Scope of phytotherapeutics in targeting ACE2 mediated Host-Viral Interface of SARS-CoV2 that causes COVID-19

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ABSTRACT

Severe acute respiratory syndrome coronavirus (SARS-CoV-2) that cause COVID-19 becomes a global threat and spread its pandemicity across the boundaries. Recent demography issued by WHO forecasting the severity of disease prevalence in more than 200 countries resulted in 16,96,588 laboratory confirmed cases excluding 1,05,952 deaths as on 12 April 2020. Countries like USA (4,92,881), Italy (1,52,271), Spain (1,61,852), Germany (1,20,479) are struggling hard to flatten their epidemic curve of COVID-19. Dynamic strategies are of utmost important in order to manage the crucial spread of SARS-CoV-2. Drug of herbal origin may offer reliable therapeutic opportunity in controlling widespread transmission. It was evident from the scientific outcomes that SARS-CoV-2 gains access in to the host cell through angiotensin-converting enzyme 2 (ACE2) receptors. Hence drugs that reveals potential binding affinity with core amino acid of ACE-2 may expected to interfere the host-viral interaction. In our present investigation 28 lead molecules from well documented medicinal herbs were subjected to molecular docking analysis targeting ACE2 receptor and their potential of impeding host-viral interface were evaluated. Results of computational analysis signifies that out of 28 ligands nearly 11 bioactive lead molecules exhibit potential binding affinity of about 100% with the target amino acid residue (31 Lys and 353 Lys).

Key words: SARS-CoV-2, COVID-19, ACE2 receptor, Medicinal plants, Bioactive lead molecules.

1.Introduction

In the scale of nomenclature coronaviruses belong to order Nidovirales, family Coronaviridae, in which SARS-CoV-2 categorized as betacoronavirus, with RNA genomic density of 29,891 nucleotides in size, encoding 9860 amino acids[1]. Novel coronal virus identified with presence of four major structural glycoproteins (spike(S), membrane (M), nucleocapsid (N) and envelope (E)) on its structural morphology[2]. It is well known that SARS-CoV-2 virus exerts its pathogenicity by binding with the Angiotensin-converting enzyme 2 (ACE2) receptor[3,4].

ACE2 belongs to type I transmembrane metallocarboxypeptidase enzyme widely expressed in variety of cell, angiotensin II considered to be an endogenous substrate for ACE2[5] additionally this receptor is being recognized as binding site for novel corona virus for its pathogenesis. This grabs the attention of researcher globally to focus more on the architecture and morphology of this enzyme for possible identification of leads to exerts if affinity. Increased affinity of the lead compounds with that of the ACE2 keeps the enzyme more occupied and less available for viral interactions,

Medicinal herbs contribute to the benefit of the mankind since several centuries. Most significant pharmacophores of the existing drugs are derived from the herbal origin. Versatile functional group and side chain moieties synergies the affinity of receptor binding and thereby offers expected pharmacological activity [6-9]. On folklore basis metabolites from numerous herbs are known for managing diseases and disorders around the globe

Traditional medicines like Nilavembu Kudineer and Kaba Sura Kudineer reasonable occupies the prescriptions of the indian medicine practitioners in the clinical management of viral infections, perhaps these two formulations are officially recommending by the government authorities for afore mentioned cause. The rationale behind selection of these Indian medicines is because of its claim in management of epidemic viral infection and also due to the existence of combinatorial makeup of their relative therapeutics components. Formulation Nilavembu Kudineer (NVK) majorly consist of *Andrographis Paniculata, Plectranthus Vettiveroides, Vetiveria Zizanioides, Zingiber Officinale, Piper Nigrum, Cyperus Rotundus, Santalum Album, Trichosanthes Cucumerina* and *Mollugo Cerviana* [10,11]. Other formulation Kaba Sura Kudineer (KSK) comprises of fifteen herbs such as *Zingiber Officinale, Piper longum, Syzygium aromaticum, Tragia involucrata , Anacyclus pyrethrum , Hygrophila auriculata , Terminalia chebula , Justicia adhatoda, Plectranthus amboinicus ,Saussurea costus ,Tinospora sinensis ,Premna herbacea, Andrographis Paniculata, Cissampelos pariera* and *Cyperus Rotundus* [12-14].

In our present investigation 28 bioactive leads (6-Shogaol, 6-Gingerol, Beta Sitosterol, Piperidine, Apigenin, Piperine, Quercetin, Chlorogenic Acid, Beta-Pinene, Alpha-Bisabolol

,Andrographolide, Bharangin, Carvacrol, Cissamine ,Costunolide, Cucurbitacin B, Gallic acid, Linoleic acid, Pellitorine, Rutin, Santalic acid, Spathulenol, Vasicine, Vetiverol , Cynaropicrin, Eugenol, Thymol and Vitexin) retrieved based on the literature search on the herbal ingredients present in both these traditional siddha medicines (NVK & KSK) were subjected to the *In-Silico* evaluation in targeting active site of the ACE2 receptor that mediates the host- viral interface.

2.Materials and Methods

2.1.Protein-ligand docking

Computational molecular investigation was performed using Auto Dock version 4 which predicts interaction binding affinity between selected therapeutic lead with that of the protein target (SARS-CoV tagged - Angiotensin-converting enzyme 2 (ACE2)- PDB- 2AJF.

2.2.Protein preparation

Three dimensional (3D) structure of SARS-CoV bounded ACE2 with protein data bank (PDB)-2AJF (Figure 1) retrieved from Research Collaboratory for Structural Bioinformatics (RCSB). Protein structure were cleaned by removing the existing lead components, water molecules cleaved, Gasteiger charges computed with inclusion of polar hydrogens, merging of non-polar and rotatable bonds were defined using Auto Dock 4 [15,16].

2.3.Active site prediciton on the target protein

Biologically active amino acid residues which are primary involved in executing the viral host interface was predicted using Ramachandran plot indicating localization of the residues on the A chain of the target enzyme. Prediction by MolProbity server and also through literature survey. Ramachandran plot signifies the sequential paradigm of 1542 residues which are present in refinement carried out in REFMAC 5.2.0019. R = 0.218; Rfree = 0.275, and the structure was solved at 2.90 Å resolution as shown in Figure 2.

