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# STUDY OF COMPUTER BASED MOLECULAR DOCKING OF CASSIA FISTULA LEAVES

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## ABSTRACT

Aim of present study is to learn the new approaches in the field of computational Chemistry. We studied here Comparative preliminary photochemical screening test of the ethanol extract and aqueous extract of leaves of Cassia fistula obtained by cold maceration process. These study antibacterial assay was performed with the help of cup plate method for ethanolic and aqueous extract. Further molecular docking is carried out for the phytoconstituents of cassia fistula and viral protein. This study helps to investigate novel use of existing molecules. As the cassia has well reported anticancerr, antibacterial, hepatoprotective, antidiabetic activities we reported by the antibacterial assay with ciprofloxacin as standard. For molecular docking we selected viral proteins such as spike protein (7BZ5) and main protease (7BUY) in COVID-19 virus. We used a docking technique to illustrate the interaction between the main viral protease and selected phytoconstituents. This enzyme represents one of the most critical targets for the antiviral pharmacological actions against COVID-19. It is essential for the virus due to its proteolysis processing of polyproteins. This enzyme is an attractive target because of its vital role in polyproteins processing that are translated from the viral RNA. In silico study showed a good interaction between the Epicatecin and Luteolin and the main protease (7BUY) with a good score.

**KEYWORDS:** Yellow Shower, Molecular Docking, Viral Protein, Flavanoides, Antiviral, Antibacterial.

## INTRODUCTION

Cassia fistula Linn. commonly known as the Golden Shower belongs to the family Fabaceae. It is a deciduous tree with greenish grey bark, compound leaves, leaf lets are each 5–12 cm long pairs. A semi-wild tree known for its beautiful bunches of yellow flowers and also used in traditional medicine for several indications. A fruit is cylindrical pod and seeds many in black, sweet pulp separated by transverse partitions. The long pods which are green, when unripe, turn black on ripening after flowers shed. Pulp is dark brown in Color, sticky, sweet and mucilaginous, odor characteristic, and somewhat disagreeable. Drug occurs in flat or curved thick pieces; outer surface smooth to rough with warty patches; greenish grey to red; inner surface rough, reddish with parallel striations; fracture, laminate; odour, sweet and characteristic; taste, astringent.[4] It is a medium size tree which is native of tropical Asia. It is widely cultivated in South Africa, East Africa, Brazil, India, West Indies, China, Mexico, etc. All parts of the plant have medicinal properties so it is a very valuable medicinal plant which is utilized in the traditional system of medicine.[1]

### 1. Taxonomic Classification

**Table 1: Classifications of Cassia Fistula Plant.**

| | |
|---|---|
| Kingdom - Plantae | Vernacular names |
| Subkingdom – Tracheobinota | Marathi - Bahava<br>Guajarati - Garmala |
| Super Division - Spermatophyta | Hindi - Sonhali, Amultus |
| Division - Mangoliophyta | Kannad - Kakkemara |
| Class – Magnoliopsida | Tamil - Shrakkonnai, Konai, Irjviruttam |
| Sub Class - Rosidae | Telegu - Kondrakayi, Raelachettu, |
| Order - Fabales | Sanskrit - Nripadruma |
| Family - Fabacae | Arab - Khayarsambhar |
| Genus - Cassia | Punjabi - Amaltaas, Kaniyaar, Girdnalee |
| Species - fistula | Urdu – Amaltaas |

### 1.2. Chemical constituents

Cassia fistula was reported to have important classes of phyto constituents like Anthraquinone glycosides, cardiac glycosides, phenolic compounds, carbohydrate, protein, fats, alkaloids, tannins, saponins, steroids, ter-penoids and phloba-tannins, linoleic acid, oleic acid, stearic acid, rhein glycosides fistulic acids, sennosides A, B, anthraquinones, flavanoid-3-olderivatives, ceryl alcohol, kaempferol, bianthraquinone glycosides, fistulin, essential oils, volatile components, phytol (16.1%), 2- hexadecanone (12%), crystals and 4-hydroxy benzoic acids hydrate etc.[5,6,7,8] Lupeol, β-sitosterol, hexacosanol, 5,7,3,40 -tetrahydroxy-6, 8-dimethoxyflavone-3-O-α-arabinopyranoside, 5,7,40 -trihydroxy-6,8,30 -trimethoxyflavone-

