nih.gov/) and PubChem ID's of selected natural compounds were collected from PubChem. All the Ligands were added in the Discovery Studio Visualizer tool one by one by removing hydrogen atoms. After removing hydrogen atoms from ligands, saved in PDB format.

Docking simulation: PatchDock was used for docking of receptors and ligands. The detected ligand-protein interaction was chosen that shows highest interaction of ligand with targeted protein.

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 interactions. 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.

Calculation of pharmacokinetic parameters: The Molinspiration online toolkit (http://www.molinspiration.com/ cgi-bin/ properties) was used to predict the drug likeness properties of the compounds. To prove the pharmaceutical fidelity of, the orally active drugs should have utilized drug likeness properties. In this project multiple parameters were calculated such as the number of hydrogen-bond donors, miLogP, the number of hydrogen-bond acceptors, TPSA, molecular mass of the compounds and the number of rotatable bonds. Violations Lipinski's rule of five (Tipnis *et al*., 2000) was also calculated.

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

- The log P 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 Veber's Rule (VR), 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.

Results and Discussion

Retrieval of identified protein and turmeric compounds: After identification of targeted proteins, the 3D structure of human ACE2 was downloaded from the 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.

Molecular docking: Consequently, docking contributed a 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 (Lagorce *et al*., 2008). 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 2 along with their PubChem ID and compound names. Ligands that show best associations with proteins on the basis of amino acids residues were selected and went for further study. 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 the ligands showed strong associations with more than one binding site of the protein.

Table 1. Compounds name and their PubChem ID's.

S. 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

Table 2. Compounds name that show best association with ACE2 protein.

S. 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

Table 3. Selected turmeric compounds with their hydrogen bond and Amino acid residue.

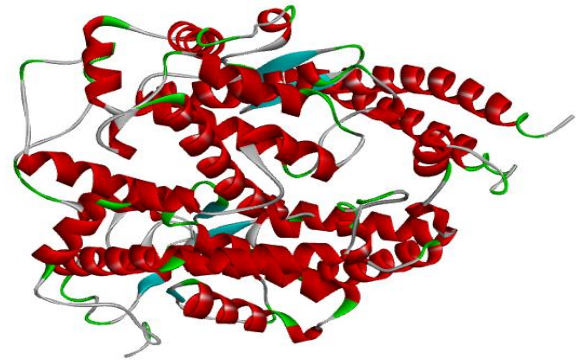
Compounds name	Ligand atoms interacting with amino acids	Amino acid interactions
Curcumin	C, H	GLN81, GLU208
Demethylcurcumin	C,H	TRP208, SER511, LYS562, GLU564
Tetrahydroxycurcumin	C.H	TYR158, LEU162, ASP615
1-(3,4-Dihydroxyphenyl)-7-(4-Hydroxy-3-Methoxyphenyl) Hepta-1, 6-diene-3, 5-Dione	C,O	GLN102, 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 (Burrell *et al*., 2005). Ace2 is found in lungs, kidneys, colon and heart as well (Tipnis *et al*., 2000).

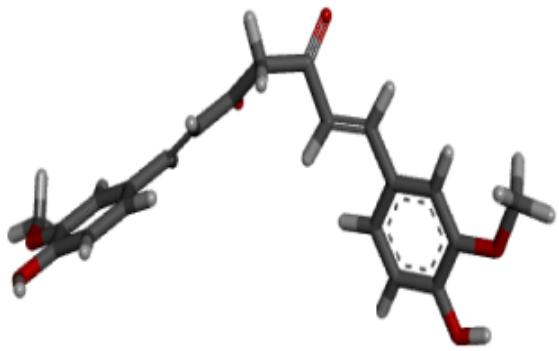
ACE2v protein shows best interaction with turmeric compounds. Selected turmeric compounds show hydrogen bonding with amino acid residues of ACE2 protein (Table 3). Vanillic acid showed four hydrogen bonds with LYS562, GLU564, and TRP566. Curcumin and (Z)-Ferulic Acid showed three hydrogen bonding with 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 showed only 1 hydrogen bond with human ACE2 (Table 3). The analysis of molecular docking of human ACE2 with different compounds is given in Figs. 2-16. In addition, the docking results with Patchdock scores, ACE values and structures of complexes are given in Table 4.

Pharmacokinetic properties: In the drug development, pharmacokinetic properties (PKs) are considered as very important because they help 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, perform 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.

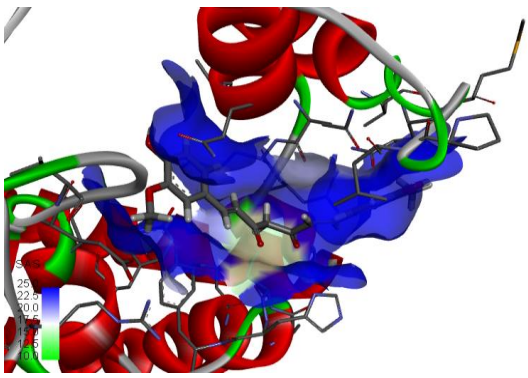
There are multiple computational approaches that are being used to determine the absorption, metabolism, distribution, excretion, and toxicity of the new compounds that have the potential of becoming drugs. Pharmacokinetics properties are determined by the Molinspiration online toolkit which is used for checking physiochemical properties of the selected 15 compounds after the docking simulation that lead towards further scrutiny (Tables 5, 6). Pharmacokinetic properties are determined on the basis of Lipinski's rule of five (Lipinski *et al*., 1997).