2.4.Ligand model preparation

Structures of the bioactive lead compounds such as 6-Shogaol, 6-Gingerol, Beta Sitosterol, Piperidine, Apigenin, Piperine, Quercetin, Chlorogenic Acid, Beta-Pinene, Alpha-Bisabolol ,Andrographolide, Bharangin, Carvacrol, Cissamine ,Costunolide, Cucurbitacin B, Gallic acid, Linoleic acid, Pellitorine, Rutin, Santalic acid, Spathulenol, Vasicine, Vetiverol , Cynaropicrin, Eugenol, Thymol and Vitexin subjected to docking investigation were outlined using ChemDraw sketch software and converted from two dimension (2D) to 3D structures. Figure 3 and 4 Summarizing 2D and 3D structure of Bio-active therapeutic ligand subjected to molecular docking Investigation against SARS-CoV tagged - Angiotensin-converting enzyme 2 - PDB- 2AJF.

2.5.Docking simulations

3D componential structure of lead molecules and protein were docked using AutoDock analytical tool version 4. Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program[17]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the programmed algorithm inbuilt with pre automation in the software. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

3.Results and Discussion

ACE2 receptors becomes primary target for SARS-CoV-2 to exert its pathogenicity in the affected individuals [18,19]. SARS-CoV-ACE2 receptors possess two promising active biding sites that residue located primly on Lys 31 and another hotspot is Lys 353. The active site Lys 31 hierarchically bridged between amino acid Lys 31 – Glu 35. Similarly, site Lys 353 bridged between ASP 38 - Lys 353. Series of amino acids (Leu455, Phe486, Ser494) lying on binding motif of SARS-CoV-2 primary mediates the biding paradigm of virus with that of the two primary hotspots (Lys 31 and Lys 353) that resides on the ACE2 receptor [20,21]. The amino terminal peptides of ACE2 glycoprotein tailed outwards targeted by receptor binding domain of SARS-CoV-2.

Results of computational analysis signifies that out of 28 ligands retrieved from two Indian medicines nearly 11 bioactive lead molecules that includes Rutin, Pellitorine, Cynaropicrin, Andrographolide, Linoleic acid, Cucurbitacin B,Vitexin, 6-Gingerol ,Beta Sitosterol, Apigenin and Quercetin exhibit potential binding affinity of about 100% with the target amino acid residue (31 Lys and 353 Lys). Other seven compounds (Eugenol, Gallic acid, Thymol, Costunolide, Bharangin, 6-Shogaol and Piperine) contributes binding affinity only with 31 Lys, whereas four compounds (Carvacrol, Santalic acid, Piperidine and Chlorogenic acid) bound only with 353 Lys. Remaining five compounds includes Vasicine, Beta-Pinene, Spathulenol, Vetiverol and Alpha-Bisabolol fails to offer expected affinity on both the amino acid present in the target site. Results of docking score along with amino acid residual interactions were summarized in Table 1 and Table 2.

4.Conclusion

Present investigation only explorate the binding potential of the valuable leads with that of the active residues of ACE2 that involved in mediating the host viral interface. Hence in future systematic investigation at *In-vitro*, *In-vivo* and clinical level become highly essential to validate the efficacy of these lead molecules prior to the recommendation.

Acknowledgements

The Ministry of AYUSH (Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy), Government of India, Indian Council of Medical Research (ICMR), Government of India, Chinese Association for Laboratory Animal Sciences (CALA), P.R.China.

Author contributions

Authors are equally involved in study design, simulation, data collection and compilation

Competing interests

The authors declare no competing interests.

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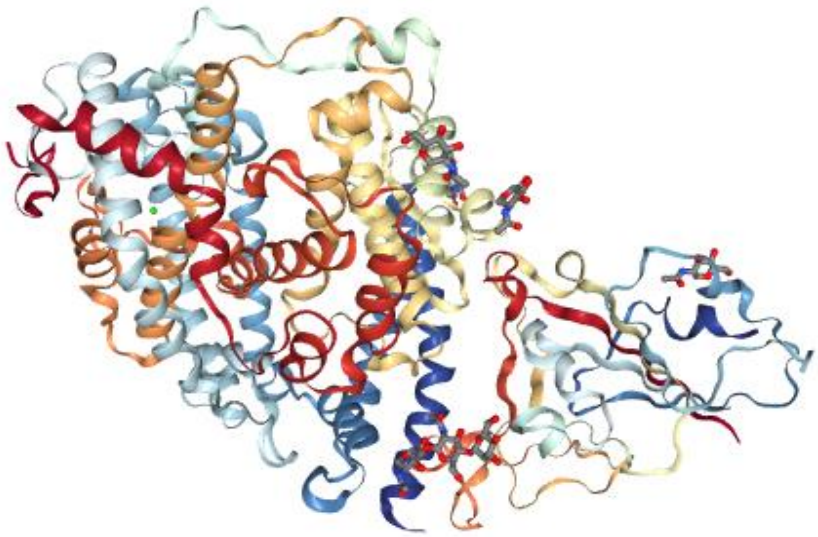
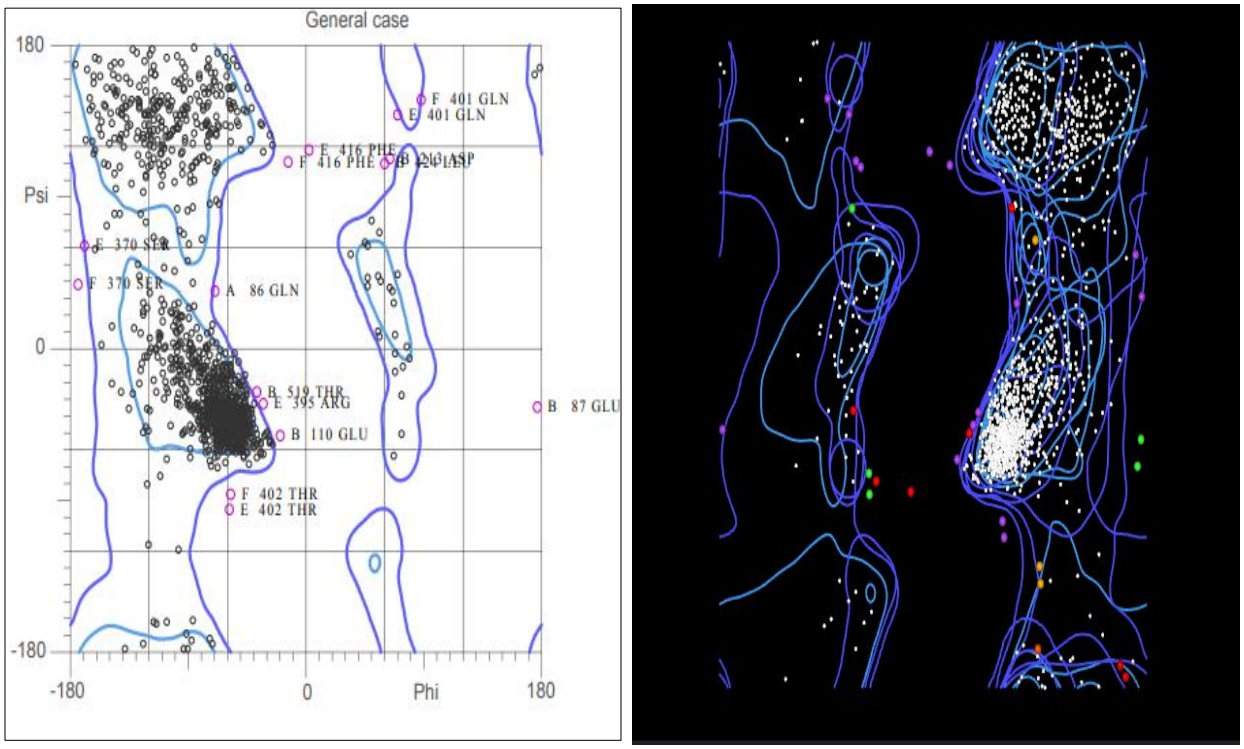


