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RATIONAL SELECTION OF PHYTOCHEMICALS BY MOLECULAR DOCKING FROM SELECTED HERBS AGAINST SARS –COV-2MAIN PROTEASE

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ABSTRACT

The present research involves herbal extracts reported against COVID 19 in the scientific literature, but not the individual phytochemicals are tested for the same, doing so is time consuming as well economically burdensome activity, in this regard rational strategy is employed by selecting of 100 herbal crude extracts already reported (but not individual phytochemicals extracted) in literature against COVID 19 with along with their IC50 value, further selecting the 10 least IC50 (potent) extracts, further, selected potent extracts, whose reported phytochemicals will be searched from literature along with their chemical structures, these chemical structures will be docked against COVID 19 main Protease active site, the Phytochemicals, whose binding will be significantly good with known drug binding amino acid residues will be selected for the study, these phytochemicals containing plants will be searched based on their feasibility of obtaining, the specific computationally good binding phytochemicals will be extracted and will be tested against COVID 19 using live human nasal swab containing COVID 19 virus using PCR method of screening, checking the viral load in the presence of extract and computationally screened and better binding phytochemical to COVID 19 main protease active site amino acid residues (specifically Known drug binding amino acid residues).

KEYWORDS: Molecular docking, Herbal drug, SARS-COV-2 Main protease.

INTRODUCTION

The emergence of a new type highly infectious respiratory syndrome started from china during December 2019 resembling shape of sun (corona) named as COVID 19 representing CO (corona)VI (Virus) D 19 (started December 2019), within a few months of occurrence,

infection spread across the world from one human to other causing more than 4 million deaths and 180 million people got infected, even though there are many theory and evidence how the disease spread across the world as well as it is manmade or naturally evolved, the current focus is rather on the treatment strategies preventing infection leading to death, in this regard many prevention methods are employed such as use of mask, social distancing, sanitization, povidone iodine mouth wash, Hydrogen peroxide nebulization, other already existing treatment strategies such as old drugs used in clinical practice; Azithromycin, Hydroxychloroquin, Ivermectin, flavipar, remdesivir, doxycycline, 2-deoxyglucose, supplements such as; vitamin D, Vitamin B12, vitamin C, quercetin, methyl prednisolone, cyproheptadine, Atorvastatin, tocilizumba and enoxaparin. The main prevention method involves vaccination such as covishield and covaxin. The Phytochemicals or phytonutrients have proved its role in treatment of diseases including viral, bacterial, cardiovascular diseases, cancer etc. From the literature, few phytochemicals have shown significant antiviral activity against COVID 19 through drug repurposing approaches like molecular docking. Also a wide range phytochemicals like alkaloids, tannins, polyphenols, flavonoids, proteins, lecithin, lignans, coumarins, and anthocyanidins possess antiviral properties against different types of corona virus including SARSCOV.[2] Since the development of new treatment with natural drugs requires time these phytochemicals also seen in common herbs can be incorporate in our daily diet which would increase the immunity to fight COVID-19. The phytochemicals possessing antiviral properties include alkaloids, polyphenols, polysaccharides, flavonoids, lecithin, proteins, terpenes, lignans, coumarins, fructans, saponins, quinones, proanthocyanidins, steroids, thiosulfonates etc. The mechanisms of action of some include Alkaloids which act by blocking the binding of virus, inhibit virus growth and reduce virus titers in lungs. Polysaccharides inhibit viral replication and viral binding to the cell, while flavonoids inhibits reverse transcriptase by preventing RNA synthesis. Terpenes inhibit virus replication and Lecithin inhibits virus penetration. Most plants possessing antiviral property have phytochemicals which have the ability to inhibit different steps of virus attack and replication, even though the data regarding the mechanism of action of particular phytochemical is reported.

MATERIALS AND METHOD

1) 100 herbal extracts are selected (Whose individual phytochemical is not tested against covid-19) COVID 19 activity is reported in the literature along with IC50 value.

2) Theoretically filtered 10 best out of 100 herbal extracts based on least IC50value reported in literature, considering them as potent herbal extracts.
3) Phytochemicals names along with their chemical structures are searched in literature for top 10 selected potent herbal extracts.
4) Phytochemical structures for 10 selected potent herbal extracts as reported in literature is drawn for using chemdraw software.
5) Phytochemical structures are added in database of MOE software. Docking was performed windows 2010 using MOE 2008.10 version (kalicharan and Srinu Bodige 2017), (parameswar Ravula, Harinadha Babu 2018). COVID 19 protease (PDB code: 6YB7) were imported from the protein data bank and enzyme were visualized using sequence option and retained dimeric state of protein as well as water molecules, but unbound water molecules, substrate and metals were deleted (Prashant and Vijay Avin 2019). The partial charge of the protein was adjusted, using the force field method AMBER 99. Later, the protein was subjected to 3D protonation at cut off 12.0, and further hydrogen was added according to standard geometry and the enzyme was energy minimized using force field MMFF94x at 0.01 KJ mole gradients. The ligand preparation was done by drawing the structure of ligands by using a builder module and adjusting the partial charges using Hamilton MMFF94 force field method and subsequently, 3D protonation and hydrogen addition was performed according to standard geometry.
6) Ligands were energy minimized at cut off 12 using MMFF94x force field at 0.01KJ mole gradient. Docking was performed using the option simulation followed by the docking on selected active site amino acids using sequence option and further docked with setting options such as: receptor and solvent, selected residues, alpha triangle, affinity, force field refinement and best 10 poses. After obtaining docking results, out of the 10 best posed resulted for each chemical structure, the best pose was retained. The resultant best pose score values in the series were used for phytochemical ranking purpose.
7) ADME properties were predicted finally selected phytochemicals based on molecular docking each phytochemicals are drawn on structure drawing window of online software preadmet.bmdrc.kr
8) Toxicity properties were predicted finally selected phytochemicals based on molecular docking, each phytochemicals are drawn on structure drawing window of online software protox.

