Figure S1: Key interacting residues between PLpro and ISG15

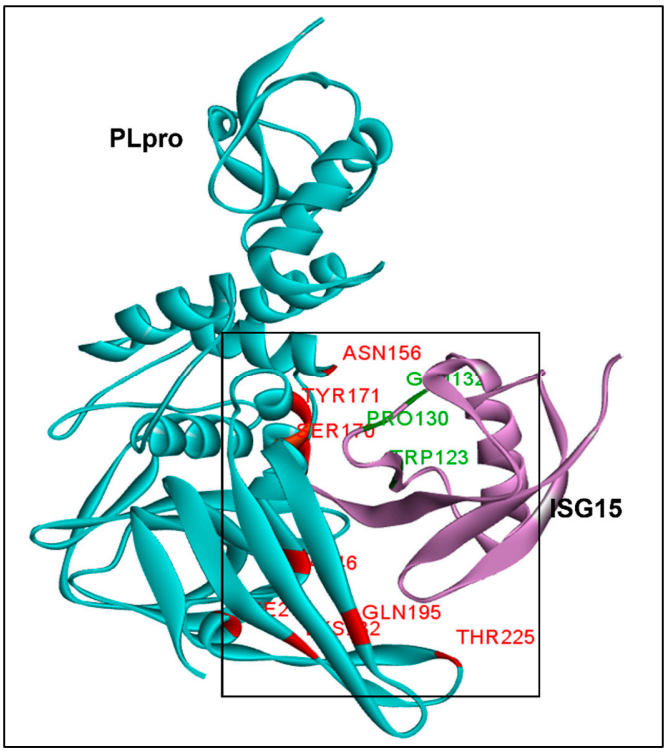


Figure S1. The key interacting residues of PLpro (Cyan) marked in Red and ISG15 (purple) marked in Green found to be critical for the improved de-ISGylation activity of PLpro (the key interacting residues adapted from (Klemm et al. 2020))

Table S1. Docking scores of various phytochemicals from *A. paniculata* (AG), *T. cordifolia (*GU), *O. sanctum* (TU) against SARS-CoV-2 (PLpro-ISG15) complex interacting site (PDB ID:6XA9)

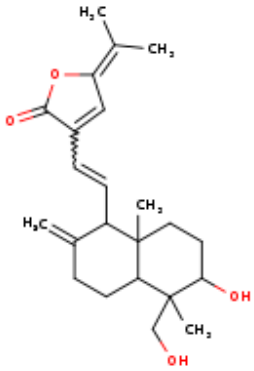
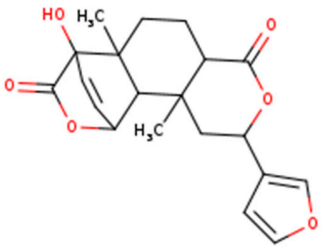
S. No.	Herb	Phytochemical	Pubchem CID	Canonical SMILES	ΔG (kcal/ mol) Plpro ISG15 interaction site at UIM (PDB: 6XA9)
1	***Andrographis paniculata***	14-deoxy-15-isopropylidene-11,12-didehydroandrographolide	637300	CC(=C1C=C(C(=O)O1)C=CC2C(=C)CCC3C2(CCC(C3(C)CO)O)C)C	-9.4