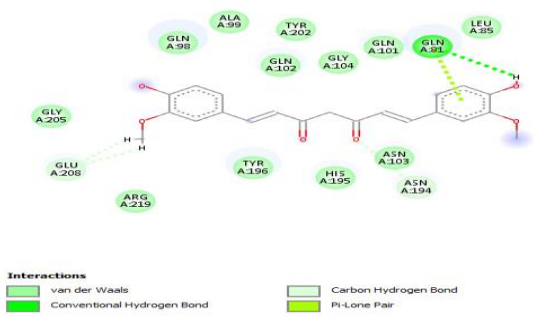
A. ACE2 protein



B. Ligand (Curcumin)

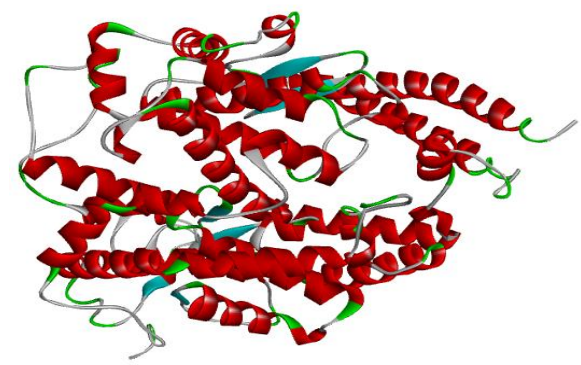


C. SAS of protein and ligand interaction

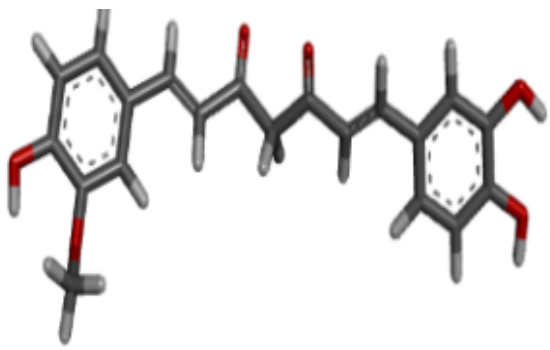


D. 2D structure with amino acid residue

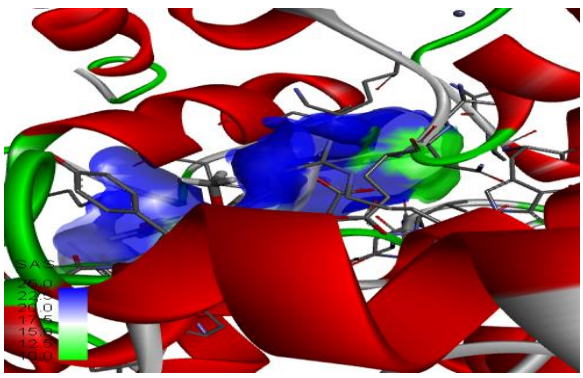
Fig. 2. Analysis of Molecular Docking of Human ACE2 and Curcumin (A) 3D structure of Human ACE2. (B) 3D structure of Curcumin. (C) SAS of Protein and ligand interaction. (D) 2D structure of active site of Ligand and amino acids residues of protein.



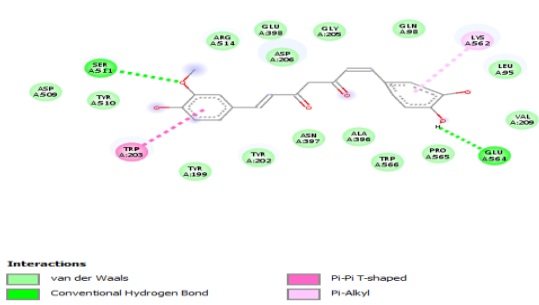
A. ACE2 protein



B. Ligand (Demethylcurcumin)

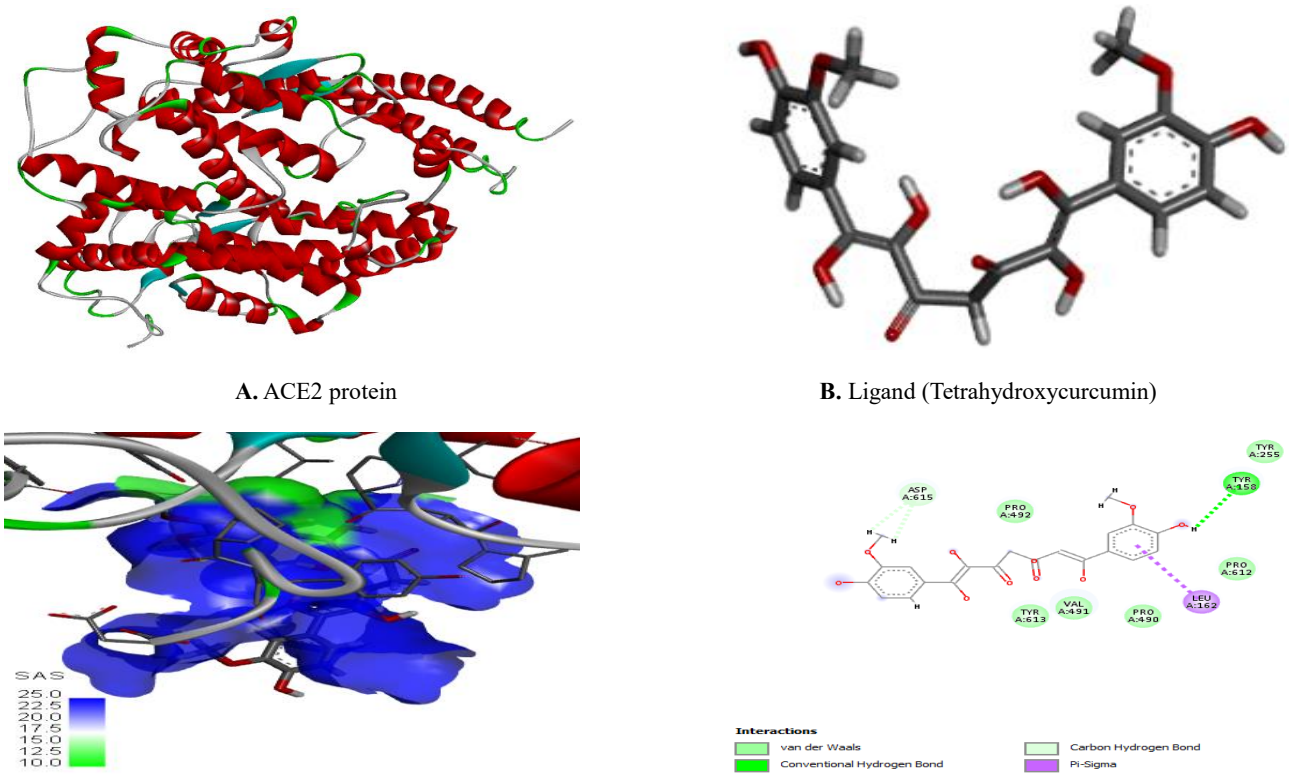


C. SAS of protein and ligand interaction



D. 2D structure with amino acid residue

Fig. 3. 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.