9) The phytochemicals showing favorable results at molecular docking, ADME properties prediction and toxicity prediction will be selected for actual extraction and purification from respective medicinal plants and further purified them through preparative HPLC.
10) The phytochemicals showing favorable results at molecular docking studies, ADME properties prediction and toxicity prediction studies and further extracted, purified and characterized phytochemicals will be subjected for in Vitro antiCOVID 19 PCR study.

RESULTS

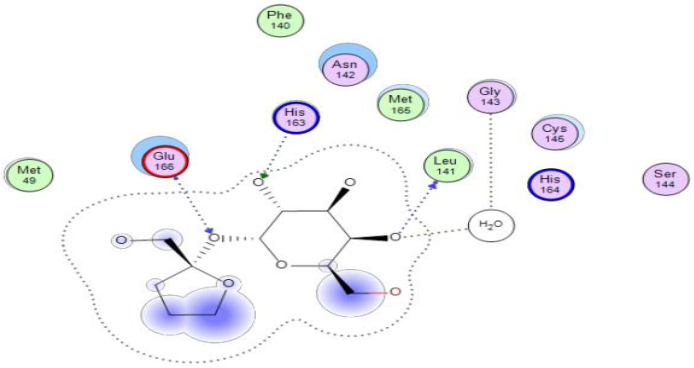

Fig. no. 1: 2D Interaction of 3, 6, diethyloyl sucrose with active site amino acid residues of covid19 main protease (PDB ID: 6YB7).

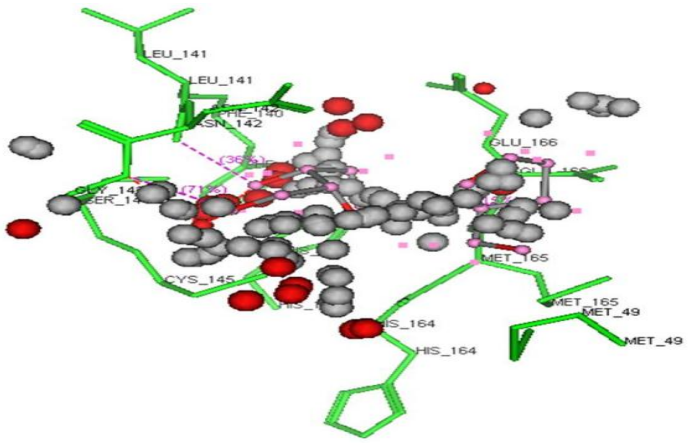

Fig. no. 2: 3D Interaction of 3, 6, diethyloyl sucrose with active site amino acid residues of covid-19 main protease (PDB ID: 6YB7).

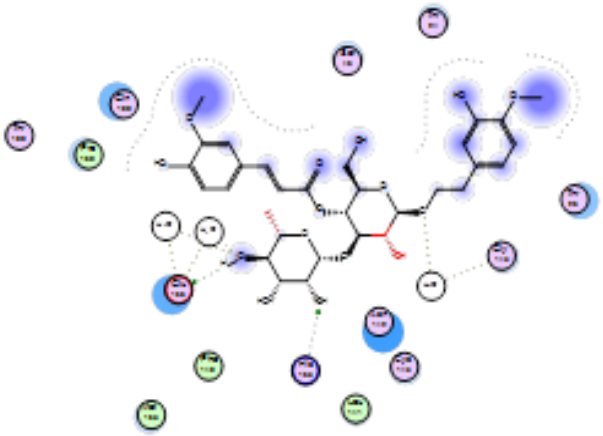

Fig. no. 3: 2D Interactions of Martinoside-14 with active site amino acid of covid-19 main Protease (PDB ID: 6YB7).

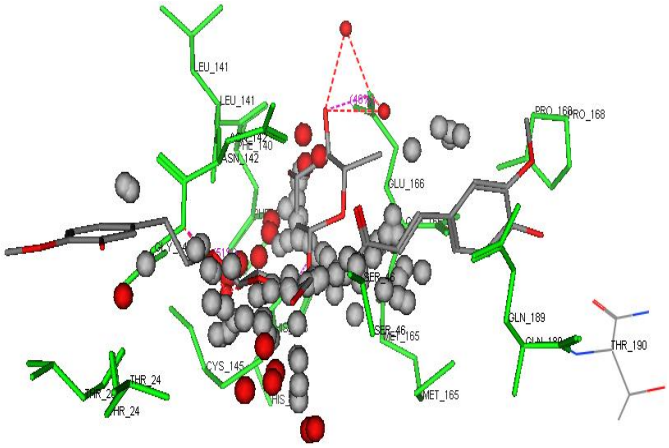

Fig. no. 4: 3D Interactions of Martinoside-14 with active site amino acid residues of covid-19 Main protease (PDB ID: 6YB7).

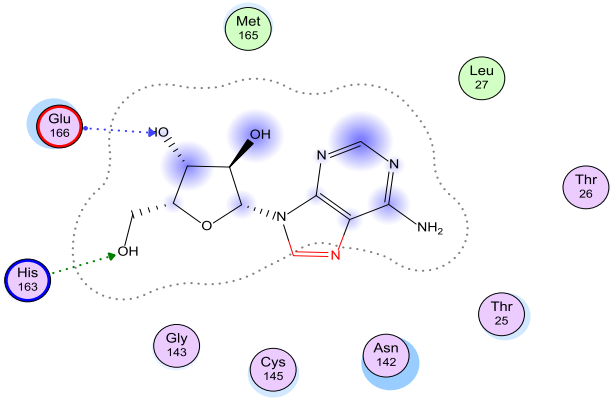

Fig. no. 5: 2D Interaction of Adenosine with active site amino acid residue of covid-19 main Protease (PDB ID: 6YB7).