Figure 1. 3D structure of SARS-CoV attached with its cellular receptor, Angiotensin-converting enzyme 2 (ACE2)- (PDB)- 2AJF



R = 0.218; Rfree = 0.275, A total of 1542 residues are present., Structure was solved at 2.90 Å resolution, 81 hetero group(s) are present.

Figure 2. Ramachandran plot indicating amino acid resides in cluster of A- Chain – Angiotensin-converting enzyme 2 (ACE2)- (PDB)- 2AJF

Chain	Res	High B	Ramachandran	Rotamer	Cβ deviation
A 31	LYS	96.1	Favored (33.64%) General / -79.6,-38.6	Allowed (0.4%) tmmt chi angles: 182.4,268.9,291.4,139.7	0.00Å
A 353	LYS	69.26	Favored (27.99%) General / 55.9,43.2	Allowed (1.4%) mttp chi angles: 305.7,228.7,171.6,49.8	0.07Å

Table 1: Summarizing sequential binding behavior of phytotherapeutic lead molecules with that of the target Amino acid residues at Angiotensin-converting enzyme 2 (ACE2)- (PDB)- 2AJF

S.No	Molecule	Docking Score (K cal/mol)	Amino Acid Residues								
1	m-Eugenol	-2.53	**31 LYS**	34 HIS	35 GLU	38 ASP					
2	Rutin	-3.41	27 THR	**31 LYS**	34 HIS	35 GLU	38 ASP	**353 LYS**			
3	Pellitorine	-3.4	**31 LYS**	34 HIS	37 GLU	38 ASP	**353 LYS**				
4	Gallic acid	-2.02	**31 LYS**	34 HIS	35 GLU						
5	Vasicine	-6.21	40 PHE	350 ASP	390 PHE	393 ARG	394 ASN				
6	p-Thymol	-2.75	**31 LYS**	34 HIS	35 GLU						
7	Carvacrol	-3.31	33 ASN	34 HIS	37 GLU	38 ASP	**353 LYS**	390 PHE	393 ARG		
8	Costunolide	-4.00	27 THR	30 ASP	**31 LYS**	34 HIS					
9	Cynaropicrin	-3.06	**31 LYS**	34 HIS	35 GLU	38 ASP	**353 LYS**				
10	Bharangin	-4.36	**31 LYS**	34 HIS	35 GLU	38 ASP					
11	Andrographolide	-4.53	**31 LYS**	34 HIS	35 GLU	37 GLU	38 ASP	**353 LYS**			
12	Cissamine	-4.77	34 HIS	37 GLU	**353 LYS**	389 PRO	393 ARG				
13	Beta-Pinene	-5.22	37 GLU	40 PHE	350 ASP	390 PHE	393 ARG				
14	Spathulenol	-4.98	30 ASP	33 ASN	34 HIS	37 GLU	390 PHE	393 ARG			
15	Vetiverol	-4.96	30 ASP	33 ASN	34 HIS	37 GLU	389 PRO	393 ARG			
16	Linoleic acid	-2.07	30 ASP	**31 LYS**	34 HIS	37 GLU	38 ASP	**353 LYS**			
17	Santalic acid	-3.41	33 ASN	34 HIS	37 GLU	**353 LYS**	393 ARG				
18	Cucurbitacin B	-5.36	30 ASP	**31 LYS**	34 HIS	37 GLU	38 ASP	**353 LYS**			
19	Vitexin	-5.71	**31 LYS**	34 HIS	35 GLU	37 GLU	38 ASP	42 GLN	**353 LYS**		
20	Alpha-Bisabolol	-5.69	40 PHE	350 ASP	390 PHE	391 LEU	393 ARG	394 ASN	562 LYS		
21	6-Shogaol	-3.33	**31 LYS**	34 HIS	35 GLU	38 ASP	39 LEU	42 GLN			
22	6-Gingerol	-3.49	**31 LYS**	34 HIS	35 GLU	37 GLU	38 ASP	**353 LYS**			
23	Beta Sitosterol	-4.88	27 THR	30 ASP	**31 LYS**	34 HIS	35 GLU	38 ASP	**353 LYS**		
24	Piperidine	-4.31	30 ASP	33 ASN	34 HIS	35 GLU	37 GLU	38 ASP	**353 LYS**	389 PRO	393 ARG
25	Apigenin	-3.75	**31 LYS**	34 HIS	35 GLU	38 ASP	**353 LYS**				
26	Piperine	-4.1	**31 LYS**	34 HIS	35 GLU	37 GLU	38 ASP				
27	Quercetin	-4.11	**31 LYS**	34 HIS	35 GLU	38 ASP	**353 LYS**				
28	Chlorogenic Acid	-2.15	37 GLU	35 GLU	37 GLU	38 ASP	**353 LYS**				