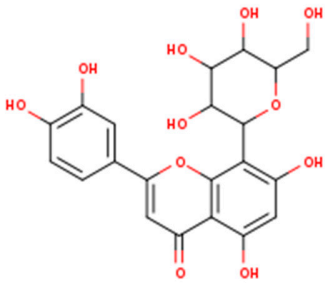
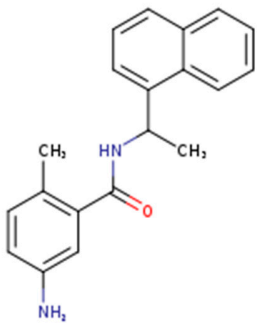
2		Andrographolactone	44206466	CC1=CC2=C(CCC1)C(=C(C(=C2)C)CCC3=CCOC3=O)C	-9.2
3		Neoandrographolide	9848024	CC1(CCCC2(C1CCC(=C)C2CCC3=CCOC3=O)C)COC4C(C(C(C(O4)CO)O)O)O	-9
4		14-Deoxy-11,12-didehydroandrographolide	5708351	CC12CCC(C(C1CCC(=C)C2C=CC3=CCOC3=O)(C)CO)O	-8.8
5		Andrographolide	5318517	CC12CCC(C(C1CCC(=C)C2CC=C3C(COC3=O)O)(C)CO)O	-8.7
6		Andrographoside	6439612	CC12CCC(C(C1CCC(=C)C2CC=C3C(COC3=O)O)(C)COC4C(C(C(C(O4)CO)O)O)O)O	-8.7
7		Andrograpanin	11666871	CC1(CCCC2(C1CCC(=C)C2CCC3=CCOC3=O)C)CO	-8.6
8		Andrographidoid D	57384563	CC1(CC(C2(C1COC(C2)O)O)O)OC(=O)C=CC3=CC=CC=C3	-8.6
9		14-deoxyandrographolide	11624161	CC12CCC(C(C1CCC(=C)C2CCC3=CCOC3=O)(C)CO)O	-8.5
10		5-hydroxy-7, 8, 2', 5'-tetramethoxyflavone	10948318	COC1=CC(=C(C=C1)OC)C2=CC(=O)C3=C(O2)C(=C(C=C3O)OC)OC	-8.4
11		Andrographidin C	5318484	COC1=C(C2=C(C(=C1)OC3C(C(C(C(O3)CO)O)O)O)C(=O)C=C(O2)C4=CC=CC=C4)OC	-8.4
12		14-deoxy-17-hydroxyandrographolide	11631693	CC12CCC(C(C1CCC(C2CCC3=CCOC3=O)CO)(C)CO)O	-8.2
13		Andrographidoid A	57384307	CC1(CC(C2(C1COC(C2)OC)O)O)OC(=O)C=CC3=CC=CC=C3	-8.2
14		Andrographidoid C	57384309	CC1(CC(C2(C1COC(C2)OC)O)O)OC(=O)C=CC3=CC=CC=C3	-8.2
15		5, 7, 2', 3'-tetramethoxyflavone	11772234	COC1=CC=CC(=C1OC)C2=CC(=O)C3=C(O2)C=C(C=C3OC)OC	-8.1
16		5-Hydroxy-2',3',7,8-Tetramethoxyflavone	5319878	COC1=CC=CC(=C1OC)C2=CC(=O)C3=C(O2)C(=C(C=C3O)OC)OC	-8.1
17		Andrographidoid B	57384308	CC1(CC(C2(C1COC(C2)OC)O)O)OC(=O)C=CC3=CC=CC=C3	-8.1
18		5-Hydroxy-7,2',3'-Trimethoxyflavone	12135219	COC1=CC=CC(=C1OC)C2=CC(=O)C3=C(C=C(C=C3O2)OC)O	-7.9
19		Dihydroskullcapflavone	12098358	COC1=C(C2=C(C(=O)CC(O2)C3=CC=CC=C3O)C(=C1)O)OC	-7.9
20		1, 8-dihydroxy-3,7-dimethoxyxanthone	5281653	COC1=C(C2=C(C=C1)OC3=CC(=CC(=C3C2=O)O)O)OC)O	-7.8
21		7-O-methylwogonin	188316	COC1=C(C2=C(C(=C1)O)C(=O)C=C(O2)C3=CC=CC=C3)OC	-7.8
22		7-O-methyldihydrowogonin	146156496	COC1=C(C2=C(CC(=O)C(O2)C3=CC=CC=C3)C(=C1)O)OC	-7.4
23		Andrographidoid E	57384564	CC1(CC(C2=C1COC(=O)C2)O)O	-6.2
24	***Tinospora cordifolia***	Isocolumbin	24721165	CC12CCC3C(=O)OC(CC3(C1C4C=CC2(C(=O)O4)O)C)C5=COC=C5	-9.9
25		Berberin	2353	COC1=C(C2=C[N+]3=C(C=C2C=C1)C4=CC5=C(C=C4CC3)OCO5)OC	-9.4
26		Ecdysterone	12304165	CC12CCC3C(=CC(=O)C4C3(CC(C(C4)O)O)C)C1(CCC2C(C)(C(CCC(C)(C)O)O)O)O	-9