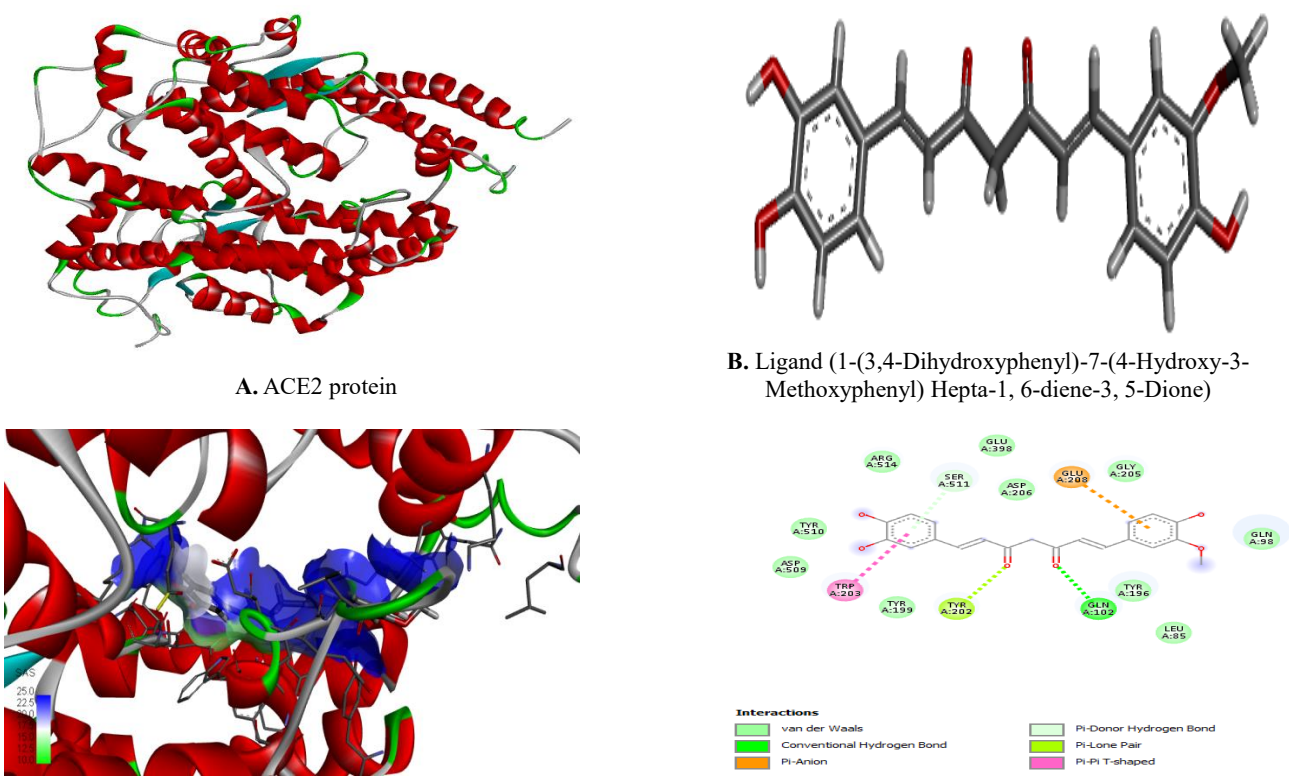
A. ACE2 protein

B. Ligand (Tetrahydroxycurcumin)

C. SAS of protein and ligand interaction

D. 2D structure with amino acid residue

Fig. 4. 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.



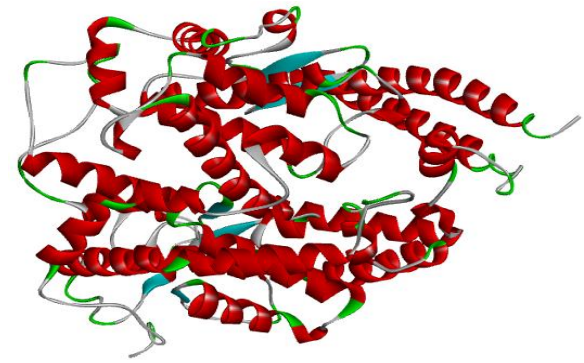
A. ACE2 protein

B. Ligand (1-(3,4-Dihydroxyphenyl)-7-(4-Hydroxy-3-Methoxyphenyl) Hepta-1, 6-diene-3, 5-Dione)

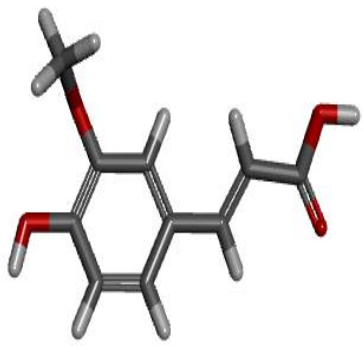
C. SAS of protein and ligand interaction

D. 2D structure with amino acid residue

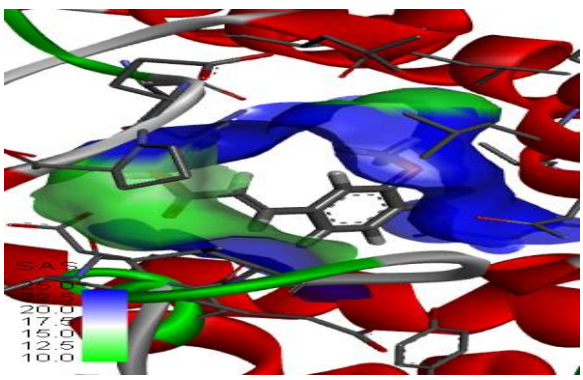
Fig. 5. Analysis of Molecular Docking of Human ACE2 and1-(3,4-Dihydroxyphenyl)-7-(4-Hydroxy-3-Methoxyphenyl)Hepta-1,6-diene-3, 5-Dione.(A) 3D structure of Human ACE2. (B) 3D structure of 1-(3,4-Dihydroxyphenyl)-7-(4-Hydroxy-3-Methoxyphenyl) Hepta-1,6-diene-3, 5-Dione. (C) SAS of Protein and ligand interaction. (D) 2D structure of active site of Ligand and amino acids residues of protein.



