INSILICO ANALYSIS OF SELECTED MEDICINAL PLANTS AND ANTIVIRAL COMPOUNDS AS POTENT DRUGS AGAINST nCOV MAIN PROTEASE

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ABSTRACT

Background: Eighteen antiviral compounds were selected from five medicinal plants (*Allium sativum, Cinnamomum verum, Ocimum basilicum, Azadirachta indica, Tinospora cordifolia)* and three standard drugs (Favipiravir, Indomethacin, Boceprevir) were docked against target protein (2019-nCoV main protease PDB : ID 7brp).Multiple approaches would be extreme necessary to handle the outbreak of corona these include statistical tools, computational modelling, and quantitative analyses to control the spread as well as the rapid expansion of a new treatment. **Objective**: To select the antiviral compounds from selected five medicinal plants against n-CoV protein. To study the 18 antiviral compounds present in the selected medicinal plants using *Insilico* analysis and the effectiveness of the compounds in treating Covid 19 by montoring the extent to which the interact with the target protein (2019-nCoV main protease PDB: ID 7brp). **Material and methods:** Eighteen antiviral compounds were selected from five medicinal plants (*Allium sativum, Cinnamomum verum, Ocimum basilicum, Azadirachta indica, Tinospora cordifolia)* were selected from pubchem compounds. Target protein (2019-nCoV main protease PDB: ID 7brp) is obtained from PDB. All the compounds were allowed for docking study with help of iGEMDOCK docking software. **Results**: The result showed that Amritoside binding energy -160 and seven hydrogen bonds when compared to Boceprevir a native ligand of the protein has binding energy -124 and six hydrogen bonds. **Conclusion:** From this study it has been concluded that Amritoside is a potent inhibitor against Covid 19 main protease enzyme.

Keywords: Potent inhibitor, nCOV main protease, Molecular Docking, Ligand, Target Protein, Antiviral Compounds.

INTRODUCTION

The herbal remedies research arm of a biomedical research institute in India has received multiple queries on the prospective use of supplementary remedies against COVID-19, such as single medicinal plants, traditional remedies, finished herbal products, supplements, food products, and medical devices, during the Movement Control Order implemented by the Indian government in March 2020 in an attempt to cure against corona virus.(Wu j *et al*., 2020) These queries have mostly been submitted directly by the general public and people who already had quick access to herbal products, or if they were found through broadly circulated messages on social media platforms. Given the existing adverse effects associated with long-term steroid use, it will be interesting to investigate the potential role of medicinal plants with anti-inflammatory properties in post-SARS-CoV-2 infection complications related to chronic inflammation, such as lung fibrosis and neuropsychiatric symptoms (wei Liu *et al*.,2020). Five medicinal plants were selected including *Azadirachta indica*, *Ocimum basilicum*, *Allium sativum.Tinospora cordifolia* and *Cinnamomum verum.* While displaying direct antiviral effects, medicinal plants previously has been reported as anti-inflammatory activities may have pleiotropic roles in COVID-19 management.

Garlic (*Allium sativum* L.) contain the two important of sulfur constitutes (~ 82%) of garlic thiosulfinates (allicin) and S-allyl cysteine sulfoxide (alliin). kaempferol, allin, allicin compounds in garlic is obtained by hydro distillation method (jan *et al*.,2014). The antiviral property of garlic against some of virueses viruses like influenza B, HIV, vesicular stomatitis virus, herpes simplex virus, coxsackievirus species, and gamma retro virus was earlier demonstrated (Chakraborty *et al*.,2020).

Compounds in *Azadirachta indica* (Neem) extract were,nimbidn, nimbolin A , azadirachtin were identified by phytochemical test (Santhosh kumar *et al.*2020).*Ocimum basilicum* contains various compounds like urolisic acid , eugenol, apigenin that have antiviral, antioxidant, antibacterial, antifungal, dermatologic, anticonvulsant and cytoprotective properties(council *et al*., 2002) (Rubab *et al*., 2017).*Tinospora cordifolia* Amritoside, isocolumbin, tinosporin B are the compounds identified by spectroscopic studies that are considerd in this study. Phyto chemistry of all these compounds were documented in the literature (Sharma *et al*., 2019).