27		Magnoflorine	73337	C[N+]1(CCC2=CC(=C(C3=C2C1CC4=C3C(=C(C=C4)OC)O)O)OC)C	-9
28		Beta-Sitosterol	222284	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	-8.4
29		Tetrahydropalmatine	5417	COC1=C(C2=C(CC3C4=CC(=C(C=C4CCN3C2)OC)OC)C=C1)OC	-8.4
30		Tinocordiside	102504931	CC1=CC(=O)C2C3C1C2(CCC3C(C)(C)OC4C(C(C(C(O4)CO)O)O)O)O)C	-8.4
31		Makisterone B	441830	CC(CC(C(C)(C1CCC2(C1(CCC3C2=CC(=O)C4C3(CC(C(C4)O)O)C)C)O)O)O)C(C)CO	-8.3
32		Tinocordifolioside	100926541	CC1=CC(=O)C2(C(CC1)C(C3C2O3)C(C)(C)OC4C(C(C(C(O4)CO)O)O)O)C	-8.3
33		Tinosporaside	14194109	CC12CCC3C(=O)OC(CC3(C1C(=O)C=CC2OC4C(C(C(C(O4)CO)O)O)O)O)C)C5=COC=C5	-8.2
34		Palmatine	19009	COC1=C(C2=C[N+]3=C(C=C2C=C1)C4=CC(=C(C=C4CC3)OC)OC)OC	-8.1
35		Cordioside	101915817	CC12CC(OC(=O)C1(CC(C3=C(CCCC23)C(=O)OC)OC4C(C(C(C(O4)CO)O)O)O)O)C5=COC=C5	-8
36		Tinocordifolin	100926540	CC1=CC(=O)C2(C(CC1)C(C3C2O3)C(C)(C)O)C	-7.9
37		Tinospinoside E	71473390	CC12CC=C3C(=O)OC(CC3(C1C4C=CC2(C(=O)O4)OC5C(C(C(C(O5)CO)O)O)O)O)C)C6=COC=C6	-7.6
38		Tinosinen	45359937	COC1=CC(=CC(=C1OC2C(C(C(C(O2)CO)O)OC3C(C(CO3)(CO)O)O)O)OC)C=CCO	-7.4
39		Syringin	5316860	COC1=CC(=CC(=C1OC2C(C(C(C(O2)CO)O)O)O)OC)C=CCO	-7.1
40		Z-syringin	9799599	COC1=CC(=CC(=C1OC2C(C(C(C(O2)CO)O)O)O)OC)C=CCO	-6.8
41	***Ocimum sanctum***	Orientin	5281675	C1=CC(=C(C=C1C2=CC(=O)C3=C(O2)C(=C(C=C3O)O)C4C(C(C(C(O4)CO)O)O)O)O)O	-9.4
42		Isoorientin	114776	C1=CC(=C(C=C1C2=CC(=O)C3=C(O2)C=C(C(=C3O)C4C(C(C(C(O4)CO)O)O)O)O)O)O	-9.2
43		Vitexin	5280441	C1=CC(=CC=C1C2=CC(=O)C3=C(O2)C(=C(C=C3O)O)C4C(C(C(C(O4)CO)O)O)O)O	-9.1
44		Isovitexin	162350	C1=CC(=CC=C1C2=CC(=O)C3=C(O2)C=C(C(=C3O)C4C(C(C(C(O4)CO)O)O)O)O)O	-9
45		Molludistin	44258315	COC1=C(C2=C(C(=C1)O)C(=O)C=C(O2)C3=CC=C(C=C3)O)C4C(C(C(CO4)O)O)O	-8.8
46		Ursolic acid	64945	CC1CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1C)C)C(=O)O	-8.7
47		Luteolin	5280445	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)O	-8.6
48		Apigenin	5280443	C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O	-8.4
49		Chlorogenic acid	1794427	C1C(C(C(CC1(C(=O)O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O	-8.3
50		Stigmasterol	5280794	CCC(C=CC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	-8.3
51		Oleanolic acid	10494	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1)C)C(=O)O)C	-8
52		Esculin	5281417	C1=CC(=O)OC2=CC(=C(C=C21)OC3C(C(C(C(O3)CO)O)O)O)O	-7.9
53		β-Guaiene	6949	CC1CCC(=C(C)C)CC2=C1CCC2C	-7.7
54		Caryophyllene oxide	1742210	CC1(CC2C1CCC3(C(O3)CCC2=C)C)C	-7.5
55		Humulene	5281520	CC1=CCC(C=CCC(=CCC1)C)(C)C	-7.5
56		beta-Gurjunene	6450812	CC1CCC2C(C2(C)C)C3C1CCC3=C	-7.4