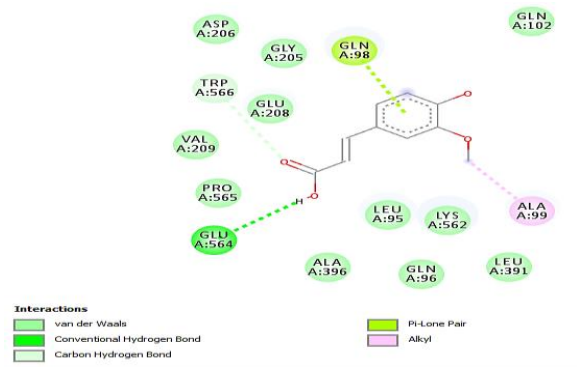
A. ACE2 protein



B. Ligand (E)-Ferulic acid

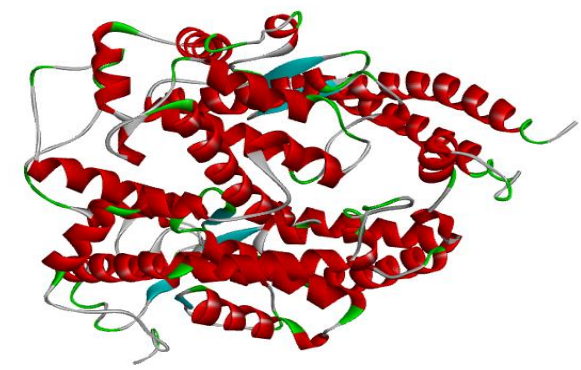


C. SAS of protein and ligand interaction

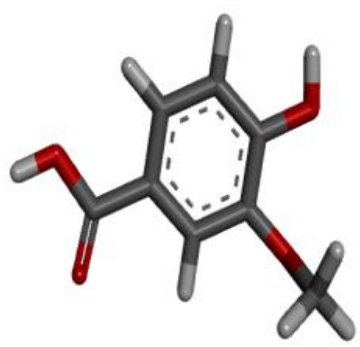


D. 2D structure with amino acid residue

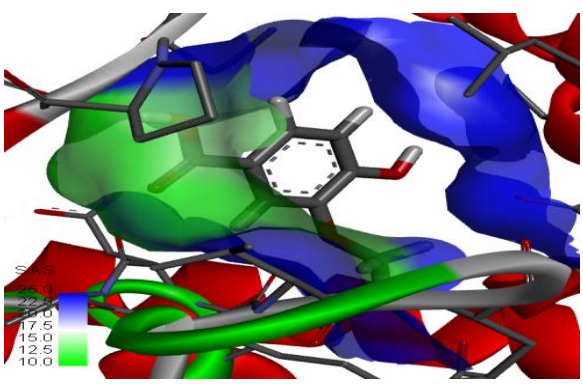
Fig. 6. Analysis of Molecular Docking of Human ACE2 and (E)-Ferulic Acid. (A) 3D structure of Human ACE2. (B) 3D structure of (E)-Ferulic Acid. (C) SAS of Protein and ligand interaction. (D) 2D structure of active site of Ligand and amino acids residues of protein.



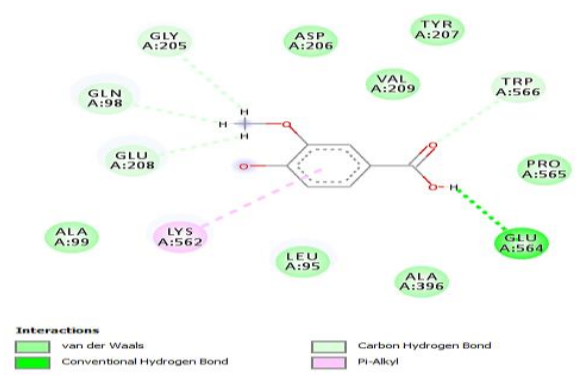
A. ACE2 protein



B. Ligand (Vanillic acid)

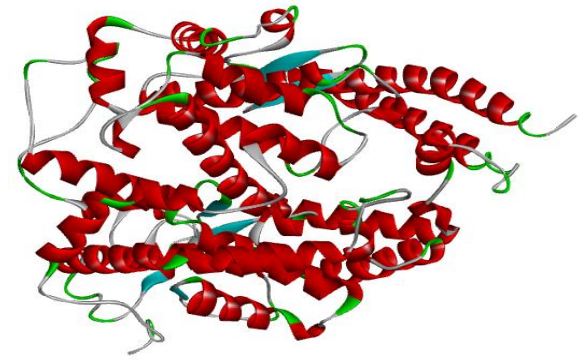


C. SAS of protein and ligand interaction

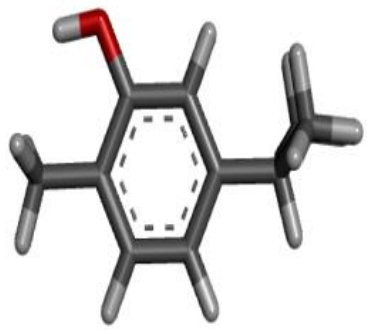


D. 2D structure with amino acid residue

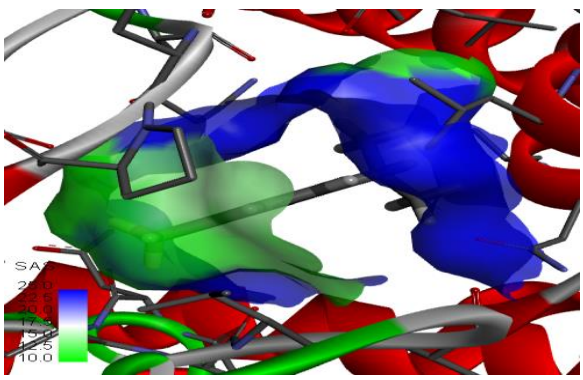
Fig. 7. Analysis of Molecular Docking of Human ACE2 and Vanillic Acid. (A) 3D structure of Human ACE2. (B) 3D structure of Vanillic Acid. (C) SAS of Protein and ligand interaction. (D) 2D structure of active site of Ligand and amino acids residues of protein