Cinnamom verum has shown several medicinal properties because it contains many phytochemical ingredients, such as cinnamic acid, cinnamaldehyde, cinnamate, and numerous polyphenols (Satya *et al.*,2012).

Purpose of study includes,to select the antiviral compounds from selected five medicinal plants against n-CoV protein ,to study the 18 antiviral compounds present in the selected medicinal plants using *Insilico* analysis and the effectiveness of the compounds in treating Covid 19 by montoring the extent to which the interact with the target protein (2019-nCoV main protease PDB : ID 7brp) and to determine the final prediction of best bioactive compound from the five plant metabolites.

MATERIALS AND METHOD

SELECTION OF ANTIVIRAL COMPOUNDS FROM FIVE MEDICINAL PLANTS

18 antiviral compounds were selected from five medicinal plants (*Allium sativum, Cinnamomum verum, Ocimum basilicum, Azadirachta indica, Tinospora cordifolia).* The antiviral compounds were apigenin, nerolidol, nevadensin, urosilic acid cinnamtannin B1, cinnamyl actate, eugenol, allicin, allin kaempferol, amirtoside, epicatechin,isocolubin, tinosporine B , Azairachtin, nimbidin, nimbolinin A, salannin.

PROTEIN PREPARATION

The target protein required for the docking studies has been retrieved from the Protein Data Bank at resolution of 1.3 Å root mean square deviations (RMSD) which represents a 3 D structure of target protein that is Crystal structure of the 2019-nCoV main protease (PDB: ID 7brp).

LIGAND PREPARATION

The ligand molecules for the docking process are prepared from the compounds obtained from the various plant-based source (selected five plants) . The compounds were obtained from the PubChem database https://pubchem.ncbi.nlm.nih.gov . The structure of the compounds was downloaded in (.sdf) format and they were converted into (.pdb) format by using open babel software searching for tautomers and steric isomers and geometry minimization of ligands

DOCKING MODULE:

Docking software iGEMDOCK was used to dock the protein Crystal structure of the 2019-nCoV main protease (PDB: ID 7bpr) of the corona virus with the drug compounds. iGEMDOCK is an integrated virtual screening (VS) environment fromed from preparations through post-screening analysis with pharmacological interactions (Lopez *et a*l., 2011). Each

compound taken from selected plants is then docked into the binding site by using the in-house docking tool iGEMDOCK. Subsequently, iGEMDOCK forms protein-compound interaction profiles of electrostatic (E), hydrogen-bonding (H), and Van der Waal's (V) interactions. Based on these profiles and compound structures that is obtained from pubchem, iGEMDOCK infers the pharmacological interactions and clusters the screening compounds for the post-screening analysis. Finally, iGEMDOCK ranks and visualizes the screening compounds by combining the pharmacological interactions, number of h bonds and energy-based scoring function of iGEMDOCK. iGEMDOCK is to facilitate steps from preparations of target protein and ligand libraries toward post-screening analysis. iGEMDOCK is specifically useful for post-screening analysis and inferring pharmacological interactions from screening compounds. iGEMDOCK is available at http://gemdock.life.nctu.edu.tw/dock/ igemdock.php (Kai-Cheng,Yen-Fu Chen , 2011).

MECHANISM OF DOCKING:

Docking was performed by iGEMDOCK molecular docking software. During Docking, at first thepreparation of molecules and bonds, bond orders, explicit hydrogen's, charges, flexible torsions were assigned to both the protein and ligands. (Huyng *et al.*, 2010).From the Docking, wizard ligands were selected and the scoring function used was iGEMDOCK score. If hydrogen bonding is possible, then the hydrogen bond energy contribution to the Docking score is assigned a penalty based on the deviations from the ideal bonding angle.

RESULT AND DISCUSSION

The main protease target protein (7BRP) was docked against 18 antiviral compounds from selected five plants, the ligands were taken from each five plants they are apigenin, nerolidol, nevadensin, urosilic acid, cinnamtannin B1 , cinnamyl actate, eugenol, allicin, kaempferol, amirtoside, epicatechin,isocolubin, tinosporine B , Azairachtin, nimbidin, nimbolinin A, salannin, and 3 standard drugs favipiravir, indomethacin, boceprevir. Binding energies, hydrogen bond energy, number of hydrogen bonds formed and amino acids involved in hydrogen bonding have been tabulated (table 1).