3-O-α-L-rhamnosyl (1→2) -O-β-D- glucopyranoside and 1,8-dihydroxy-3, 7-dimethoxyxanthone-4-O- α-L-rhamnosyl (1→2)-Of-D-glucopyranoside are present in the stem bark of the plant (see chemical structure in table 4). A major anthraquinone derivative called rhein and 1,8-dihydroxy-3- anthraquinone found in fruit pulp of the plant. Fistucacidin, an optically inactive leucoanthocyanidin which is a phenolic compound from the heart wood of the plant. A bianthoquinone glycoside, fistulin together with kaempferol and rhein found in the flowers of the plant. A major carbohydrate called galactomannan consisting of 8 different type of sugar moieties are reported from the seeds of the plant. The anthraquinones like Rhein, Chrysophanol and Physcion, 9-(-)-epiafzelechin, 3-O-B-D-Glucopyranoside, 7 bioflavonoids and two triflavonoids together with (-) – epiafzelechin, (-)-epicatechin and procyanidin B-2 from the leaves of the plant. 3B-hydroxy-17-norpimar- 8(9)-en-15-one, 3-formyl-1-hydroxy-8- methoxy anthraquinone and fistulic acid from pods of the plant. The young and old leaves of the plant contain highest amount of phenolic, flavonoid and proanthocyanidin contents. Rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid) is a lipophilic anthraquinone extensively found in medicinal plants (see chemical structure in Table 4). It is a major bioactive compound reported in Cassia fistula for many therapeutic activities. The aim of the present study was to recommend the suitable substituent for those drugs which are uses in huge amount.[2]

## 2. MATERIAL AND METHODS

Cassia fistula Linn. Leaves were procured from Regional area of Kolhapur.

## 3. Preliminary phytochemical analysis

Preliminary phytochemical screening results showed the presence or absence (Table 2) of certain phytochemicals in the Cassia fistula sample. 4g of the sample was taken in a glass stoppered 250 ml flask. 100 ml of absolute ethanol was added. The flasks were shaken occasionally for 6 hours and allowed to stand for 18 hours. The extract was filtered and evaporated to dryness. The same procedure was followed for aqueous extraction. The extracts were collected, dried, weighed and stored separately for preliminary phytochemicals screening. The tests performed using alcoholic extract and aqueous extract to different types of qualitative test for the identification of phytoconstituents present in the leaves of Cassia fistula.[21, 22, and 23]

**Table 2: Phytochemicals analysis tests.**

| Name of the phytochemical | Qualitative test | Ethanolic | Aqueous |
|---|---|---|---|
| **Alkaloids** | Mayers reagents | + | + |
| | Dragendorff's reagents | – | - |
| | Hager's reagents | – | + |
| | Wagner's reagents | – | - |
| **Carbohydrates** | Molisch's test | - | - |
| | Fehling's test | + | + |
| | Bendict test | + | + |
| **Tannin and phenolic compound** | With ferric chloride | + | + |
| | With lead acetate | - | + |
| | With gelatin solution | - | - |
| **Protein and amino acids** | Biuret test | + | + |
| | Ninhydrin test | + | + |
| | Xanthoprotein test | - | - |
| | Milons test | - | - |
| **Flavonoids** | With NaOH | + | + |
| | With H2SO4 | + | + |
| | With Mg/Hcl | + | + |
| **Oils and fats** | With filter paper | - | - |
| | With alkaline KOH | - | - |
| **Vitamin C** | With indophenols | - | - |
| | Sodium nitroprusside | - | - |
| *present + | *Absent - | | |

**4. Antibacterial Assay**

The antibacterial potential of the extracts was first assessed with the help of agar streak plate method. The extracts which were found to inhibit bacteria were further subjected to micro dilution method to find the minimum inhibitory concentration. The solutions of minimum inhibitoryconcentrationwere furthersubjectedto agar cup plate assay to determine the potency of the extracts.

**Table 3: Zone of inhibition and its comparisons.**

| Sample concentration | Aqueous extract | Ethanolic extract | Standard Solution of ciprofloxacin |
|---|---|---|---|
| **( Zone of inhibition)in mm** | | | |
| 5ml | 0 | 0.50 | 0.50 |
| 10ml | 0 | 1.0 | 0.75 |
| 15ml | 0.1 | 1.30 | 1.30 |
| 20ml | 0.2 | 1.75 | 1.50 |
| 25ml | 0.4 | 2.0 | 1.75 |