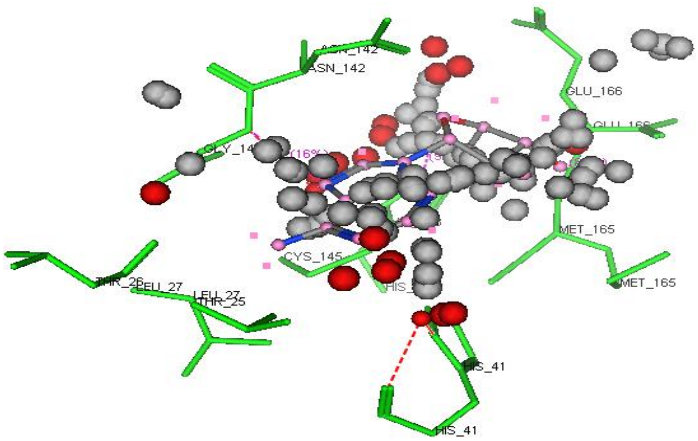

Fig. no. 6: 3D Interaction of Adenosine with active site amino acid residues of Covid-19 main protease (PDB ID: 6YB7).

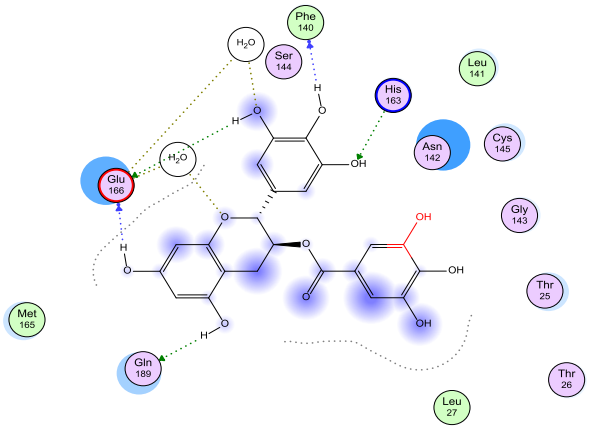

Fig. no. 7: 2D Interaction of Epigallactocatechine gallate with active site amino acid residues of covid-19 main protease (PDB ID: 6YB7).

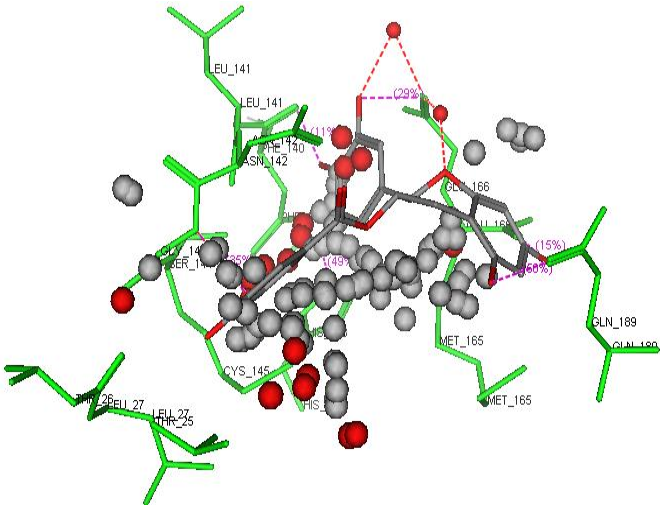

Fig. no. 8: 3D Interaction of Epigallactocatechine gallate with active site amino acid residue of covid-19 main protease (PDB ID: 6YB7).

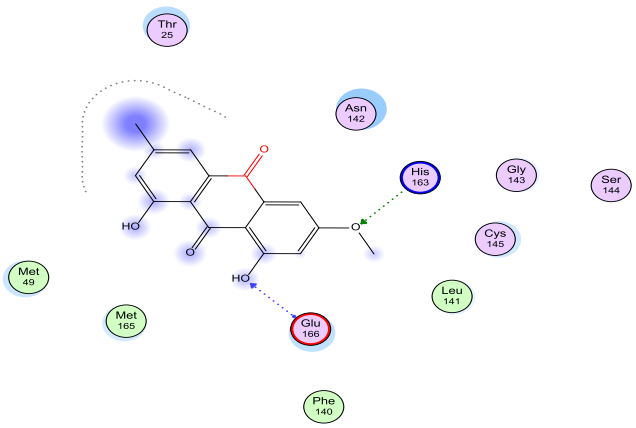

Fig. no. 9: 2D Interaction of physcion with active site amino acid residues of covid-19 main Protease (PDB ID: 6YB7).

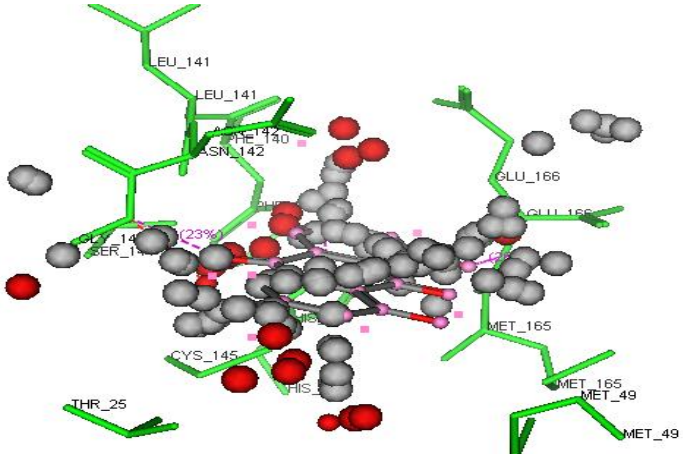

Fig. no. 10: 3D Interaction of physcion with active site amino acid residue of covid-19 main (PDB ID: 6YB7)