Figure 3: 2D structure of Bio-active therapeutic ligand subjected to molecular docking Investigation against angiotensin-converting enzyme 2 (ACE2)- 2AJF (PDB)

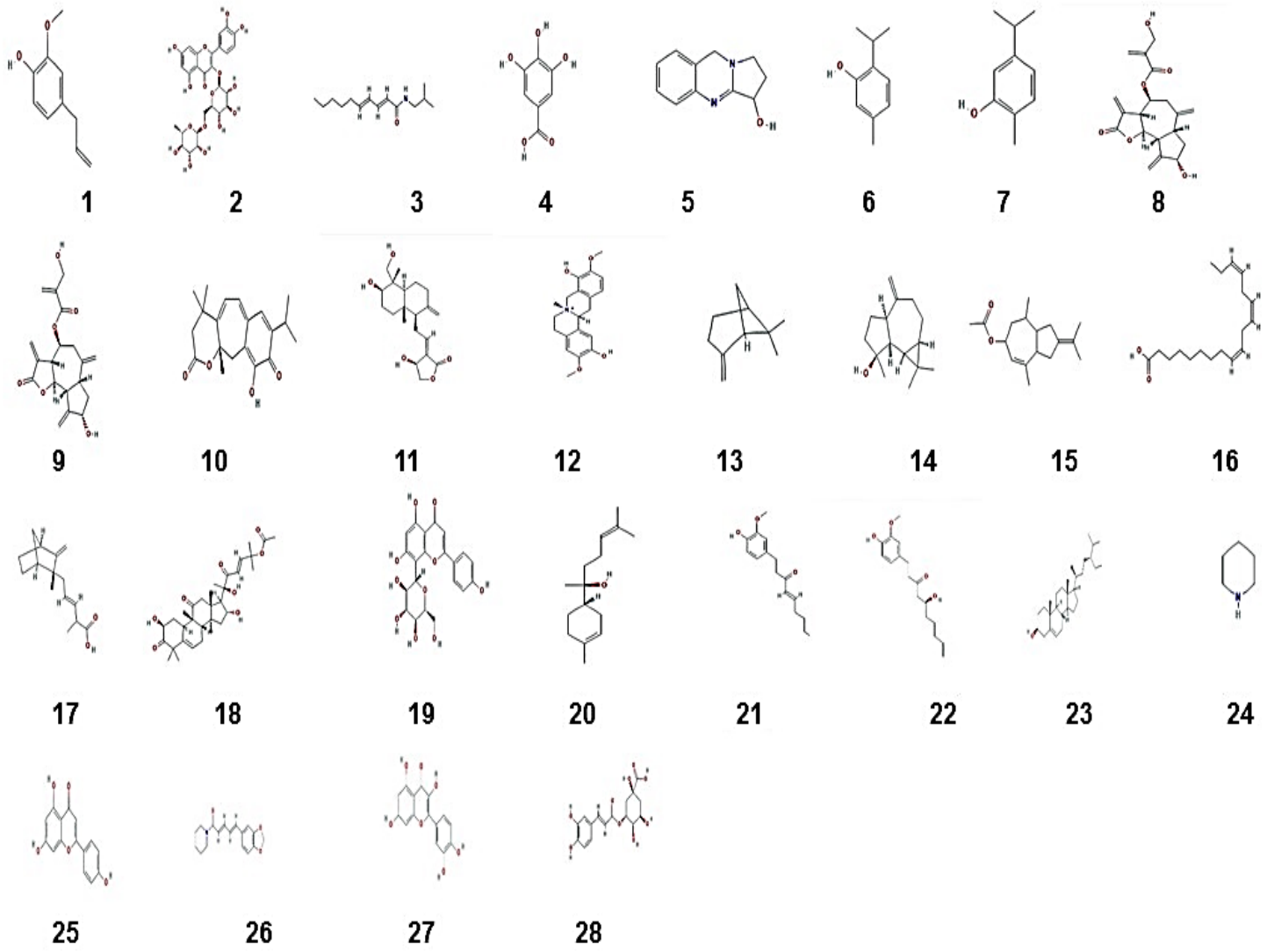


Figure 4: 3D structure of Bio-active therapeutic ligand subjected to molecular docking Investigation against angiotensin-converting enzyme 2 (ACE2)- 2AJF (PDB)