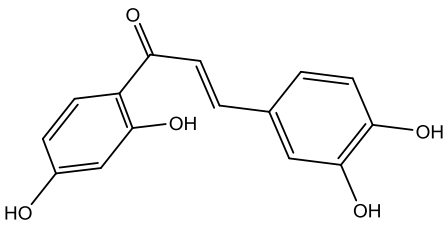
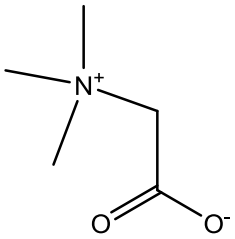
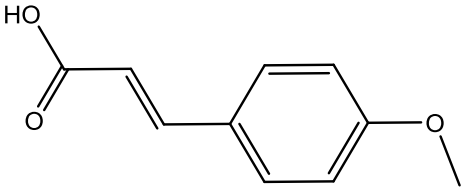
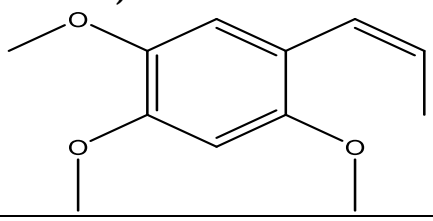
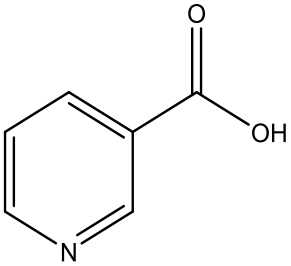
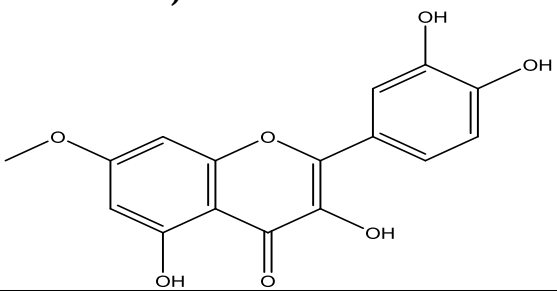
### 5. Molecular docking study

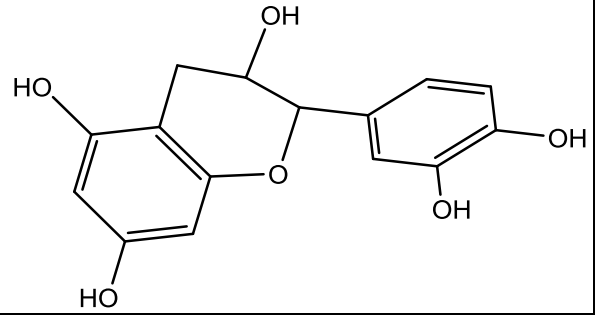
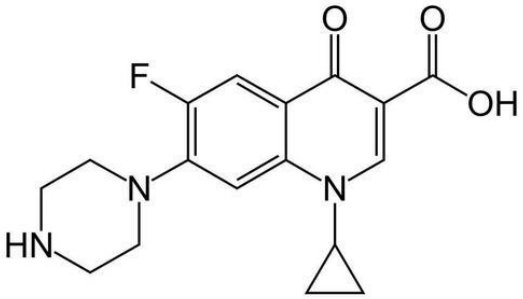
An assessment of the binding affinities of specific chemicals found in the Ethanolic leaves extract of Cassia Fistula against several target proteins has been conducted using computer-based strategies. A group of applications including Pyrex, PyMol 2.3, Discovery Studio 4.5, and Swiss PDB viewer has been utilized to complete the process.

### 5.1 Ligand preparation

The 3D SDF structure of identified compounds mentioned in Table 4 was hunted and downloaded from Pub hem (https://pubchem. ncbi.nlm.nih.gov/ (accessed on 7 March 2024)). Additionally, the 3D SDF structures of standard Ciprofloxacin (PubChem CID_2764) were also downloaded from the website 4. The compounds and standards were systematically imported into Discovery Studio 4.5 to generate a ligand library. As a result, the Pm6semiempirical technique is used to optimize all compounds, which improves the docking precision.[3]

**Table 4: 2D structures of ligands.**

| **5.2 Ligand Structure:** | |
|---|---|
| **1)BUTEIN**:  | **2)BETAINE**:  |
| **3) 4-METHOXYCINAMIC ACID:**  | **4) B-ASARONE:**  |
| **5) NICOTINIC ACID:**  | **6) RHAMNETIN:**  |

| 9)EPICATECHIN: | 10) CIPROFLOXACIN (STD): |
|---|---|
|  |  |

### 5.3 Target protein selection

11 compounds found from the ethanol fractions of the extract of Cassia fistula leaves were tested by computerized docking to identify their potential activity of antimicrobial properties. To evaluate the antiviral potential, the 3D crystal structure of the Viral protein [PDB ID: 7BUY & 7BZ5][3] was downloaded from the protein data bank ((https://www.rcsb.org/(accessed on 5 March 2024)).