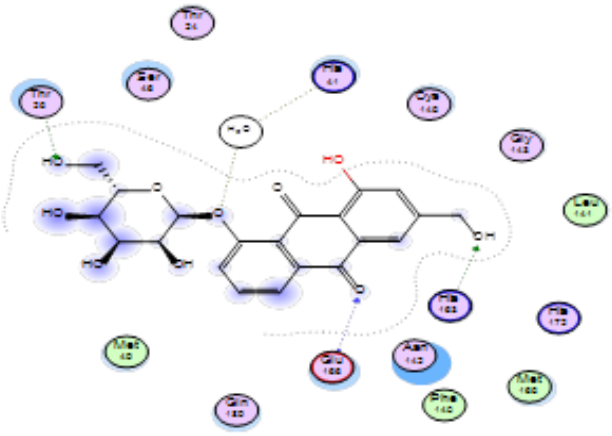

Fig. no. 11: 2D Interaction of aloeemodine-8-glucoside with active site amino acid residues of covid-19 main protease (PDB ID: 6YB7).

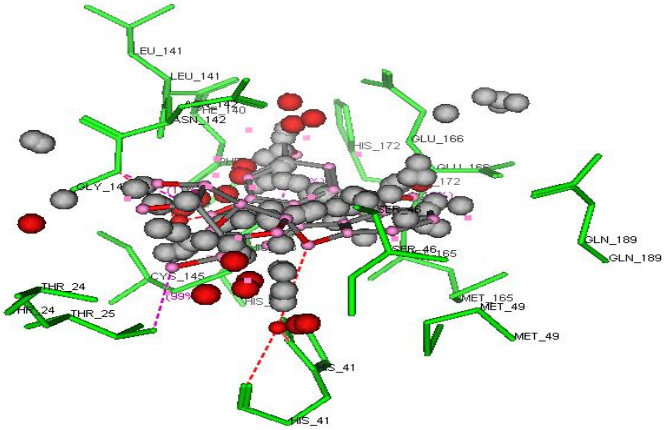

Fig. no. 12: 3D Interaction of aloeemodine-8-glucoside with active site amino acid residues of covid-19 main protease (PDB ID: 6YB7).

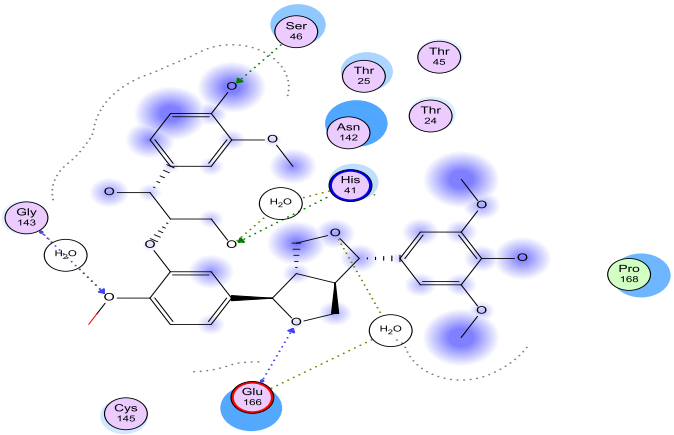

Fig. no. 13: 2D Interaction of Ficusesquciligan-B with active site amino acid residues of covid -19 main protease (PDB ID: 6YB7).

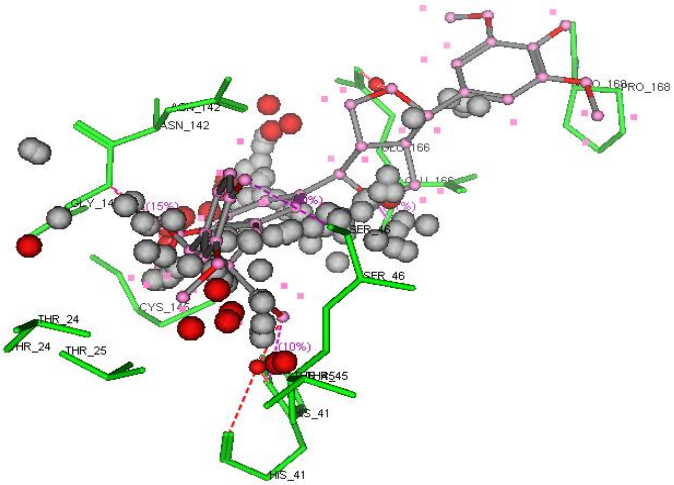

Fig. no.14: 3D Interaction of Ficusesquciligan-B with active site amino acid residues of COVID-19 main protease (PDB ID: 6YB7).

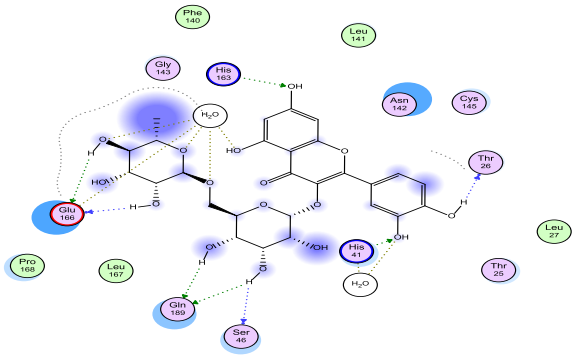

Fig. no. 15: 2D Interaction of Rutin with active site amino acid residues of COVID-19 main protease (PDB ID: 6YB7).