Eighteen plant based compounds have been selected to examine the binding affinity towards the Crystal structure of the 2019-nCoV main protease (PDB ID: 7BRP). The selected compounds were tested using iGEMDOCK molecular docking software to perform molecular

inhibition analysis of protein and ligand. The binding energy values obtained after performed molecular docking technique using iGEMDOCK molecular docking software. Binding energies were tabulated (table 1) with number of hydrogen bonds (H bonds), amino acids involved in H bonds and drug likeliness of the compounds. Binding energies are one of the important criteria for selecting a potent inhibitor against the selected drug target to map the inhibition efficiency. Compounds are ranked according their binding energy, higher the binding energy indicated higher the binding affinity of protein ligand complex (Muegge *et al*., 2003). The result showed the amirtoside from *Tinospora cordifolia* having high binding energy -160.141 with 7 hydrogen bonds and for compounds like Cinnamyl acetate, Allin and Nimbolin A having no intraction as they are not having H bond formation. Since *van der waals* force are weak thus they are neglected.

Another important criteria is the H bonds formation, based on the H bonds the binding complex are considered to be more interactive and strong. Hydrogen bonds play a major role in the stabilization of protein-ligand complexes (Tobias,Mathias 2019)

Binding energy and hydrogen bonds data were taken into consideration for selecting a potent inhibitor against Covid 19. The binding energies were also compared with standard drugs Favipiravir Indomethacin and protein native ligand Boceprevir. The scores are tabulated for comparison and elimination of compounds (table 1).