57		Cubenol	519857	CC1CCC(C2C1(CCC(=C2)C)O)C(C)C	-7.4
58		Germacrene a	9548705	CC1=CCCC(=CCC(CC1)C(=C)C)C	-7.2
59		Retinol	445354	CC1=C(C(CCC1)(C)C)C=CC(=CC=CC(=CCO)C)C	-7.2
60		alpha-Selinene	10123	CC1=CCCC2(C1CC(CC2)C(=C)C)C	-7.1
61		Bornyl Acetate	6448	CC(=O)OC1CC2CCC1(C2(C)C)C	-6.7
62		Esculetin	5281416	C1=CC(=O)OC2=CC(=C(C=C21)O)O	-6.6
63		Farnesol	445070	CC(=CCCC(=CCCC(=CCO)C)C)C	-6.4
64		Caffeic acid	689043	C1=CC(=C(C=C1C=CC(=O)O)O)O	-6.3
65		Phytol	5280435	CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C	-6.3
66		Camphor	2537	CC1(C2CCC1(C(=O)C2)C)C	-6.1
67		Borneol	64685	CC1(C2CCC1(C(C2)O)C)C	-6
68		Eucalyptol	2758	CC1(C2CCC(O1)(CC2)C)C	-6
69		Farnesene	5281516	CC(=CCCC(=CCC=C(C)C=C)C)C	-6
70		Gallic acid	370	C1=C(C=C(C(=C1O)O)O)C(=O)O	-6
71		Linolenic acid	5280934	CCC=CCC=CCC=CCCCCCCCC(=O)O	-6
72		Eugenol	3314	COC1=C(C=CC(=C1)CC=C)O	-5.9
73		Methyl chavicol	8815	COC1=CC=C(C=C1)CC=C	-5.9
74		Ascorbic acid	54670067	C(C(C1C(=C(C(=O)O1)O)O)O)O)O	-5.8
75		Camphene	6616	CC1(C2CCC(C2)C1=C)C	-5.6
76		D-Limonene	440917	CC1=CCC(CC1)C(=C)C	-5.6
77		Linoleic acid	5280450	CCCCCC=CCC=CCCCCCCCC(=O)O	-5.6
78		β-Pinene	14896	CC1(C2CCC(=C)C1C2)C	-5.6
79		alpha-Pinene	6654	CC1=CCC2CC1C2(C)C	-5.5
80		Sabinene	18818	CC(C)C12CCC(=C)C1C2	-5.5
81		Galuteolin	5280637	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)OC4C(C(C(C(O4)CO)O)O)O)O)O)O	-5.4
82		alpha-Thujene	17868	CC1=CCC2(C1C2)C(C)C	-5.3
83		Linalool	6549	CC(=CCCC(C)(C=C)O)C	-5.3
84		Oleic acid	445639	CCCCCCCCC=CCCCCCCCC(=O)O	-5.3
85		Palmitic acid	985	CCCCCCCCCCCCCCCC(=O)O	-5.3
86		(E)-beta-Ocimene	5281553	CC(=CCC=C(C)C=C)C	-5.1
87		Benzaldehyde	240	C1=CC=C(C=C1)C=O	-4.9
88		alpha-Myrcene	519324	CC(=C)CCCC(=C)C=C	-4.8
89		2-Furaldehyde	7362	C1=COC(=C1)C=O	-4.3
90		Heptan-1-ol	8129	CCCCCCCO	-4.3
	Positive control	GRL0617	24941262	CC1=C(C=C(C=C1)N)C(=O)NC(C)C2=CC=CC3=CC=CC=C32	-8.5