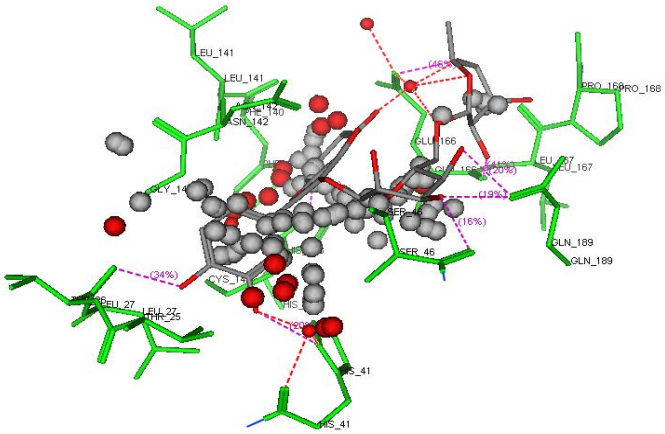

Fig. no. 16: 3D Interaction of Rutin with active site amino acid residues of COVID-19 main protease (PDB ID: 6YB7).

ORAL TOXICITY PREDICTION STUDIES FOR PHYTOCHEMICALS BY PROTOX ONLINE SOFTWARE.

PHYTOCHEMICAL NAME	TOXICITY PARAMETERS																		
	TOXISITY SCALE	$LD_{50MG/KG}$	HEPATOTOXICITY	CARCINOTOXICITY	IMMUNOTOXICITY	MUTAGENESITY	CYTOTOXICITY	Aryl hydrocarbon Receptor (AhR	Androgen Receptor (AR)	Androgen Receptor Ligand Binding Domain (AR-LBD	Aromatase	Estrogen Receptor Alpha (ER)	Estrogen Receptor Ligand Binding	Proliferator Activated Receptor Gamma	Proliferator Activated Receptor Gamma	Heat shock factor response element (HSE)	Mitochondrial Membrane Potential (MMP)	Phosphoprotein (Tumor Supressor) p53	ATPase family AAA domain-containing protein 5 (ATAD5)
3,6-O-DIETHYLOYL SUCROSE	6	29700	NT (0.94)	NT (0.90)	NT (0.99)	NT (0.81)	NT 0.67	NT 0.99	NT 0.85	NT 098	NT 0.95	NT 0.94	NT 0.95	NT 0.97	NT 0.99	NT 0.99	NT 0.97	NT 0.96	NT 0.96
MARTYNOSIDE-14	5	5000	NT 0.90	NT 0.73	T 0.99	NT 0.79	NT 0.73	NT 094	NT 0.94	NT 0.98	NT 0.85	NT 0.85	NT 0.91	NT 0.94	NT 0.92	NT 0.92	NT 0.82	NT 0.82	NT 0.94
ADENOSINE	2	8	NT 0.84	NT 0.71	NT 0.99	NT 0.87	T 0.74	NT 0.93	NT 1.0	NT 1.0	NT 0.92	NT 0.91	NT 1.0	NT 0.83	NT 1.0	NT 1.0	NT 0.97	NT 0.88	NT 0.73
EPIGALLACTOCATECHINE GALLATE	6	9150	NT 0.58	NT 0.61	T 0.61	NT 0.67	NT 0.76	NT 0.9	NT 0.97	NT 0.99	NT 0.92	NT 0.93	NT 0.98	NT 0.95	NT 0.98	NT 0.98	NT 0.90	NT 0.74	NT 0.91
PHYSCION	5	5000	NT 0.71	NT 0.63	T 0.87	T 0.94	NT 0.92	T 0.59	NT 0.99	NT 1.0	NT 0.96	T 0.84	NT 0.56	NT 0.92	NT 0.93	NT 0.93	T 0.84	NT 0.65	NT 0.87
ALOEEMODINE –B-GLUCOSIDE	5	3000	NT 0.87	NT 0.80	T 0.99	T 0.87	NT 0.75	NT 0.93	NT 0.79	NT 0.95	NT 0.98	NT 0.78	NT 0.99	NT 0.97	NT 0.96	NT 0.96	NT 0.87	NT 0.60	NT 0.99
FICUSESALILIGNANS-B	4	1500	NT 0.85	NT 0.70	T 0.98	NT 0.74	NT 0.75	NT 0.57	NT 0.96	NT 0.97	NT 0.88	NT 0.81	NT 0.94	NT 0.93	NT 0.84	NT 0.84	NT 0.64	NT 0.61	NT 0.86
RUTIN	5	5000	NT 0.86	NT 0.81	T 0.95	NT 0.68	NT 0.62	NT 0.88	NT 0.90	NT 0.98	NT 0.95	NT 0.85	NT 0.99	NT 0.92	NT 0.92	NT 0.92	NT 0.90	NT 0.79	NT 0.98

Fig. no. 17: Oral toxicity prediction studies for phytochemicals by protox online software.