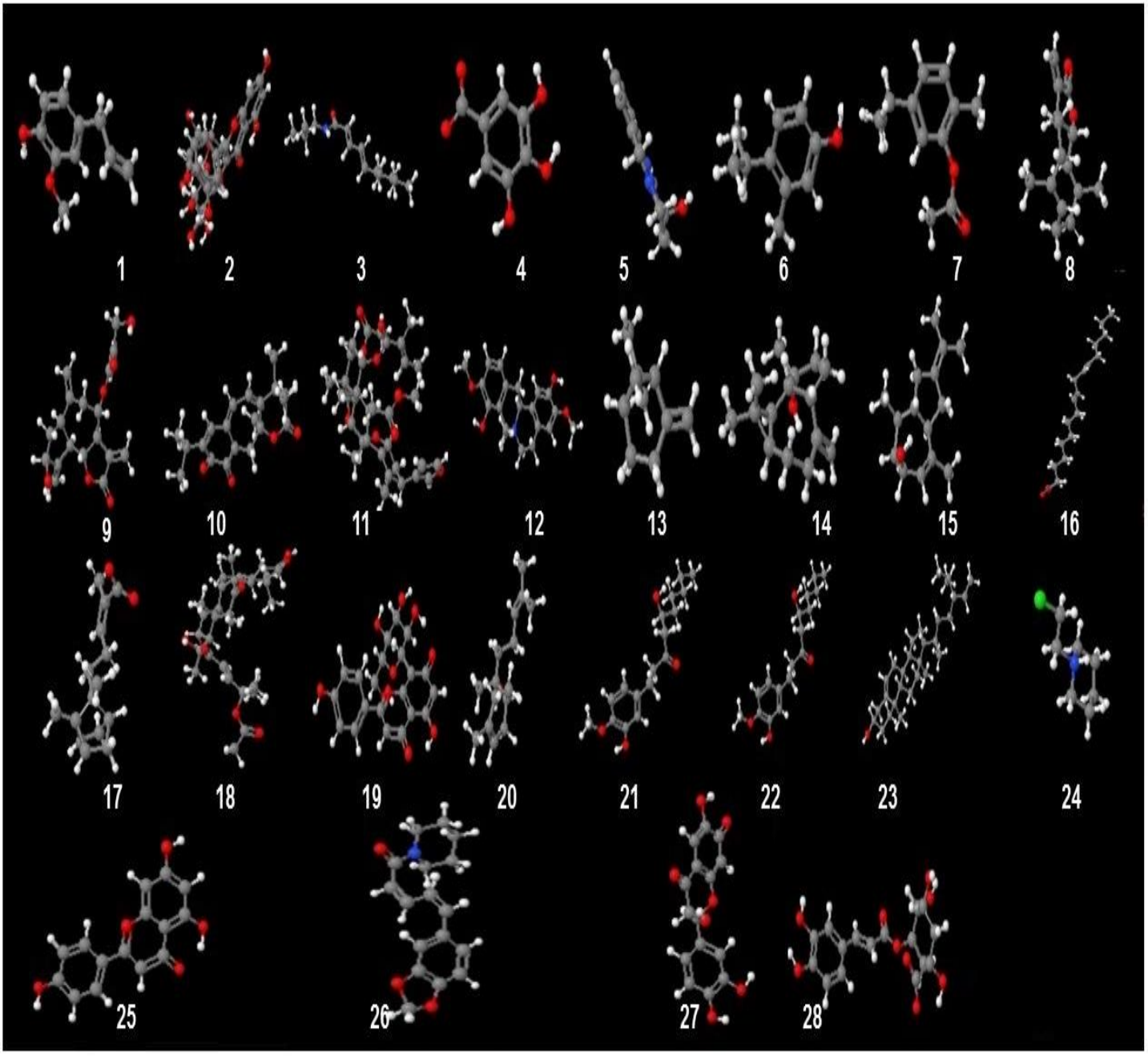
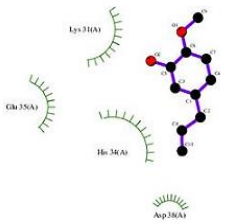
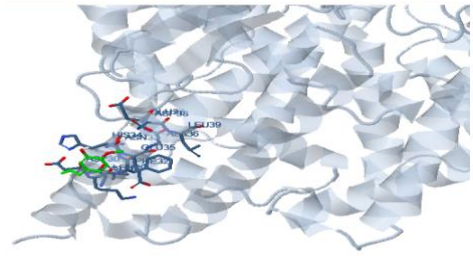
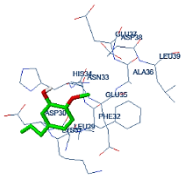
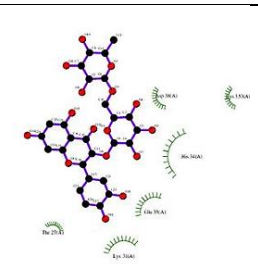
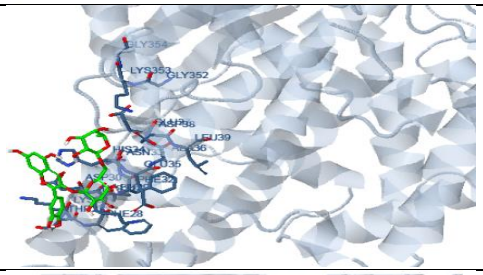
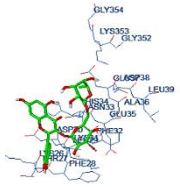
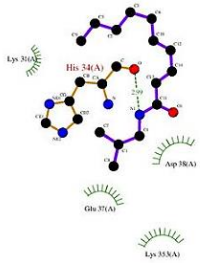
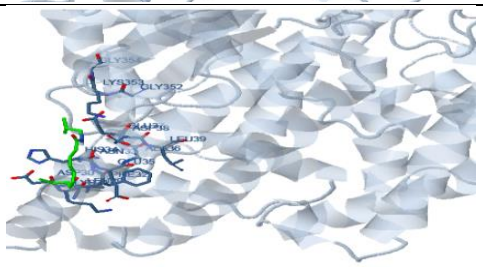
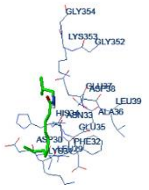
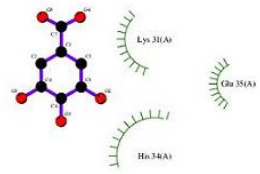
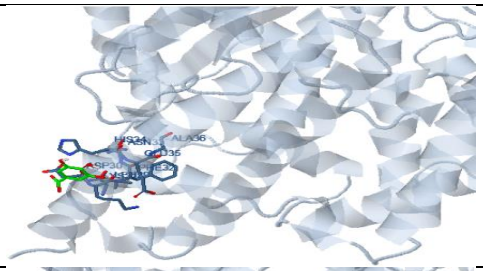
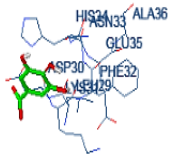
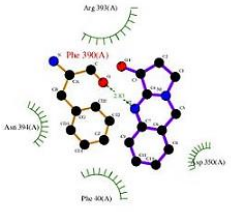
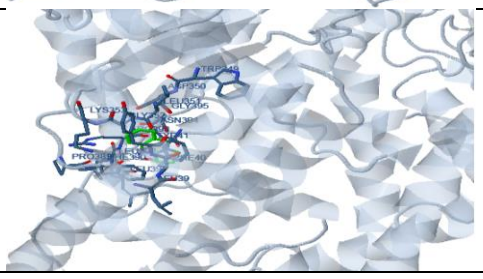
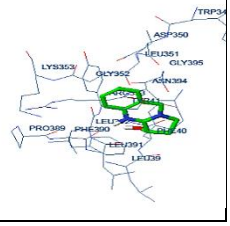
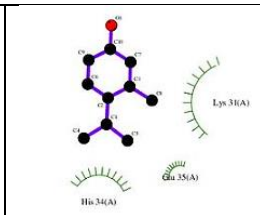
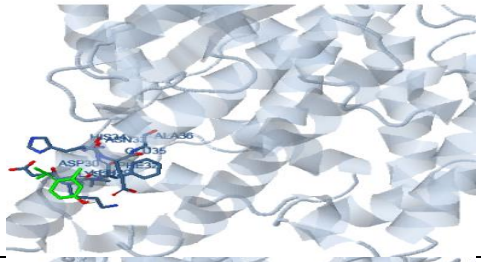
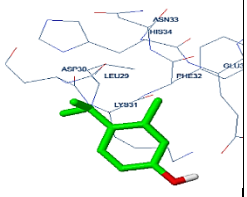
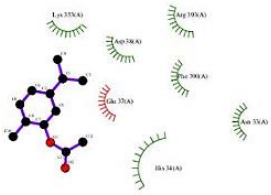
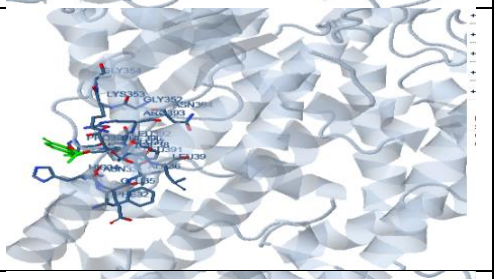
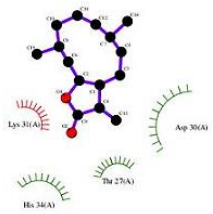