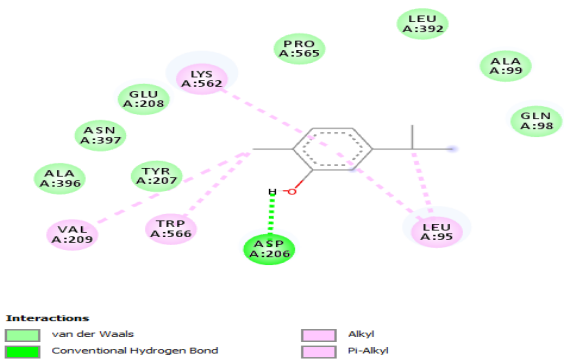
A. ACE2 protein



B. Ligand (Carvacrol)

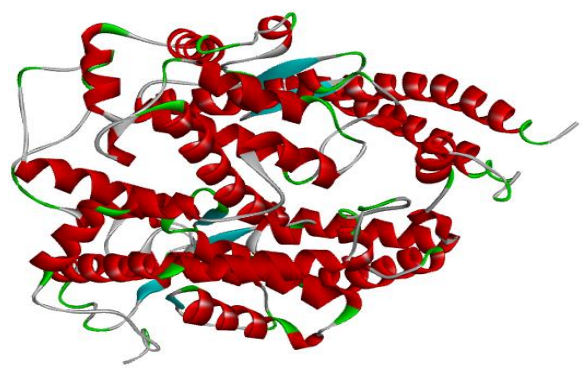


C. SAS of protein and ligand interaction

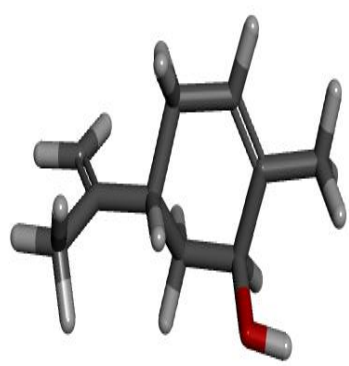


D. 2D structure with amino acid residue

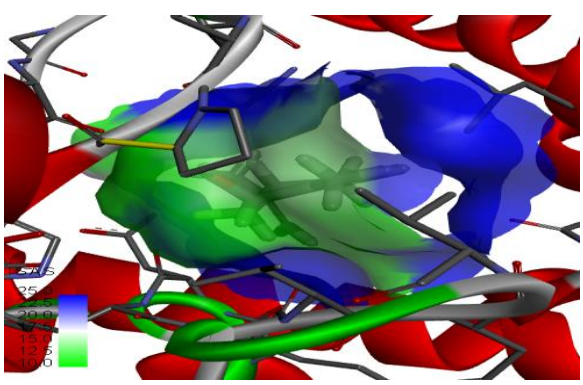
Fig. 8. Analysis of Molecular Docking of Human ACE2 and Carvacrol. (A) 3D structure of Human ACE2. (B) 3D structure of Carvacrol. (C) SAS of Protein and ligand interaction. (D) 2D structure of active site of Ligand and amino acids residues of protein.



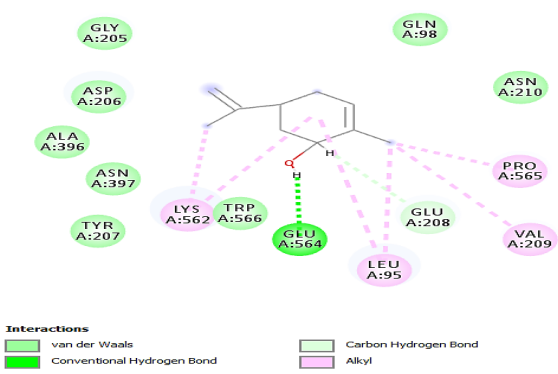
A. ACE2 protein



B. Ligand ((E)-Carveol)

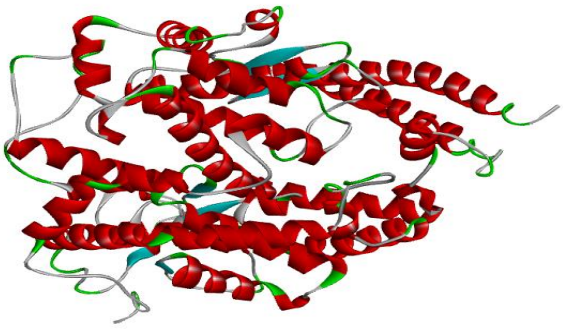


C. SAS of protein and ligand interaction

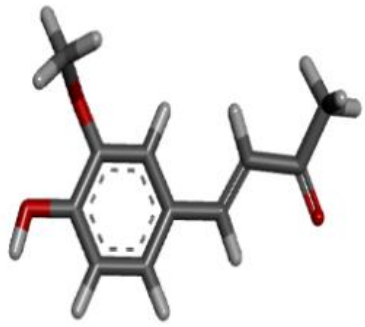


D. 2D structure with amino acid residue

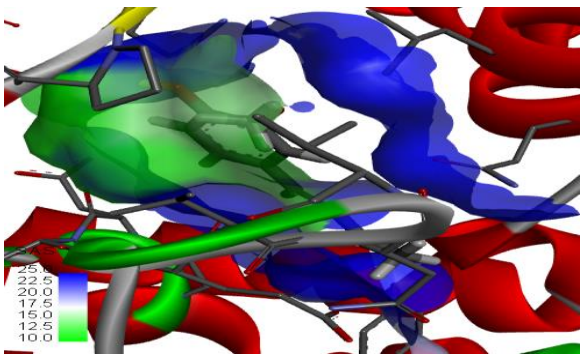
Fig. 9. Analysis of Molecular Docking of Human ACE2 and (E)-Carveol. (A) 3D structure of Human ACE2. (B) 3D structure of (E)-Carveol. (C) SAS of Protein and ligand interaction. (D) 2D structure of active site of Ligand and amino acids residues of protein.



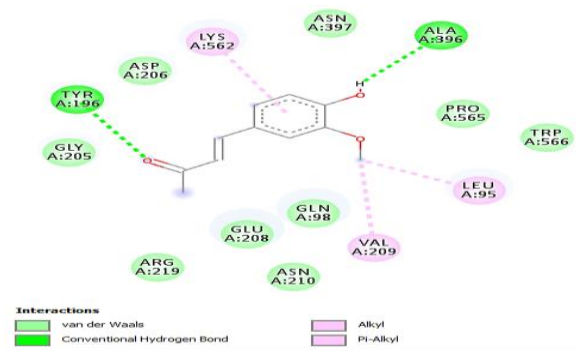
A. ACE2 protein



B. Lihand (E)-4-(4-Hydroxy- 3Methoxyphenyl) But-3-En-2-One

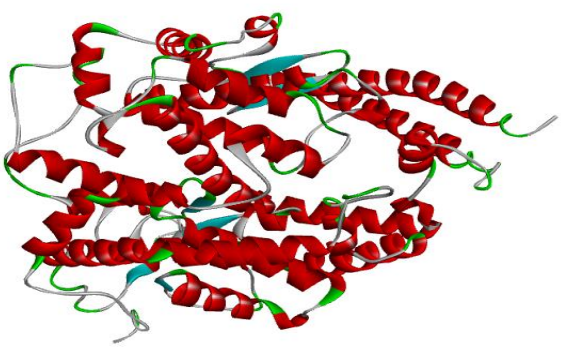


C. SAS of protein and ligand interaction

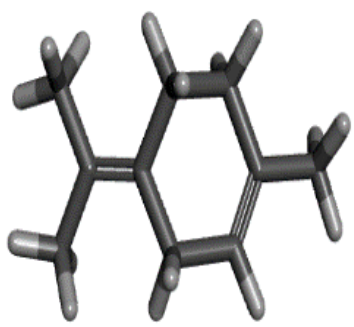


D. 2D structure with amino acid residue

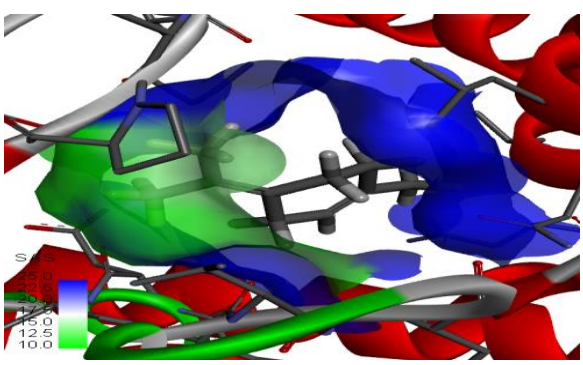
Fig. 10. Analysis of Molecular Docking of Human ACE2 and (E)-4-(4-Hydroxy- 3Methoxyphenyl) But-3-En-2-One. (A) 3D structure of Human ACE2. (B) 3D structure of (E)-4-(4-Hydroxy- 3Methoxyphenyl) But-3-En-2-One. (C) SAS of Protein and ligand interaction. (D) 2D structure of active site of Ligand and amino acids residues of protein.