ADME PREDICTION STUDIES FOR PHYTOCHEMICALS BY PROTOX ONLINE SOFTWARE

PHYTOCHEMICAL NAME	ADME PARAMETERS																	
	BBB	Buffer_solubility	Caco2	CYP_2C19_inhibition	CYP_2C9_inhibition	CYP_2D6_inhibition	CYP_2D6_substrate	CYP_3A4_inhibition	CYP_3A4_substrate	HIA	MDCK	Pgp_inhibition	Plasma_Protein_Binding	Pure_water_solubility_mg_L	Skin_Permeability	SKlogD_value	SKlogP_value	SKlogS_buffer	SKlogS_pure
3,6-O-DIETHYLOYL SUCROSE	0.078517	3507 78.6	16.9513	IN	IN	NON	NON	IN	WK	22.907599	2.09501	NON	22.947727	880221	-5.06694	2.19 4050	2.19 4050	-0.923750	0.475800
ADENOSINE	0.115402	7669 .69	1.64447	NON	NON	NON	NON	IN	WK	48.243228	0.711411	NON	9.331696	5217.44	-5.17183	-1.93 4180	-1.93 4180	-1.542130	-1.709450
EPIGALLACTOCATECHIN GALLATE	0.875288	1743 7.9	12.0421	IN	IN	NON	NON	IN	WK	20.712498	0.044 6203	IN	100.000000	552.794	-3.99546	3.54 9160	3.54 9160	-1.419730	-2.918660
PHYSCION	0.624171	1.91 9128	20.748	IN	IN	NON	NON	NON	WK	91.606037	39.1496	NON	86.666996	37.2738	-3.80681	1.83 7240	1.83 7240	-5.178520	-3.888440
MARTINOSIDE -14	0.31007	0.33 4544	16.9621	IN	IN	NON	NON	IN	WK	87.271301	0.043 4385	IN	89.100373	0.270404	-2.75209	4.43 8570	4.43 8570	-6.274030	-6.366470
ALOEEMODINE –B-GLUCOSIDE	0.032702 7	29.8 063	13.4232	IN	IN	NON	NON	NON	WK	37.017688	1.6 6293	NON	51.249784	52.3973	-4.722926	-0.46 4010	-0.46 4010	-4.161560	-3.916560
FICUSESALILIGNANS-B	0.038451 4	676. 34	42.9146	IN	IN	NON	NON	IN	SUB	93.505321	0.045 4137	IN	86.252250	1.20763	-3.11601	3.66 5740	3.66 5740	-2.935240	-5.683470
RUTIN	0.028254 3	3.22 38	15.1137	IN	IN	NON	NON	IN	WK	3.1657771	0.069 9765	NON	42.300580	948.043	-4.66989	-2.24 2170	-2.24 2170	-5.027200	-2.818740

NON STANDS FOR NO INHIBITION, IN STANDS FOR INHIBITOR, WK STANDS FOR WEAKLY , SUB STANDS FOR SUBTRATE

Fig. no. 18: ADME prediction studies for phytochemicals by protox online software.

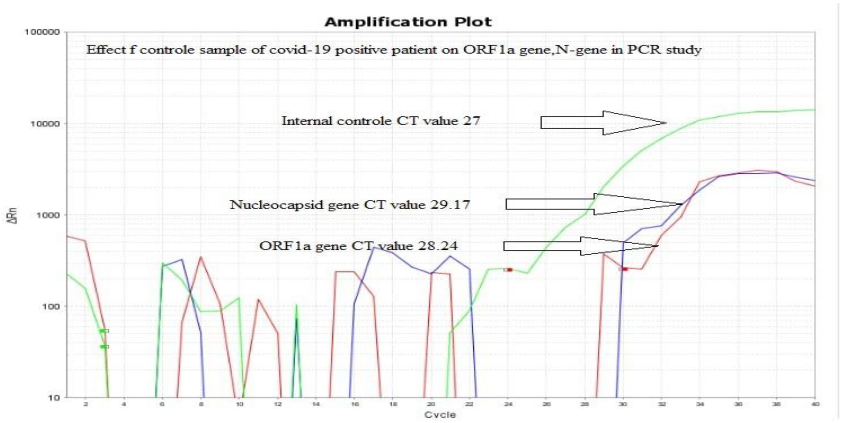

Fig. no. 19: Effect of control sample on covid 19 gene (ORF, n-gene) in RT- PCR study.

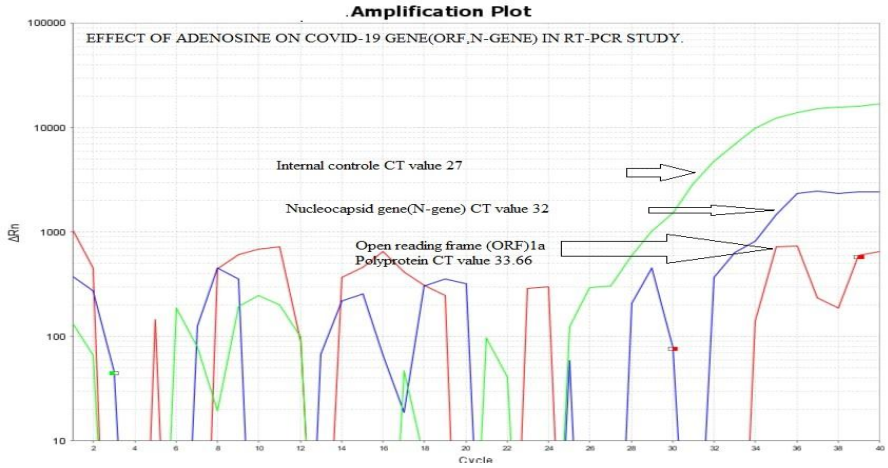

Fig. no. 20: Effect of Adenosine on covid 19 gene (ORF, n-gene) in RT- PCR study.

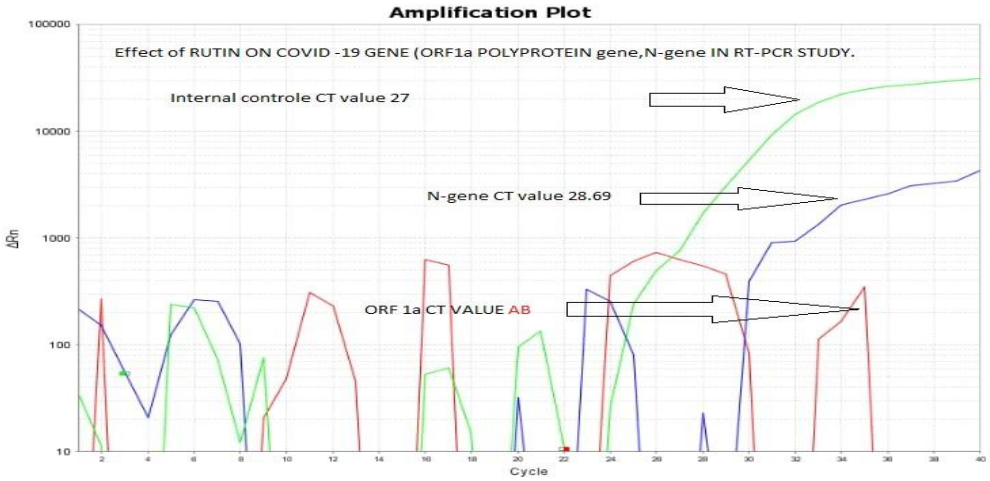

Fig. no. 21: Effect of rutin on covid 19 gene (ORF, n-gene) in RT- PCR study.