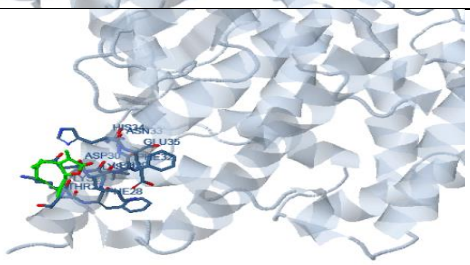
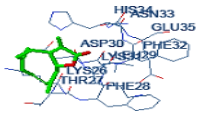
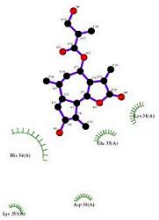
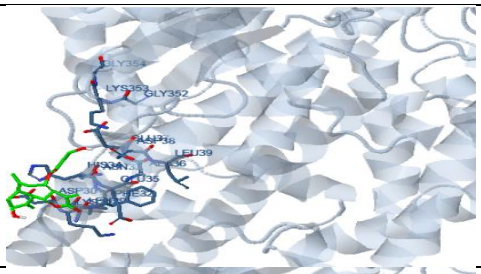
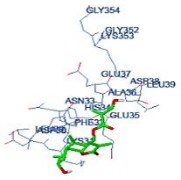
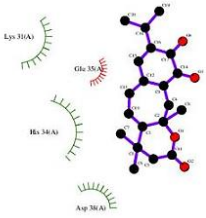
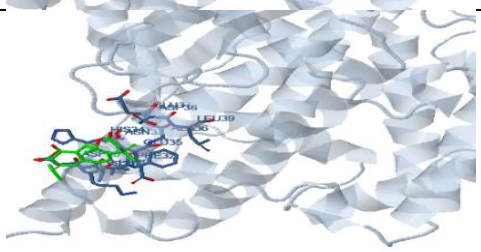
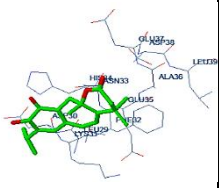
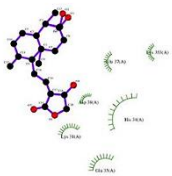
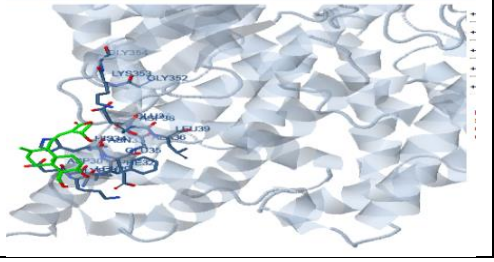
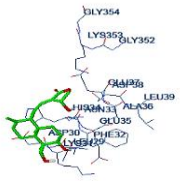
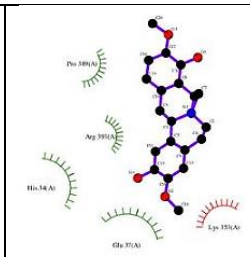
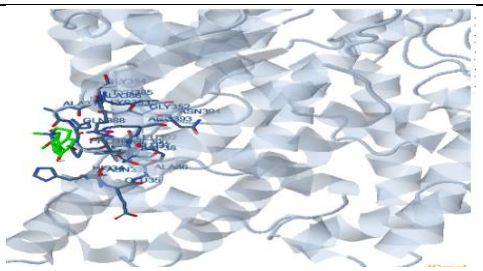
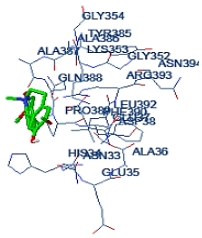
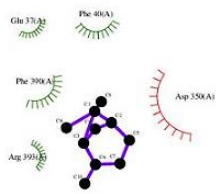
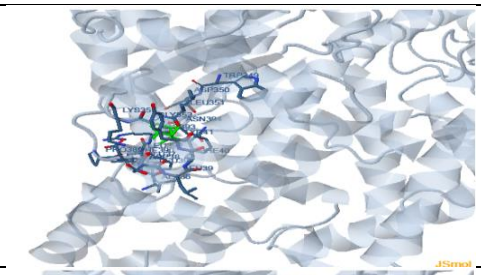
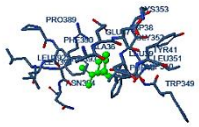
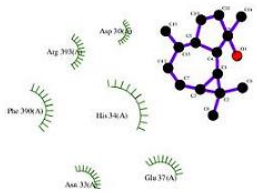
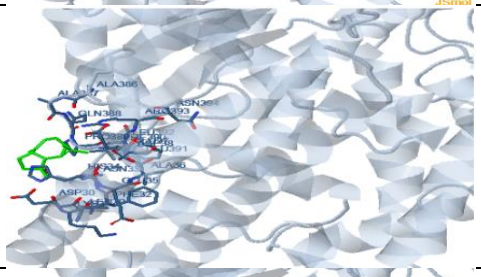
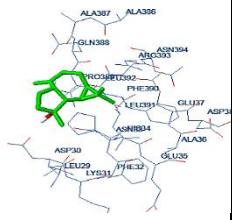
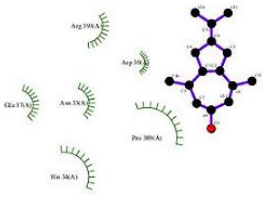