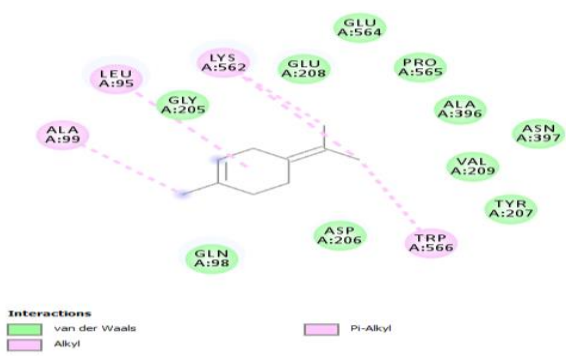
A. ACE2 protein



B. Ligand (Terpinolene)

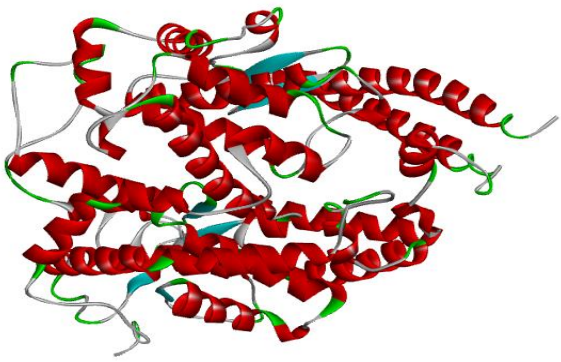


C. SAS of protein and ligand interaction

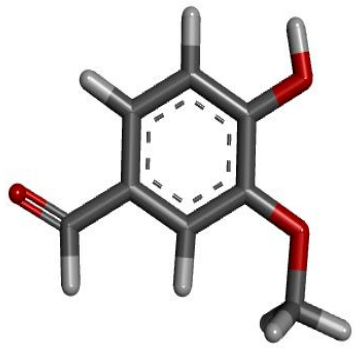


D. 2D structure with amino acid residue

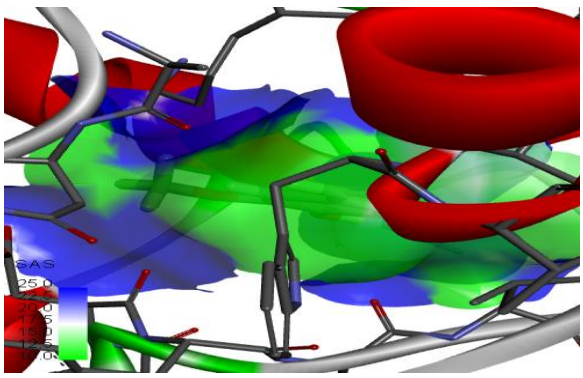
Fig. 11. Analysis of Molecular Docking of Human ACE2 and Terpinolene. (A) 3D structure of Human ACE2. (B) 3D structure of Terpinolene. (C) SAS of Protein and ligand interaction. (D) 2D structure of active site of Ligand and amino acids residues of protein.



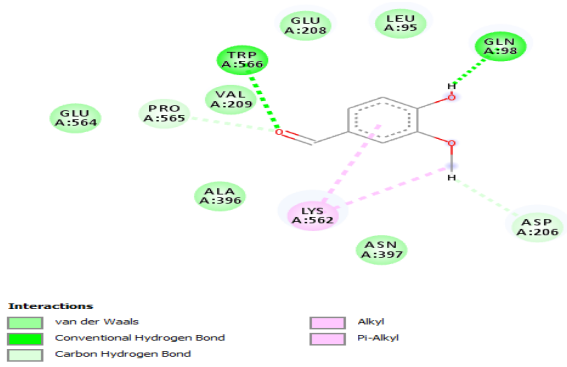
A. ACE2 protein



B. Ligand (Vanillin)

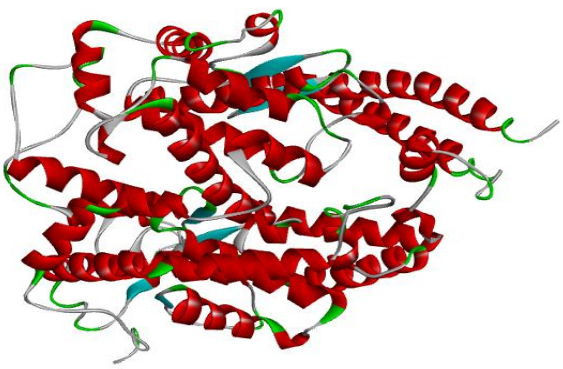


C. SAS of protein and ligand interaction

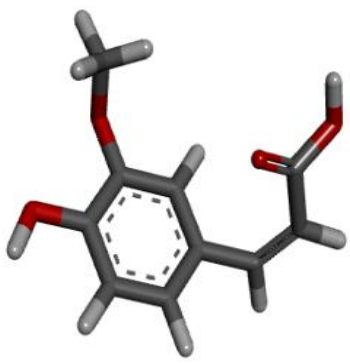


D. 2D structure with amino acid residue

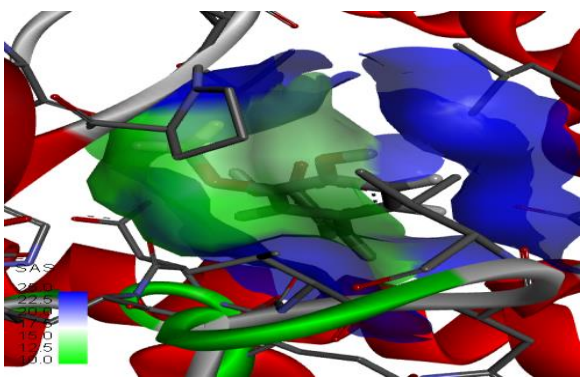
Fig. 12. Analysis of Molecular Docking of Human ACE2 and Vanillin. (A) 3D structure of Human ACE2. (B) 3D structure of Vanillin. (C) SAS of Protein and ligand interaction. (D) 2D structure of active site of Ligand and amino acids residues of protein.