Table no. 1: Effect of Control sample, Rutin and Adenosine on covid 19 gene (ORF, n-gene) in RT- PCR study.

Sr. no.	CT VALUE FOR IC(green)	Ct value for N-gene	CT value for ORF(RED)
Controle	27	29.17	28.24
adenosine	27	32	33.66
rutin	27	28.69	AB(o)

DISCUSSION

In molecular docking analysis 3,6, diethyloyl sucrose showed multiple interaction in molecular docking study at the active site of COVID 19 main protease of COVID 19 main protease, such as glycine 143 formed hydrogen acceptor (H-acc) bond with water and in turn with hydroxyl group bonded (71.7%) at distance (2.8A0), whereas histadine163(His-163) formed hydrogen acceptor bond (27.5%) at 2.51 A0 with aliphatic alcoholic group of the ligand and third hydrogen bond was also formed between Glutamic acid (Glu-166) and ethereal oxygen of the phytochemical by forming hydrogen acceptor bond (14.8%) at 3.21 A^0 as shown in figure 1 and 2 . Martinoside-14 (figure no.3 and 4) displayed binding interaction in molecular docking studies at the active site of COVID 19 main protease including Glutamic acid (Glu-166) formed hydrogen donor (H-don) bond with water via hydroxyl group bond (39.4%) at distance 2.43 A0 considering that glycine-143 (Gly-143) formed hydrogen acceptor (H-acc) bond 50.6% at 2.92 A0 with water molecule, apart from this, Histamine -163 (His-163) formed by hydrogen acceptor (H-acc) with 23.3% at distance 2.99 A0 with the help of hydroxyl (OH-) group. Adenosine displayed significant interaction in molecular docking study at the active site of COVID 19 main protease such as histamine -

163(His-163) formed hydrogen acceptor (H-acc) bond with water via hydroxyl group with 94.5% at a distance 2.50 A0 whereas Glutamic acid 166 formed bond with ethereal oxygen via water in (87.6%) at a distance 2.72 A^0(figure no.5 and 6). Epigallactocatechine gallate showed better interaction in molecular docking studies at the active site of COVID 19 main protease such as phenylalanyine-140 (Phe-140) formed hydrogen donor (H-don) bond with water via hydroxyl group bound 11.0% at a distance 1.68 A0. whereas Glutamic acid -166 bound with water and binding with hydroxyl group and ethereal oxygen with hydrogen donor at a distance 1.51 A0 with 15.3 %. Also histidine -163 bound with hydrogen and interacted with hydroxyl group with hydrogen acceptor (H-acc)at a distance 2.80 A0 with 49.2%.(figure no.7 and 8). Physcion showed stronger interaction in molecular docking studies at the active site of COVID 19 main protease such as Histadine- 163 (His -163) formed hydrogen acceptor (H-acc) bonded along with water (38.8%) at a distance 2.98 A0, whereas Glutamic acid-166(Glu-166) formed hydrogen acceptor (H-acc) bond with water with 26.0% at a distance 2.96 A^0. (figure no.9 and 10). aloeemodine-8-glucoside showed multiple interaction in molecular docking studies at the active site of COVID 19 main protease such as Histidine -163(His-163) formed hydrogen acceptor (H-acc) bound via water with 48.9% binding at a distance 2.77 A0 and along with hydroxyl group, also histadine-41(His-41) formed by weak bond with 0.0% at a distance 3.89 A0 and also with water molecule. It has another interaction with Glutamic acid-166 (Glu-166) by hydrogen acceptor (H-acc) bond with 15.5% at a distance 3.15 A^0 (figure no.11 and 12). Ficusesquciligan-B revealed significant interaction in molecular docking studies at the active site of COVID 19 main protease, such As Glutamic acid -166 (Glu-166) formed hydrogen acceptor (H-acc) bound to water (10.8%) and interacted with ethereal oxygen and water at a distance 3.44 A0 .also histadine- 41(His -41) formed hydrogen acceptor (H-acc) bound with water at distance 2.94 A0 And interacted with water with 10.3% also Glycine -143 formed hydrogen acceptor (H-acc) bound with water and interacted with water at distance 3.07 A0 with 15.4%. Apart from this, serine -46 (Ser-46) formed hydrogen acceptor (H-acc) bond with water with 39.7% at a distance 2.83 A^0|as shoen in (figure 13 and 14). Rutin showed multiple interaction in molecular docking studies at the active site of COVID 19 main protease such as histidine -41(His-41) formed hydrogen acceptor (H-acc) bond with water with 20.0% at a distance 2.93 A0 and interaction with water (H2o) and hydroxyl (OH-) group, also histidine 163 (His -163) formed hydrogen acceptor (H-acc) bond with water with (50.5%) at a distance 2.82 A0 and interacted with hydroxyl (OH-) group, whereas Glutamic acid-166 (Glu-166) formed hydrogen donor with 46.2% at a distance 2.49 A0 and interacted with water (H2o). Also Glycine 189 (Gln-189)

formed hydrogen donor with19.7% at distance 2.58 A0 and serine -46 (Ser-46) found to show weaker hydrogen bonding at a distance 3.83 A^0 (figure no.15 and 16).