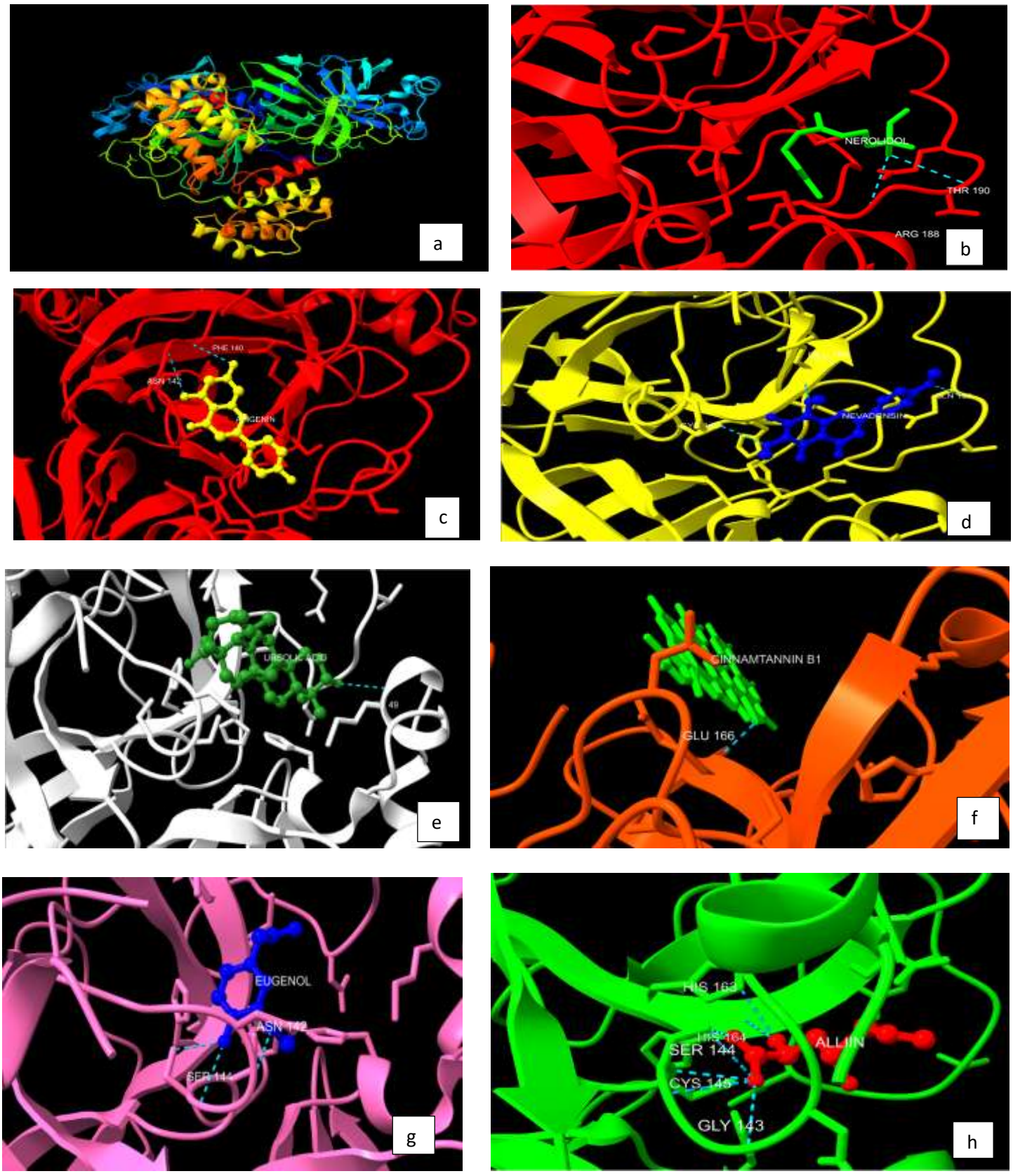

Figure :1 a)Crystal structure of the 2019-nCoV target protein(7PBRP) docked against b)apigenin c)nerolindol d)nevadensin e)urolisic acid f)cinnamatin B1 g)euginol h)allin