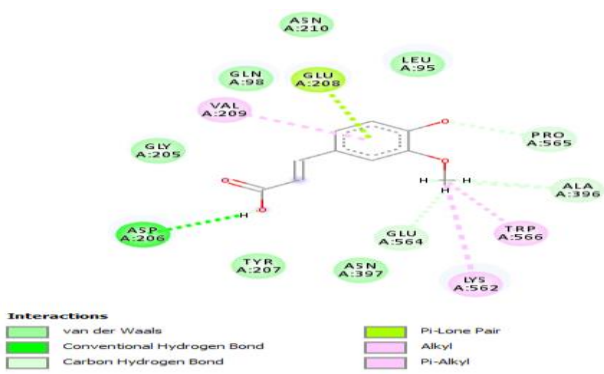
A. ACE2 protein



B. Ligand ((Z)-Ferulic acid)

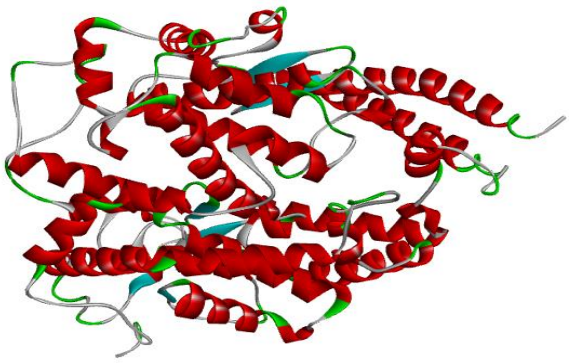


C. SAS of Protein and Ligand interaction

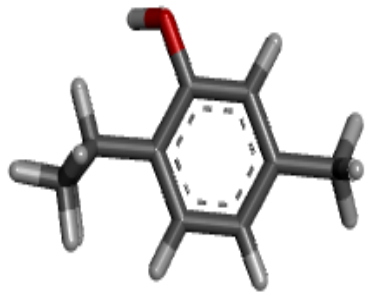


D. 2D structure with amino acid residue

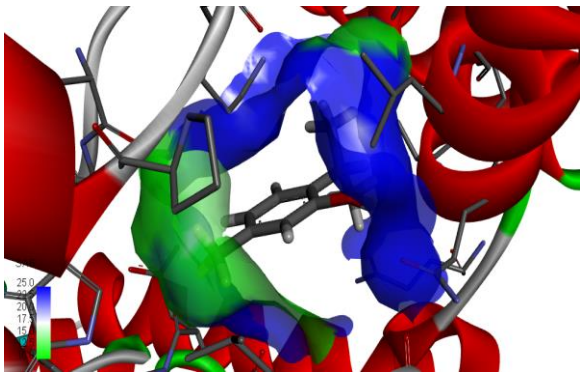
Fig. 13. Analysis of Molecular Docking of Human ACE2 and (Z)-Ferulic Acid (A) 3D structure of Human ACE2. (B) 3D structure of (Z)-Ferulic Acid. (C) SAS of Protein and ligand interaction. (D) 2D structure of active site of Ligand and amino acids residues of protein.



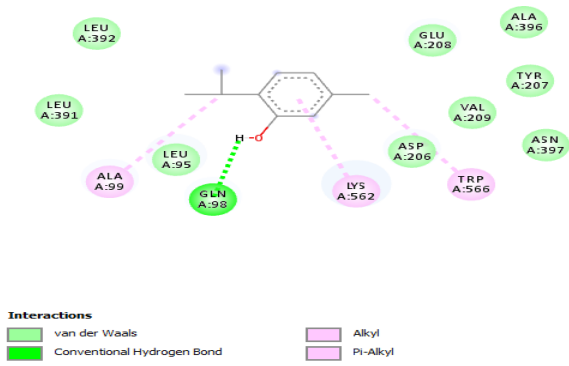
A. ACE2 protein



B. Ligand (Thymol)

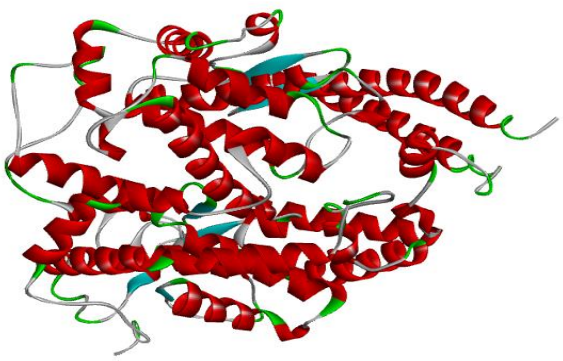


C. SAS of Protein and Ligand interaction

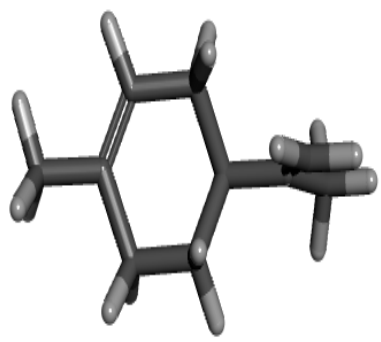


D. 2D structure with amino acid residue

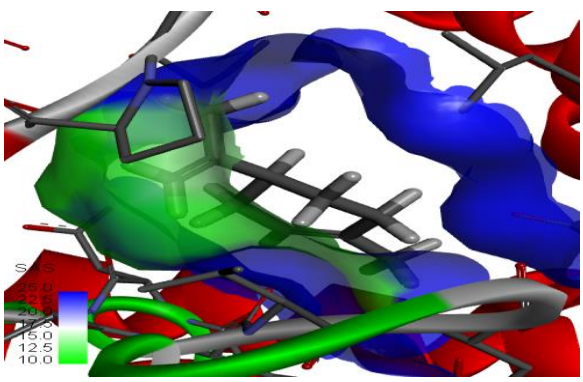
Fig. 14. Analysis of Molecular Docking of Human ACE2 and Thymol. (A) 3D structure of Human ACE2. (B) 3D structure of Thymol. (C) SAS of Protein and ligand interaction. (D) 2D structure of active site of Ligand and amino acids residues of protein.