Table S2. (i). Drug-likeness properties of the top-ranking phytochemicals from ***A. paniculate***, ***T. cordifolia, O. sanctum, and*** **GRL0617.**

S.No.	Herb	Phytoconstituents	Molecular formula	ADME properties (Lipinski's Rule of Five)		Drug Likeness
				Properties	Value	
1	***A. paniculata***	14-deoxy-15-isopropylidene-11,12-didehydroandrographolide (AG1) 	C23H32O4	Molecular weight (<500 Da)	372.50 g/mol	YES
				LogP (<5)	3.52	
				H-Bond donor (<5)	2	
				H-Bond acceptor (<10)	4	
				Violations	0	
2	***T. cordifolia***	Isocolumbin (GU1) 	C20H22O6	Molecular weight (<500 Da)	358.39 g/mol	YES
				LogP (<5)	2.12	
				H-Bond donor (<5)	1	

				H-Bond acceptor (<10)	6	
				Violations	0	
3	***O. sanctum***	Orientin (TU1) 	C21H20O11	Molecular weight (<500 Da)	448.38 g/mol	NO
				LogP (<5)	1.27	
				H-Bond donor (<5)	8	
				H-Bond acceptor (<10)	11	
				Violations	2	
4	**GRL0617**	GRL0617 	C20H20N2O	Molecular weight (<500 Da)	304.39 g/mol	YES
				LogP (<5)	2.69	
				H-Bond donor (<5)	2	

				H-Bond acceptor (<10)	1	
				Violations	0	

Table S2. (ii). Pharmacokinetic properties (ADMET) prediction for the top-ranking phytoconstituents from ***A. paniculate***, ***T. cordifolia, O. sanctum, and*** **GRL0617.**

Property	Model Name	AG1	GU1	TU1	GRL0617
Absorption	Buffer solubility (mg/L)	0.53	32388.1	562.68	5.81
	Caco2 permeability (nm/sec)	22.12	21.91	3.00	21.65
	Human Intestinal Absorption (%)	93.75	95.26	14.99	94.85
	MDCK cell permeability (nm/sec)	0.06	55.59	0.70	9.90
	P-glycoprotein inhibition	Inhibitor	Non	Non	Non
	Plasma Protein Binding (%)	98.92	88.65	63.12	86.11
	Pure water solubility (mg/L)	3.06	23.84	256.36	1.42
	Skin Permeability (logKp, cm/hour)	-1.73	-3.47	-4.69	-2.59
Distribution	BBB (C.brain/C.blood)	1.22	0.22	0.03	1.40
Metabolism	CYP2C19 inhibition	Non	Non	Inhibitor	Non
	CYP2C9 inhibition	Inhibitor	Inhibitor	Inhibitor	Non
	CYP2D6 inhibition	Non	Non	Non	Non
	CYP2D6 substrate	Non	Non	Non	Non
	CYP3A4 inhibition	Inhibitor	Inhibitor	Inhibitor	Non
	CYP3A4 substrate	Substrate	Substrate	Weakly	Substrate
Toxicity	acute algae toxicity	0.0129867	0.10331	0.0208	0.028122
	Ames test	mutagen	mutagen	non-mutagen	mutagen
	hERG inhibition	medium risk	medium risk	high risk	medium risk

Abbreviations: 14-deoxy-15-isopropylidene-11,12-didehydroandrographolide (AG1), Isocolumbin (GU1), Orientin (TU1), ADMET, absorption, distribution, metabolism, excretion, and toxicity; BBB, blood-brain barrier; CYP, cytochrome P; hERG, human ether-a-go-go-related gene.