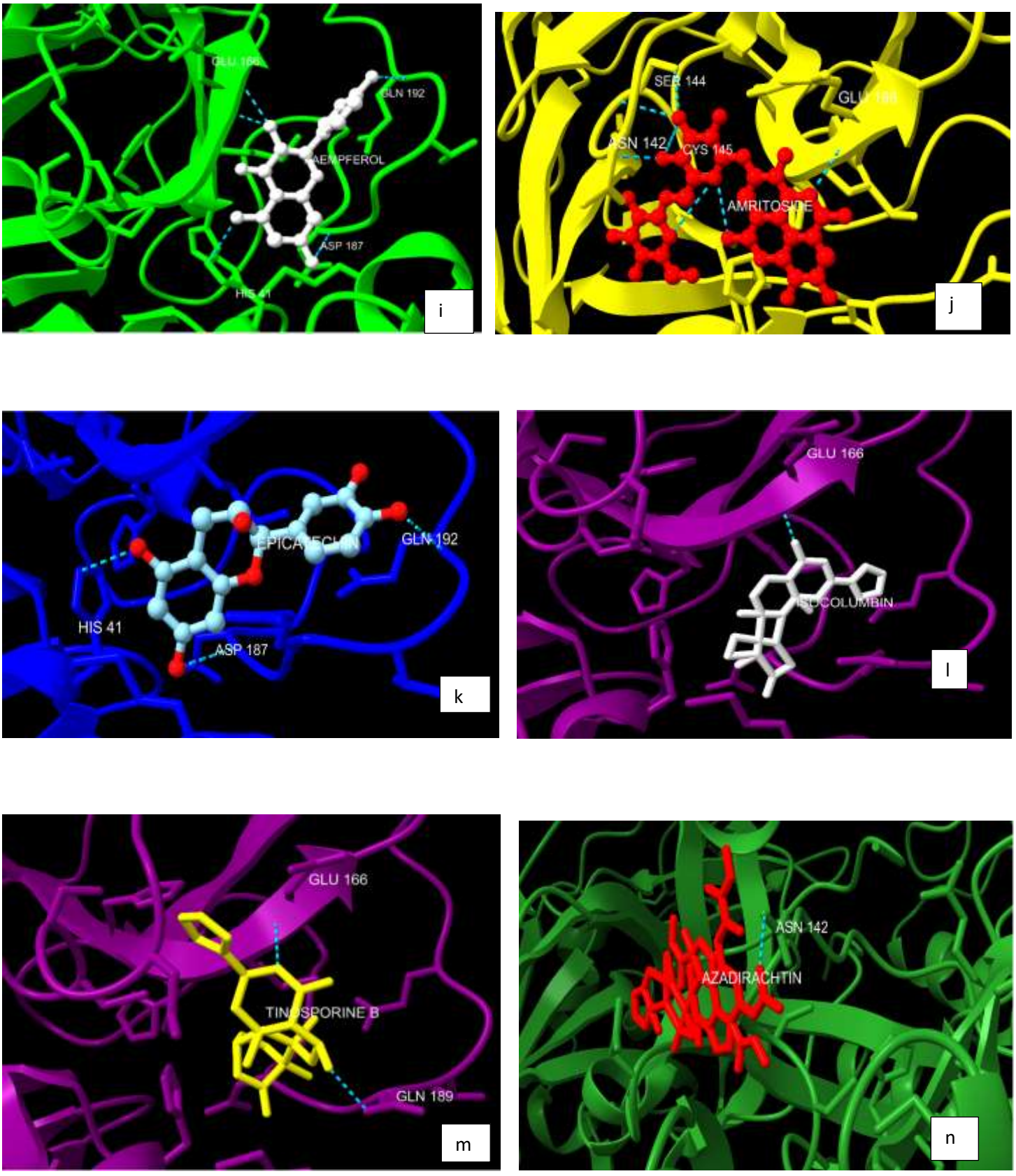

Figure:2 Target protein (7brp) against i)kaempferol j)amirtoside k)epicatechin l)isocolumbin m)tinosporin B n)azadirachtin