**Table 5: Crystallographic properties of Viral proteins [PDB ID-7BUY and 7BZ5].**

| Protein and enzyme | PDB code | Classification | Expression system | Resolution | Method | Total structure weight (kDa) | Chain |
|---|---|---|---|---|---|---|---|
| COVID-19 Main protease | 7BUY | Viral protein | Escherichia coli B121(DE3) | 1.60A | X-ray Diffraction | 34.36 | A |
| COVID-19 Main protease | 7BZ5 | Viral protein /Immune system | Spodoptera frugiperda, Homo sapiens | 1.84A | X-ray Diffraction | 34.36 | A |

The target molecules' propensities and probable adherence profiles of plant constituents have been estimated using the software driven ligand-receptor interaction diagram. The highly advanced PyRx Autodock Vina was used to carry out this molecular drug protein linking approach, and semi-flexible modeling was used for the molecular docking. We have selected the amino acids from the literature and their corresponding IDs. Prior to loading and formatting the protein to the appropriate macromolecule, we verified that the ligands would only bind to that specific biomolecules During the docking process, amino acids including Leucine 718, Valine 726, Alanine 743, Lysine 745, Methionine 766, Lysine 775, Arginine 776, Leucine 777, Leucine 788, Threonine 790, Glutamine 791, Leucine 792, Methionine 793, Glycine 796, Cysteine 797, Leucine 799, Aspartic acid 800, Arginine 803, Leucine 844, Threonine 854, Aspartic acid 855, and Phenylalanine 856 were specifically targeted for Viral Protein.

The ligands' SD files were imported into PyRx Auto-Dock Vina's Open Bable tool, and then converted to pdbqt format so that docking against these chosen macromolecules could get the greatest possible match. Additionally, these functional amino sites were contained within a grid box using grid mapping. For Example center x =-23.828, center y =18.214, center z =60.692 were kept during Viral protein docking.

## RESULT

**Table 6: In Config files presented X, Y &Z Dimensions.**

| Docking No. | Config Dimension | | |
|---|---|---|---|
| | X | Y | Z |
| 1. | -23.828 | 18.214 | 60.692 |
| 2. | -23.383 | 16.221 | 62.089 |
| 3. | -15.881 | 18.835 | 66.780 |
| 4. | -19.915 | 20.221 | 64.310 |
| 5. | -42.595 | -26.221 | 0.685 |
| 6. | -20.454 | 18.835 | 66.780 |
| 7. | -45.756 | -31.547 | 3.492 |
| 8. | -45.756 | -31.547 | 3.492 |
| 9. | -45.121 | -31.553 | 5.886 |
| 10. | -20.719 | 18.019 | 65.272 |
| 11. | -48.979 | -31.612 | -1.485 |

After that, a final docking using AutoDock Vina (version 1.1.2) was performed to determine the ligands' affinity for the macromolecule. Water molecules and heteroatoms were eliminated from the proteins. The nonpolar hydrogens and Gasteiger charges were retained using preset option during the protein preparation process. All proteins were minimised using UCSFChimaera to reach their lowest energy state. The normal residues were handled with AMBERff14sB, while additional residues were processed using Gasteiger mode for further analysis. The final step involved conceptualizing the outcome and utilizing BIOVIA Discovery Studio version 4.5 to forecast the best-fitting 2D and 3D models.[3]

**Table 7: Shows binding energy of protein & ligand interactions and its Interacting residues.**

| Sr. No | Proteins | Ligands | Binding Energy kcl/mol | Interacting Residues |
|---|---|---|---|---|
| 1 | 7BZ5 | BUTEIN | 0.0 | PHE(A:342),LEU(A:368), PHE(A:338),GLY(A:339) |
| 2 | 7BUY | BETAINE | -4.0 | HOH(A:531),HIS(A:164,41),CYS(A:145),MET(A:49) |
| 3 | 7BUY | 4-METHOXYCINAMIC ACID | -4.7 | HOH(A:531,661,630,682),ASN(A:142), HOH(A:682),HIS(A:164,41),CYS(A:145),DMS(A:402), MET(A:165,49),ASP(A:187),SER(A:144),GLY(A:143) |