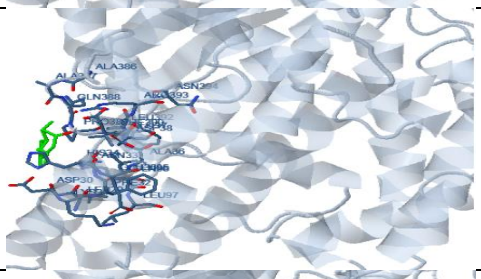
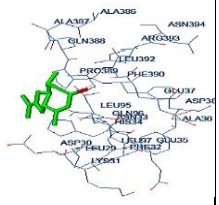
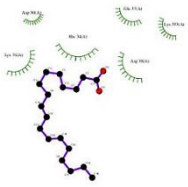
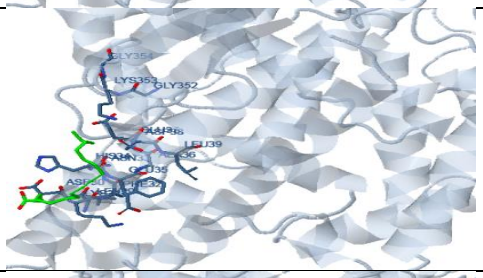
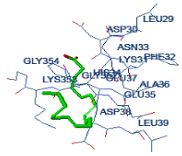
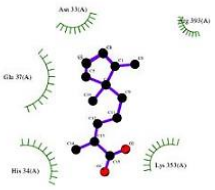
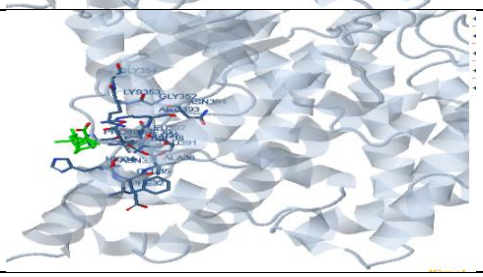
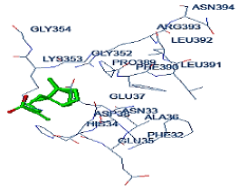
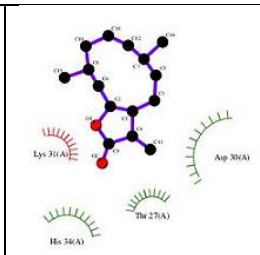
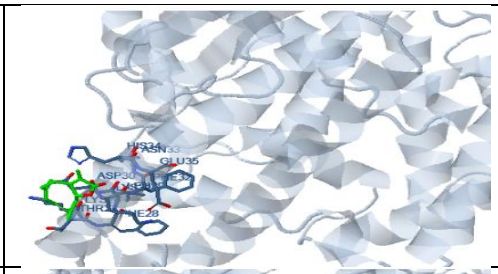
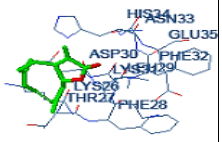
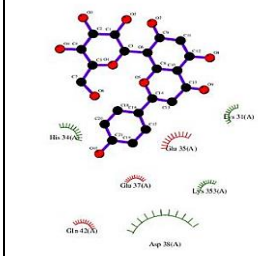
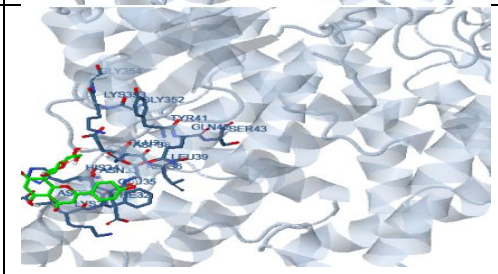
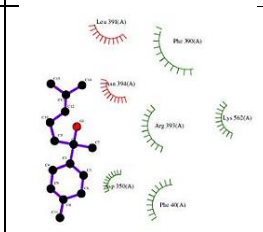
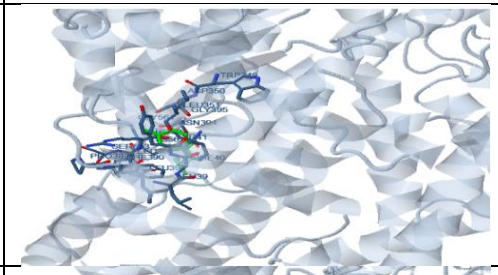
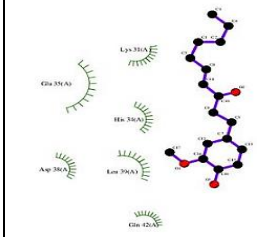
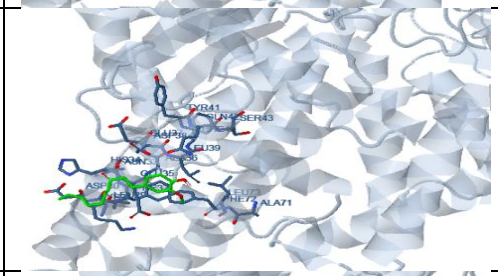
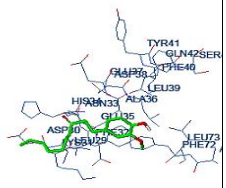
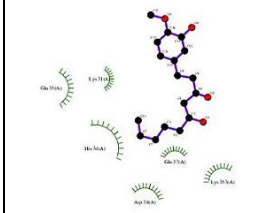
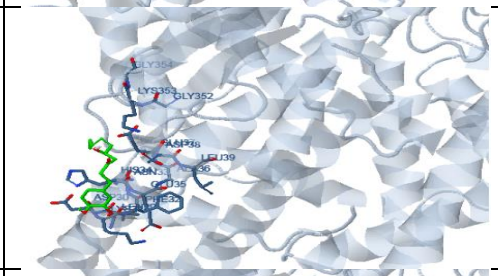
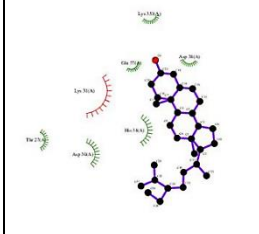
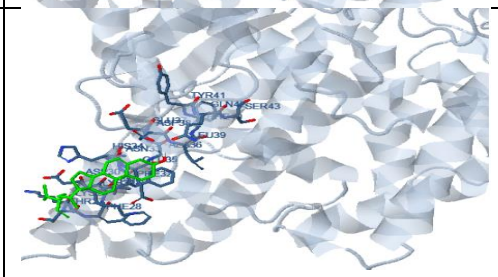