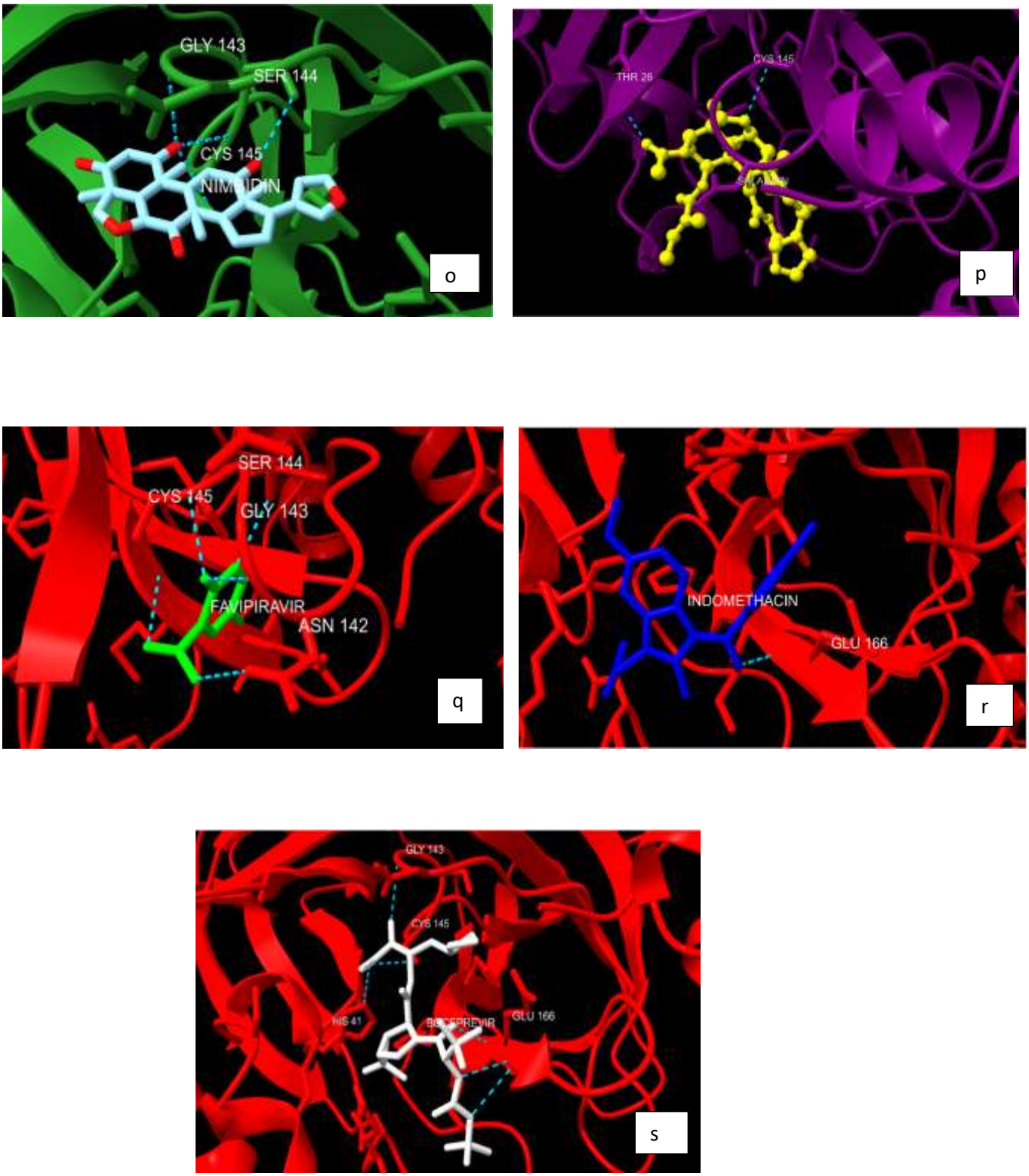

Figure:3 Target protein (7brp) against o)nimbidin p)salannin q)favipiravir r)indomethacin s)boceprevir

Table 1.

S.NO	COMPOUND	ENERGY	VDW	HBOND	NO.OF HBONDS	AA-HBONDS
1	Apigenin	-94.0681	-67.9256	-26.1425	2	PHE 140 ASN 142
2	Nerolidol	-69.0905	-59.9724	-9.11811	2	THR 190 ARG 188
3	Nevadensin-	-96.5659	-80.55	-16.0159	3	CYS 146, GLU 166 SLN132
4	Ursolic acid	-84.03	-67.8448	-16.1852	1	MET 49
5	Cinnamtannin B1	-43.2137	-42.8751	-0.338567	1	GLU 166
6	cinnamyl acetate	-65.1159	-61.6228	-3.49306	0	
7	eugenol	-64.6148	-44.3654	-20.2494	3	ASN 142,SER 144
8	Allicin	-51.8885	-48.4284	-3.46008	0	
9	Allin	-70.6062	-35.5855	-35.0207	7	HIS 163,HIS 164,SER 144,CYS 145 AND GLY 143
10	kaempferol	-103.419	-80.3354	-23.0835	5	GLU 166,GLN 192,HIS 41 AND ASP 187
11	Amritoside	-160.141	-118.223	-41.9175	7	SER 144,ASN 142,CYS 145 AND GLU 166
12	Epicatechin	-95.1649	-79.2061	-15.9588	3	HIS 41 ,ASP 187 AND GLN 192
13	Isocolumbin	-111.246	-98.8448	-12.4008	1	GLU 166
14	Tinosporine B	-112.088	-88.554	-23.5338	2	GLU 166 AND GLN189
15	Azadirachtin	-15.8125	21.7347	-5.92218	1	ASN 142
16	Nimbidin	-135.673	-113.53	-22.1425	4	CYS 146,GLY 143 AND SER 144
17	Nimbolinin A	-94.245	-91.6847	-2.5603	0	
18	Salannin	-82.0901	-66.3854	-15.7047	2	CYS 145 AND THR 26
19	Favipiravir	-70.7868	-38.2804	-32.5064	5	CYS 145, SER 144,GLY 143 AND ASN 142
20	Indomethacin	-88.6987	-74.3881	-14.3106	1	GLU 166
21	Boceprevir	-124.278	-101.456	-22.8218	6	CYS 14,HIS 41, GLY 143 AND GLU 166

From the above (table 1) it shows amirtoside (j) from *Tinospora cordifolia* having high binding energy -160.141 with 7 hydrogen bonds. And for compounds like Cinnamyl acetate, Allin and

Nimbolin A having no intraction as they are not having H bond formation. Since *van der waals* force are weak thus they are neglected.