| | | | | |
|---|---|---|---|---|
| 4 | 7BUY | B-ASARONE | -4.7 | HOH(A:531,661,630,682),ASN(A:142), HOH(A:682),HIS(A:164,41),CYS(A:145),DMS(A:402), MET(A:165,49),ASP(A:187),SER(A:144),GLY(A:143) |
| 5 | 7BZ5 | NICOTINIC ACID | -5.5 | HOH(A:375), HOH(439) |
| 6 | 7BUY | RHAMNETIN | -6.4 | HOH(A:531),HIS(A:164,41),CYS(A:145),MET(A:49) |
| 7 | 7BZ5 | 4-HYDROXYCOUMARIN | -6.7 | HOH(A:800,729),GLY(A:339) |
| 8 | 7BZ5 | LUTEOLIN | -8.1 | PHE(A:342),LEU(A:368), PHE(A:338),GLY(A:339),HOH(A:800) |
| 9 | 7BZ5 | EPICATECHIN | -8.4 | HOH(A:L375) |
| 10 | 7BZ5 | CIPROFLOXACIN(STD) | -9.8 | LEU(A:20),THR(A;49),VAL(A:6),ALA(A:7),ILE(A:14) SER (A:108). |

In the Table 7 shows binding affinity of protein 7BUY and Epicatechin scoring is better than other ligand and show it's have Antiviral activity. In silico study showed a good interaction between the Epicatecin and Luteolin and the main protease (7BUY) with a good score. Epicatechin and luteolin is shows the better score as compare with STD Ciprofloxacin (-9.8).

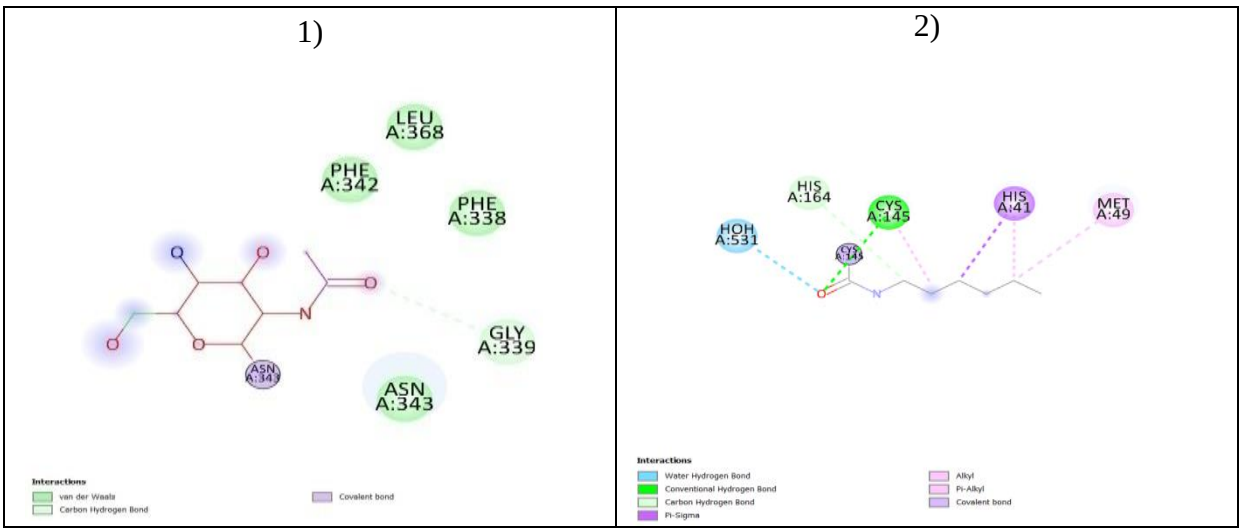
**In silico investigation (molecular docking)**