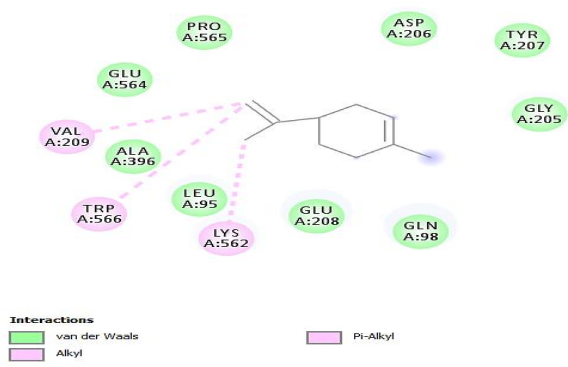
A. ACE2 protein



B. Ligand (Limonene)



C. SAS of protein and ligand interaction

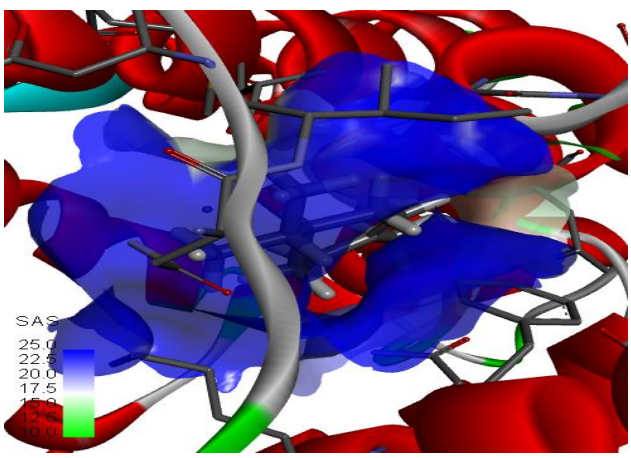


D. 2D structure with amino acid residue

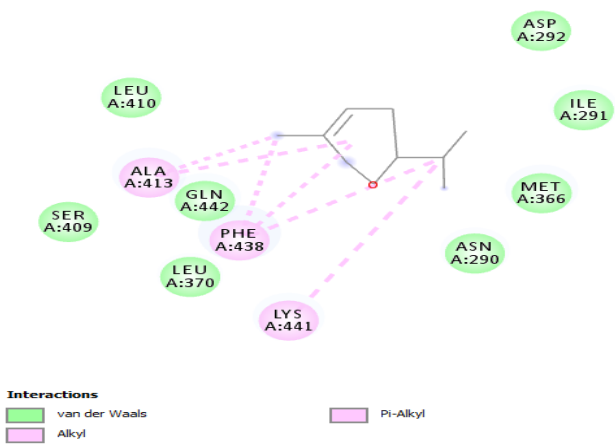
Fig. 15. Analysis of Molecular Docking of Human ACE2 and Limonene. (A) 3D structure of Human ACE2. (B) 3D structure of Limonene. (C) SAS of Protein and ligand interaction. (D) 2D structure of active site of Ligand and amino acids residues of protein.

A. ACE2 protein

B. Ligand (Terpinen-4-Ol)



C. SAS of protein and ligand interaction



D. 2D structure with amino acid residue

Fig. 16. 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.

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 (Laskowski *et al*., 2006). Based on the Veber's 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 (Hou *et al*., 2007). If any drug is violating any of the above given rules then it will have problems regarding bioavailability.

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 have been selected as lead compounds as they have been identified as the most active from all molecules shown interactions with the target receptors (Table 6). These compounds fulfill all the requirements that are required for an oral bioavailable drug.

Conclusion

30 Turmeric compounds mined from the literature were docked for protein and best turmeric compounds were selected for targeted proteins. Turmeric compounds showing strong interaction with protein were selected. 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 rules that are important for the formation of any drug such as absorption, toxicity, metabolism and excretion. All the resulting 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.

Table 4. Docking results with Patchdock scores, ACE value and structures of complex.

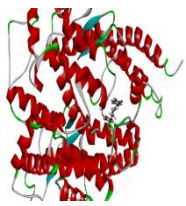
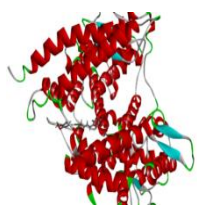
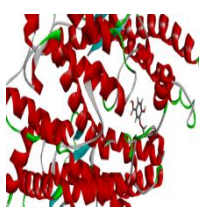
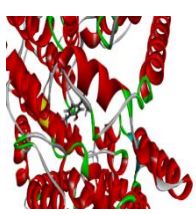
S. No.	Compounds name	PubChem ID	Patchdock scores	ACE value	Structures of complex
1.	Curcumin	969516	4970	-87.13	
2.	Demethylcurcumin	5469426	4610	-123.80	
3.	Tetrahydroxycurcumin	129762283	5050	-290.99	
4.	1-(3,4-Dihydroxyphenyl)-7-(4-Hydroxy-3-Methoxyphenyl) Hepta-1,6-diene-3, 5-Dione	390474	4684	-106.43	
5.	(E)-Ferulic Acid	445858	3212	-96.14	
6.	Vanillic Acid	8468	2818	-92.52	
7.	Carvacrol	10364	3188	-95.88	

Table 4. (Cont'd.).

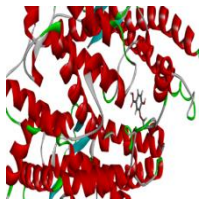
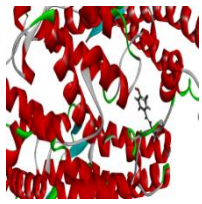
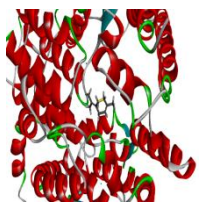
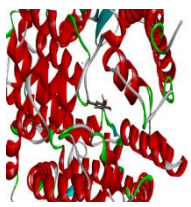
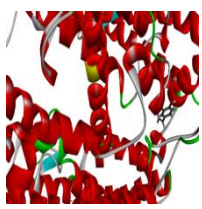
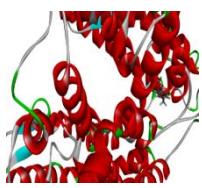
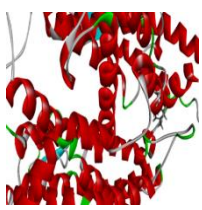
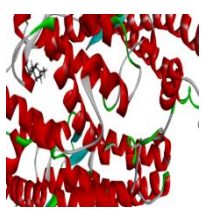
S. No.	Compounds name	PubChem ID	Patchdock scores	ACE value	Structures of complex
8.	(E)-Carveol	94221	2862	-103.12	
9.	E-4-(4-Hydroxy-3-Methoxyphenyl)-3-Buten-2-One	5354238	3326	-56.96	
10.	Terpinolene	11463	3100	-90.87	
11.	Vanillin	1183	2776	-77	
12.	(Z)-Ferulic Acid	1548883	3252	-112.96	
13.	Thymol	6989	2996	-63.36	
14.	Limonene	22311	3056	-116.72	
15.	Terpinen-4-Ol	11230	29.34	-60.34	

Table 5. 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	20.2	154.25	2.60	1	1	1

Table 6. 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

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