Supplementary Data

This supplementary data is a part of paper entitled "Exploring the Potency of *Nigella sativa* seed in Inhibiting SARS-CoV-2 Main Protease Using Molecular Docking and Molecular Dynamics Simulations".

Table S1. Secondary metabolites in *Nigella sativa* seeds curated from the literature [1-8]

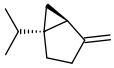
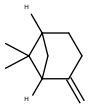
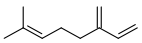
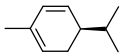
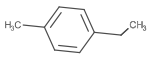
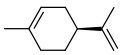
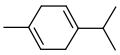
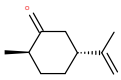
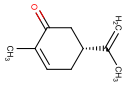
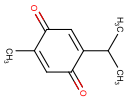
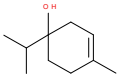
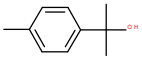
No.	Secondary Metabolites	2D Structure	Charge*	Ref.
	Monoterpenoid hydrocarbons			
1	*α*-Thujene		0	1
2	*α*-Pinene		0	1
3	Sabinene		0	1
4	*β*-Pinene		0	1
5	Myrcene		0	1
6	*α*-Phellandrene		0	1
7	*p*-Cymene		0	2
8	Limonene		0	1
9	*γ*-Terpinene		0	1
	Monoterpenoid ketones			
10	Fenchone		0	1
11	Dihydrocarvone		0	1
12	Carvone		0	2
13	Thymoquinone		0	2
	Monoterpenoid alcoholss			
14	*α*-Terpineol		0	2
15	*p*-Cymene-8-ol		0	1

Table S1. Secondary metabolites in *Nigella sativa* seeds curated from the literature [1-8] (*Continued*)

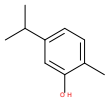
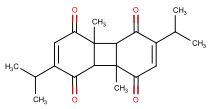
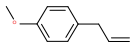
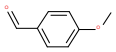
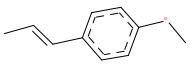
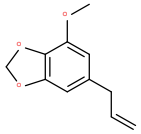
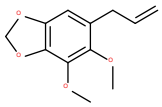
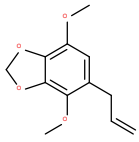
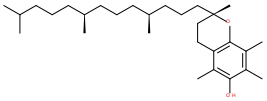
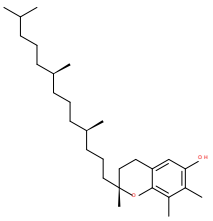
No.	Secondary Metabolites	2D Structure	Charge*	Ref.
16	Carvacrol		0	2
17	Thymol		0	3
	Diterpenoids			
18	Dithymoquinone		0	2
	Sesquiterpenoid hydrocarbones			
19	α-Longipinene		0	1
20	Longifolene		0	1
	Phenyl propanoid compounds			
21	Estragole		0	1
22	Anisaldehyde		0	1
23	*trans*-Anethole		0	2
24	Myristicin		0	1
25	Dillapiole		0	1
26	Apiole		0	1
	Vitamin E			
27	α-Tocopherol		0	3
28	γ-Tocopherol		0	3

Table S1. Secondary metabolites in *Nigella sativa* seeds curated from the literature [1-8] (*Continued*)

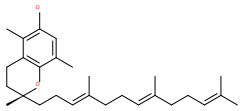
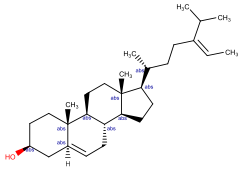
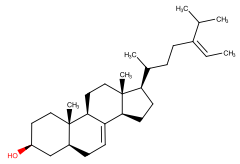
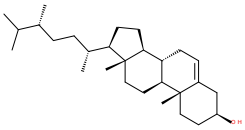
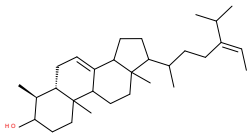
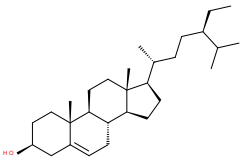
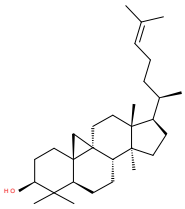
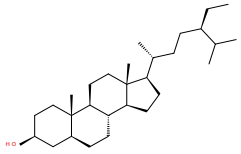
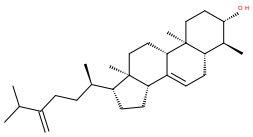
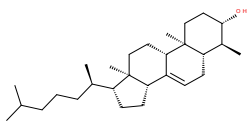
No.	Secondary Metabolites	2D Structure	Charge*	Ref.
29	*β*-Tocotrienol		0	3
	Phytosterols			
30	Avenasterol-5-ene		0	4
31	Avenasterol-7-ene		0	4
32	Campesterol		0	4
33	Citrostadienol		0	4
34	*β*-sitosterol		0	3
35	Cycloartenol		0	4
36	Stigmastanol		0	4
38	Gramisterol		0	4
37	Lophenol		0	4