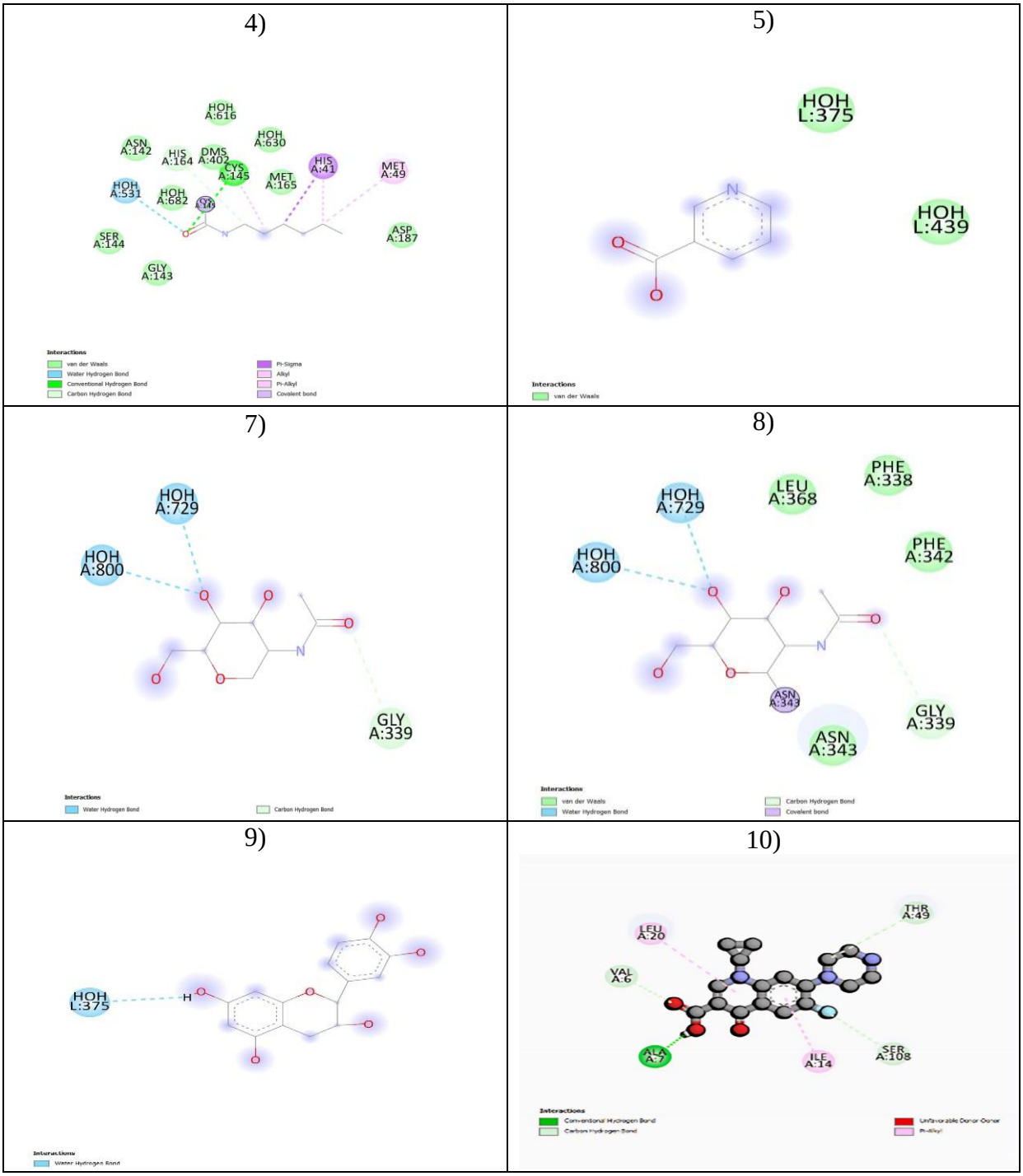
According to the data presented in Table 7, Compound exhibited the highest binding affinity 10 for the Viral Protein, scoring – 8.4kcal/ mol. Notably, Compounds 8 and 9 also demonstrated a significant affinity for binding to the Viral Protein, with values of −8.1 and -8.4 kcal/ mol, respectively.

In comparison, the standard Ciprofloxacine had a binding affinity of – 9.8 kcal/mol, indicating its stronger binding capacity. On the other hand, compounds 3, 4, and 5 displayed relatively lower binding affinities of − 5.5kcal/mol against this receptor.

**2D Structure**

**Table 8: Shows Interacting Residues in 2D structures.**






## DISCUSSION

Photochemical constituents collected from the extract of C.Fistula leaves and evaluated for various phytoconstituents by using various evaluation testes. Further by using Cup plate method antibacterial study was carried for various bacteria with use of Ciprofloxacin as standard. To investigate novel compounds with novel use important Phytochemicals are chosen for in silico analysis. Among the various compounds two flavonoids such as Epicatechin and Luteolin found effective in molecular binding against various receptors like

Viral Protein (7BUY,7BZ5) provides good binding affinity towards Viral protein receptor. This molecular investigation helps in In-Vitro findings of the plant extract which might offer an entirely new view point on controlling Viral Infections, Anti-bacterial and cytotoxicity infections. However, there is a need for further study to investigate the mechanism of action.

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