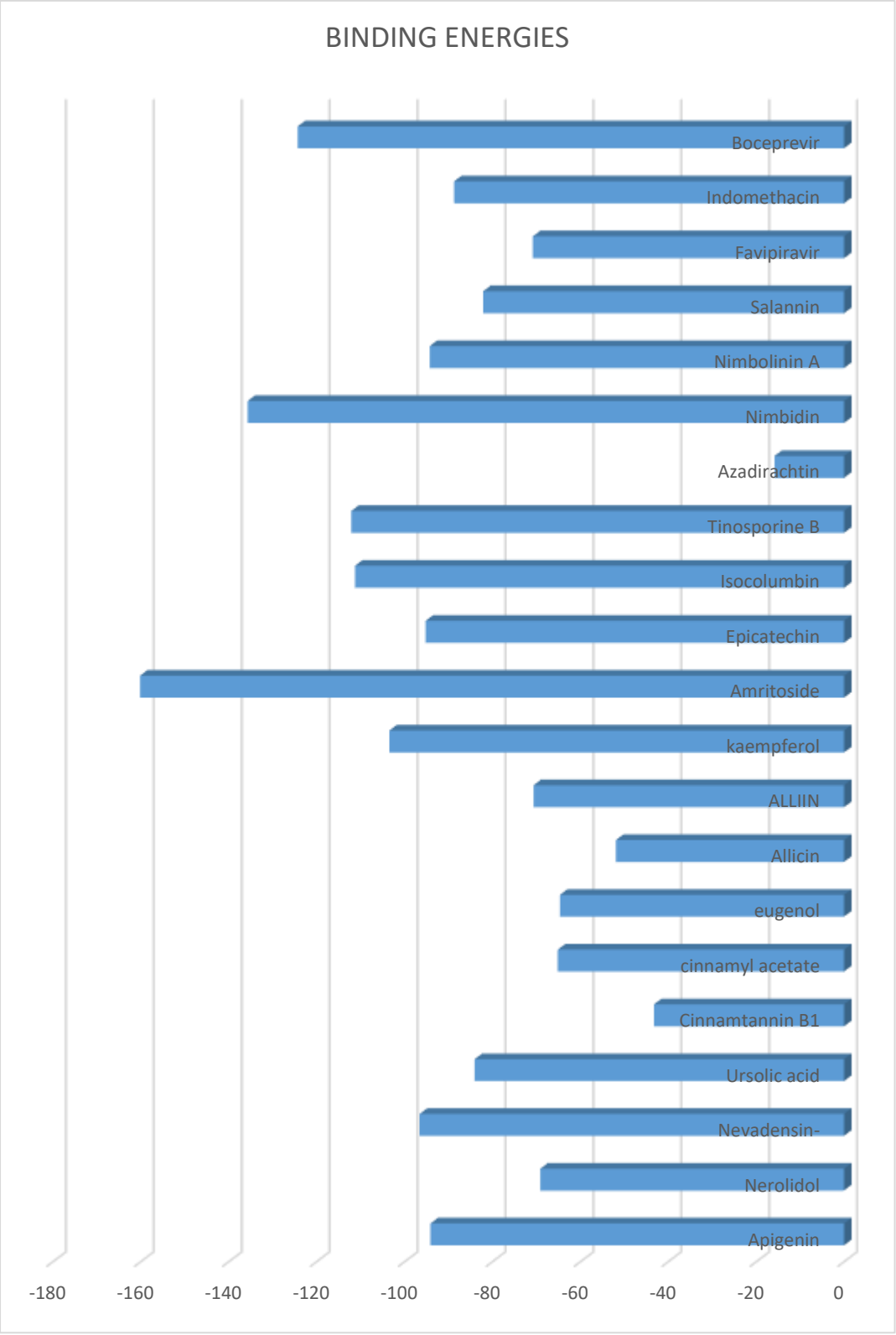

Figure 4. binding energies against ligand molecule.

CONCLUSION

There are compounds from many plants that is available in market that is in use against corona, other than finding new compound against corona it is necessary to evaluate which is best active against corona virus. So, it is required to find the best ligand that inhibit action of main protease . From this study it has been concluded that Amritoside is a potent inhibitor against covid 19 main protease enzyme. This conclusion is obtained by analysing 18 antiviral compounds and three synthetic drugs.

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