Table S1. Secondary metabolites in *Nigella sativa* seeds curated from the literature [1-8] (*Continued*)

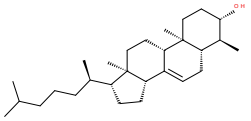
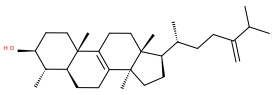
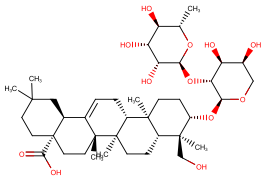
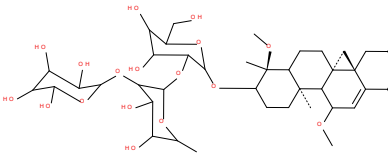
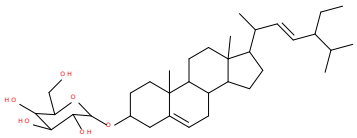
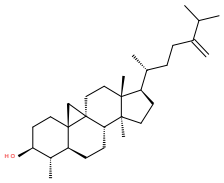
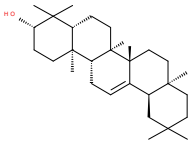
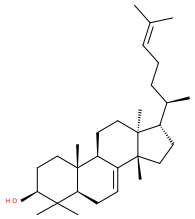
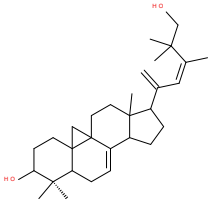
No.	Secondary Metabolites	2D Structure	Charge*	Ref.
38	Lophenol		0	4
39	Obtusifoliol		0	4
	Saponin			
40	*α*-Hederin		-1	2
41	3-O-[*β*-D-xylopyranosyl-(1→2)-alpha-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl]-11-methoxy-16,23-dihydroxy-28-methyl olean-12-enoate		0	5
42	Stigma-5,22-dien-3-*β*-D-glucopyranoside		0	5
	Triterpenoids			
43	Cycloeucalenol		0	4
44	*β*-amyrin		0	4
45	Butyrospermol		0	4
46	Cycloart-23-methyl-7,20,22-triene-3*β*,25-diol		0	5

Table S1. Secondary metabolites in *Nigella sativa* seeds curated from the literature [1-8] (*Continued*)

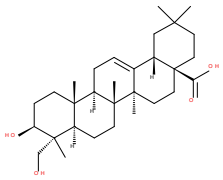
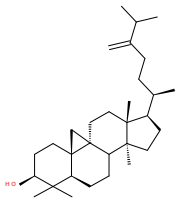
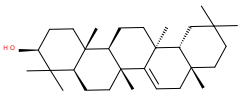
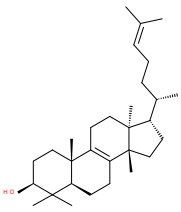
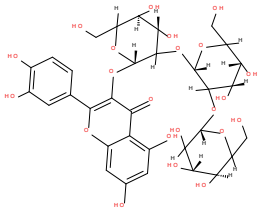
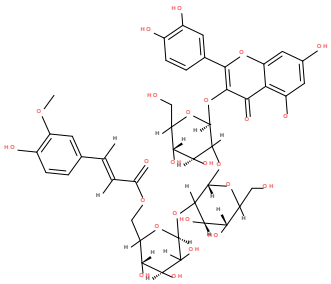
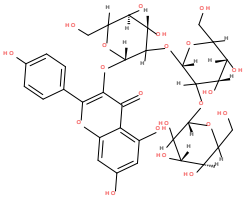
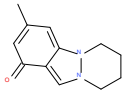
No.	Secondary Metabolites	2D Structure	Charge*	Ref.
47	Melanthigenin		-1	4
48	24-Methylene-cycloartanol		0	4
49	Taraxerol		0	4
50	Tirucallol		0	4
	Flavonols			
51	Quercetin 3-glucosyl-(1→2)-galactosyl-(1→2)-glucoside		-1	6
52	Quercetin 3-(6""-feruloylglucosyl)-(1→2)-galactosyl-(1→2)-glucoside		-1	6
53	Kaempferol 3-glucosyl-(1→2)-galactosyl-(1→2)-glucoside		-1	6
	Alkaloids			
54	Nigeglanine		0	7