Principle component analysis (PCA)

The molecular dynamics trajectories were used for PCA to identify the conformational motions relevant to protein functions. Eigenvalues were used to calculate the conformational changes due to the movement of atoms (Khan et al., 2016). The eigenvalues were generated by diagonalizing the covariance matrix of the Cα atomic fluctuations against the equivalent Eigenvectors' (EV) indices. The first 10 modes were taken into consideration in the analysis of the essential subspace as they cover >95% variance of the protein where an exponentially decaying curve of eigenvalues is obtained against the EVs (Supplementary Figure- 2). In this study, PC1 and PC2 that dominate the protein conformational fluctuations were also used for the analysis of PLpro without ligand and in the complexes. For the first two PCs taken into consideration, simulation results revealed the subspace dimension for PLpro in the unbound state and in the complex is comparable, with no noticeable large difference in the dimension (Supplementary Figure- 1). This is also reflected in their 2D projection plots of trajectories, with similar trace values of the covariance matrix for both.

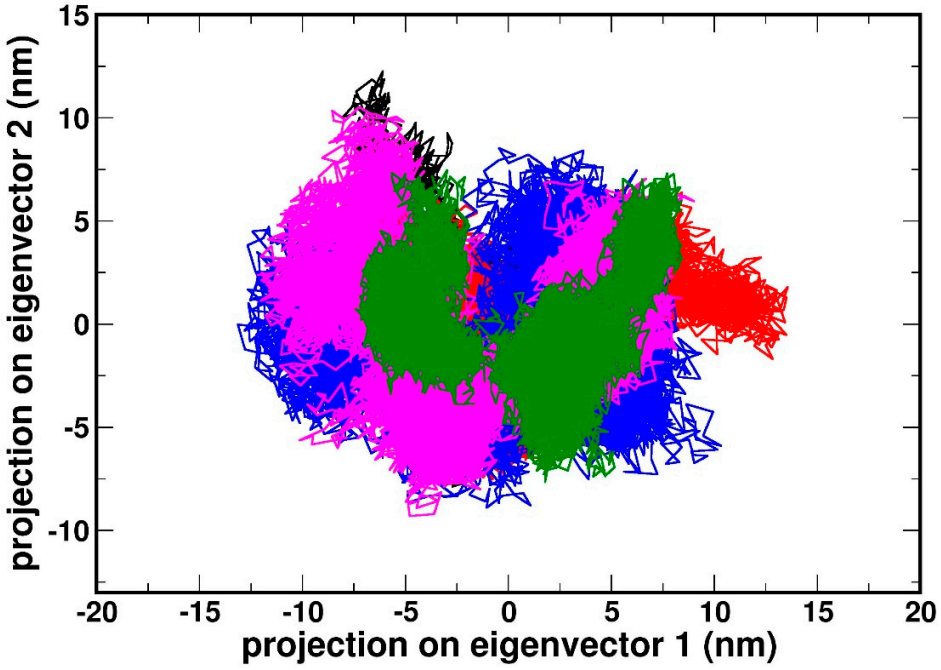


Figure S2. Principal component analysis (PCA) of (PLpro-ISG15) in the unbound state (Black color) and in the (PLpro-ISG15) complex-AG1 (Red color), (PLpro-ISG15) complex-GU1(Blue color), (PLpro-ISG15) complex-TU1(magenta color) and (PLpro-ISG15) complex-GRL0617 (Green color). The 2D projection plot of the first two principal eigenvectors