Oral toxicity prediction studies for phytochemicals by protox online software was predicted for finally selected eight phytochemical molecule using online toxicity prediction software "protox" where structure of the each phytochemical is drawn on the structure drawing window and toxicity is predicted. Toxicity prediction involves score from 1 to 6 scale, where 1 is highest toxicity and 6 score represent least toxicity ,the software also provide prediction about LD50 value least value of LD50 value more will be the toxicity, whereas more the LD50 value least will be the toxicity. Toxicity model report involves organ specific toxicity, such as hepatotoxicity, cardio toxicity, and target specific toxicity; such as Aryl hydrocarbon receptor, androgen receptor, aromatase, estrogen receptor alpha, proliferator activated gamma (PPAR-GAMA), heat shock factor response element (HSR), mitochondrial membrane potential (MMP), phosphoproteins (p53), ATPase (ATAD5) with toxicity prediction accuracy probability is varying from 0-1, 0 is least predictable, whereas 1 is highest level of prediction. The toxicity scale for finally selected phytochemical observed to range from 2-6, adenosine showed higher toxicity ,whereas 3,6-O-DIETHYLOYL SUCROSE and epigallactocatechin showed lesser toxicity, martynoside predicted probability of toxicity is 0.99, epigallactocatechin also showed immunotoxicity with the prediction probability of 0.61, aloeemodine –B-glucoside showed immunotoxicity with prediction probability 0.99 and rutin also displayed immunotoxicity with the prediction probability level of 0.95. FICUSESALILIGNANS-B reveals immunotoxicity with prediction probability of 0.98, whereas adenosine predicted to show cytotoxicity with probability of 0.74.

ADME prediction studies for phytochemicals by protox online software was predicted to finally selected eight phytochemical molecules using online ADME prediction software "Preadmet.bmdrc.kr" where structure of the each phytochemical is drawn on the structure drawing window and ADME is predicted. ADME prediction involves multiple ADME parameters drug absorption such as BBB, buffer solubility, pure water solubility, Caco2 (intestinal absorption model), Skin absorption model and the skin permeability sk log D value, sk log P value, sk log S buffer value and sk log S (skin permeability parameters), HIA (passive gastrointestinal absorption), MDCK (drug efflux by p-gp (p glycoprotein)) and p-gp inhibition (efflux transport protein for drug transport). Drug distribution parameters mainly involves plasma protein binding prediction value varying from 0-100%, more the drug

protein predicted binding better will be the drug distribution and lesser will be free drug available for pharmacological action and vice versa.

Metabolism prediction parameters involves such as CYP2C19 (causes oxidation of aromatic and aliphatic carbon) inhibition, CYP2C9 (aliphatic carbon oxidation such as steroids and fatty acids), CYP2d6 (causes hydroxylation, demethylation and dealkylation) substrate, CYP3a4 (causes hydroxylation, epoxidation of olefins, aromatic oxidation, hetro atom oxidation, N –AND- P –dealkylation, aldehyde oxidation and dehydrogenation) inhibition and CYP3a4 substrate. All the phytochemicals under the study are predicted to show fairly well intestinal absorption, distribution and metabolism. The phytochemicals showed predicted percentage of drug protein binding varying from 9 to 100. Adenosine showed least protein binding 9, whereas epigallactocatechin gallate displayed highest predicted drug protein binding 100%.

The amplifycation curve for internal control (IC) ct is ≤ 35 i.e, 27 hence the detection is valid. Furthermore a typical S-type (sigmoidal) amplification curve is detected by the ORF1ab gene(FAM) and nucleoprotein/nucleocapsid N gene (HEX) channel ct for adenosine is 33.66which is little bit less than 35 and for Rutin this value is absent and for controle ORF1ab is 28.24<35.behalf of this ct for nucleocapsid gene for control, adenisine and Rutin is 29.17, 32 and 28.69 respectively(figure no.19,20,21) Hence, the result revealed that Adenosine showes little bit of inhibition on the other hand Rutin knockout the COVID19 virus as it is absent in ORF1ab gene.

CONCLUSION

Phytochemicals in molecular docking study were ranked based on the number of hydrogen bonds formed with amino acid residues of COVID-19 main protease active site, more the number of interaction, better will be the inhibition predicted for Phytochemical between active site amino acid of COVID-19 main protease, drug binding amino acid interactions will be given preference over non drug interacting amino acid interactions at active site.

Percentage of binding of Phytochemical with active site amino acid will also be given preference, Phytochemical and COVID -19 main protease active site interaction distances in terms of A0 will also be considered, shorter the distance, better will be the binding between Phytochemical and enzyme.

Considering all the above factors Phytochemicals was ranked in molecular docking study as given below;

I. Adenosine
II. Epigallactocatechin gallate
III. Rutin
IV. Aloeemodine-8-glyciside
V. Martiniside-14
VI. 3, 6-o-diethyloyl sucrose
VII. Physcion
VIII. Ficusesquciligan-B

Compound 2 an 3 also showed better predicted pharmacokinetic activity and least toxicity at prediction studies, hence these two compounds were selected for in vitro COVID19 antiviral studies.

Among of these adenosine and rutin were selected for testing with drug and without drug (control) using, positive corona patients oral and nasal swab samples of positive COVID-19 patients using PCR (polymerase chain reaction) in vitro study. The In Vitro is study is under progress to correlate molecular docking and In vitro anti COVID 19 activity using PCR method the result revealed that Adenosine showes little bit of inhibition on the other hand Rutin knockout the COVID19 virus as it is absent in ORF1ab gene.

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