Table S1. Secondary metabolites in *Nigella sativa* seeds curated from the literature [1-8] (*Continued*)

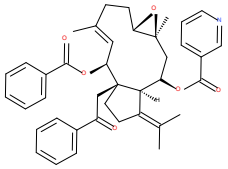
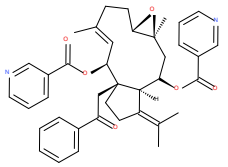
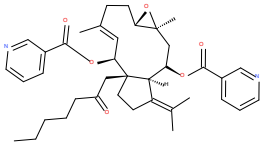
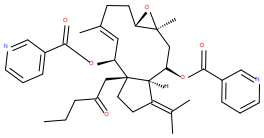
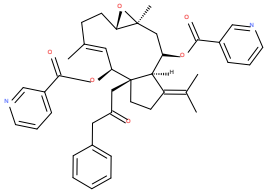
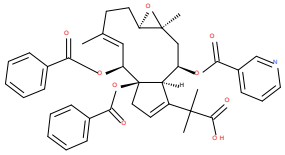
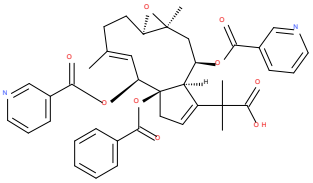
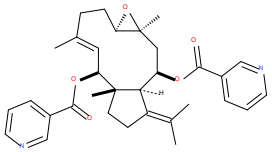
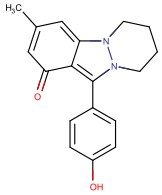
No.	Secondary Metabolites	2D Structure	Charge*	Ref.
55	Nigellamine A1		0	2
56	Nigellamine A2		0	2
57	Nigellamine A3		0	2
58	Nigellamine A4		0	2
59	Nigellamine A5		0	2
60	Nigellamine B1		-1	8
61	Nigellamine B2		-1	8
62	Nigellamine C		0	2
63	Nigellidine		-1	2

Table S1. Secondary metabolites in *Nigella sativa* seeds curated from the literature [1-8] (*Continued*)

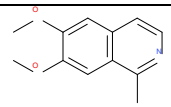
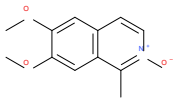
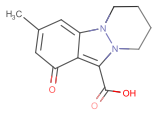
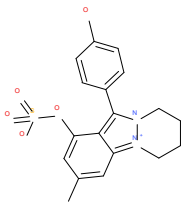
No.	Secondary Metabolites	2D Structure	Charge*	Ref.
64	Nigellicimine		0	2
65	Nigellicimine-N-oxide		0	2
66	Nigellicine		-1	2
67	Nigellidine-4-O-sulfite		0	4

Table S2. Lipinski's rule of five results for secondary metabolites in *N. sativa* seeds

No.	Secondary Metabolites	Lipinski's Rule of Five				
		MW (g/mol)	MlogP	H-bond Acceptor(s)	H-bond Donor(s)	Violation(s)
Monoterpenoid hydrocarbons						
1	*α*-Thujene	136.23	4.29	0	0	1
2	*α*-Pinene	136.23	4.29	0	0	1
3	Sabinene	136.23	4.29	0	0	1
4	*β*-Pinene	136.23	4.29	0	0	1
5	Myrcene	136.23	3.56	0	0	0
6	*α*-Phellandrene	136.23	3.27	0	0	0
7	*p*-Cymene	134.22	4.47	0	0	1
8	Limonene	136.23	3.27	0	0	0
9	*γ*-Terpinene	136.23	3.27	0	0	0
Monoterpenoid ketones						
10	Fenchone	152.23	2.30	1	0	0
11	Dihydrocarvone	152.23	2.20	1	0	0
12	Carvone	150.22	2.10	1	0	0
13	Thymoquinone	164.20	1.08	2	0	0
Monoterpenoid alcoholss						
14	*α*-Terpineol	154.25	2.50	1	1	0
15	*p*-Cymene-8-ol	150.22	2.11	1	1	0
16	Carvacrol	150.22	2.82	1	1	0
17	Thymol	150.22	2.82	1	1	0
Diterpenoids						
18	Dithymoquinone	328.4	1.74	4	0	0
Sesquiterpenoid hydrocarbons						
19	*α*-Longipinene	204.35	5.65	0	0	1

Table S2. Lipinski's rule of five results for secondary metabolites in *N. sativa* seeds (*Continued*)