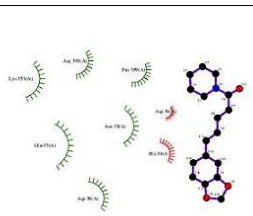
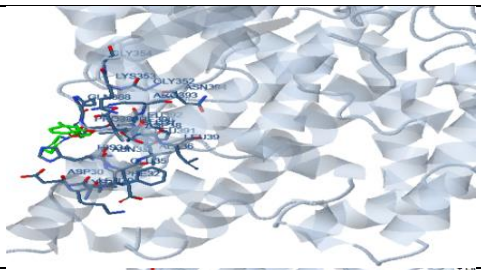
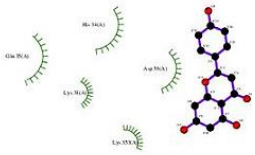
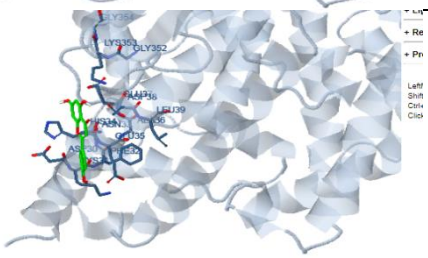
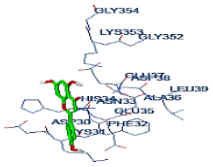
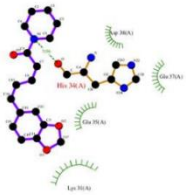
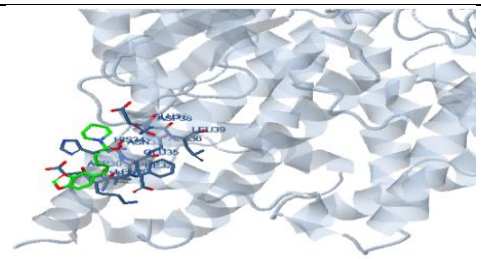
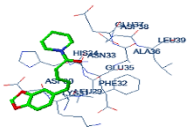
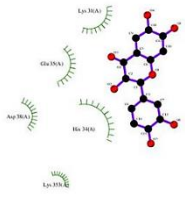
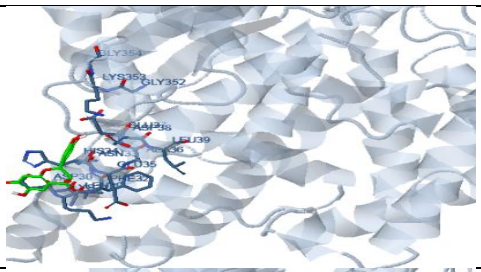
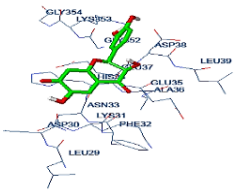
Table 2: Summarizing the docking score and representing the interaction analysis plot with best binding docking pose of phytochemicals from the potentials herbs against Angiotensin-converting enzyme 2 (ACE2)- (PDB)- 2AJF

S.No	Name of the Ligand	2D Plot	Docking Pose	Interaction Surface
1.	Eugenol			
2.	Rutin			
3.	Pellitorine			
4.	Gallic acid			
5.	Vasicine			

6.	Thymol			
7.	Carvacrol			
8.	Costunolide			
9.	Cynaropicrin			
10.	Bharangin			
11.	Andrographolide			

12.	Cissamine			
13.	β-pinene			
14.	Spathulenol			
15.	Vetiverol			
16.	Linoleic acid			
17.	Santalic acid			

18.	Cucurbitacin B			
19.	Vitexin			
20.	Alpha-Bisabolol			
21.	6-Shogaol			
22.	6-Gingerol			
23.	Beta Sitosterol			

24.	Piperidine			
25.	Apigenin			
26.	Piperine			
27.	Quercetin			
28.	Chlorogenic Acid