No.	Secondary Metabolites	Lipinski's Rule of Five				
		MW (g/mol)	MlogP	H-bond Acceptor(s)	H-bond Donor(s)	Violation(s)
20	Longifolene	204.35	5.65	0	0	1
Phenyl propanoid compounds						
21	Estragole	148.20	2.67	1	0	0
22	Anisaldehyde	137.14	1.12	2	0	0
23	*trans*-Anethole	148.20	2.67	1	0	0
24	Myristicin	192.21	1.70	3	0	0
25	Dillapiole	222.24	1.40	4	0	0
26	Apiole	222.24	1.40	4	0	0
Vitamin E						
27	α-Tocopherol	430.71	6.14	2	1	1
28	γ-Tocopherol	416.68	5.94	2	1	1
29	β-Tocotrienol	410.63	5.68	2	1	1
Phytosterols						
30	Avenasterol-5-ene	412.69	6.62	1	1	1
31	Avenasterol-7-ene	412.69	6.62	1	1	1
32	Campesterol	400.68	6.54	1	1	1
33	Citrostadienol	426.72	6.82	1	1	1
34	β-sitosterol	414.71	6.73	1	1	1
35	Cycloartenol	426.72	6.92	1	1	1
36	Stigmastanol	416.72	6.88	1	1	1
37	Gramisterol	412.69	6.62	1	1	1
38	Lophenol	400.68	6.54	1	1	1
39	Obtusifoliol	426.72	6.82	1	1	1
Saponin						
40	α-Hederin	750.96	1.46	12	7	3
41	3-O-[β-D-xylopyranosyl-(1→2)-alpha-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-11-methoxy-16,23-dihydroxy-28-methyl olean-12-enoate	989.15	-2.27	20	10	3
42	Stigma-5,22-dien-3-β-D-glucopyranoside	574.83	3.85	6	4	1
Triterpenoids						
43	Cycloeucalenol	426.72	6.92	1	1	1
44	β-amyrin	426.72	6.92	1	1	1
45	Butyrospermol	426.72	6.82	1	1	1
46	Cycloart-23-methyl-7,20,22-triene-3β,25-diol	442.72	6	2	2	1
47	Melanthigenin	472.70	4.97	4	3	1
48	24-Methylene-cycloartanol	440.74	7.12	1	1	1
49	Taraxerol	426.72	6.92	1	1	1
50	Tirucallol	426.72	6.82	1	1	1
Flavonols						
51	Quercetin 3-glucosyl-(1→2)-galactosyl-(1→2)-glucoside	788.66	-6.64	22	14	3
52	Quercetin 3-(6""-feruloylglucosyl) -(1→2)-galactosyl-(1→2)-glucoside	964.83	-6.01	25	14	3

Table S2. Lipinski's rule of five results for secondary metabolites in *N. sativa* seeds (*Continued*)

No.	Secondary Metabolites	Lipinski's Rule of Five				
		MW (g/mol)	MlogP	H-bond Acceptor(s)	H-bond Donor(s)	Violation(s)
53	Kaempferol 3-glucosyl-(1→2)-galactosyl-(1→2)-glucoside	772.66	-6.2	21	13	3
Alkaloids						
54	Nigeglanine	202.25	1.91	1	0	0
55	Nigellamine A1	633.77	8.13	7	0	2
56	Nigellamine A2	634.76	7.52	8	0	1
57	Nigellamine A3	628.80	7.79	8	0	1
58	Nigellamine A4	600.74	7.01	8	0	1
59	Nigellamine A5	648.79	7.45	8	0	1
60	Nigellamine B1	679.75	6.77	10	1	2
61	Nigellamine B2	680.74	6.17	11	1	2
62	Nigellamine C	530.65	6.27	7	0	1
63	Nigellidine	294.35	3.28	2	1	0
64	Nigellicimine	203.24	2.56	3	0	0
65	Nigellicimine-N-oxide	219.24	1.80	3	0	0
66	Nigellicine	246.26	1.60	3	1	0
67	Nigellidine-4-O-sulfite	374.41	3.32	5	1	0

Table S3. Binding energy scores of secondary metabolites in *N. sativa* to M^{pro}. Binding energy scores were obtained from molecular docking

No.	Ligand	Affinity/kcal.mol^{-1}
1	N3	-9.1
2	Nigellidine-4-O-sulfite	-8.2
3	Taraxerol	-7.8
4	Nigellidine	-7.8
5	Nigellamine A2	-7.7
6	Nigellamine A3	-7.7
7	Melanthigenin	-7.7
8	Leupeptine	-7.6
9	Nigellamine A5	-7.5
10	Butyro-spermol	-7.5
11	Stigma-5,22-dien-3-beta-D-glucopyranoside	-7.5
12	*β*-amyrin	-7.4
13	Dithymoquinone	-7.3
14	Nigellamine A4	-7.2
15	*β*-Tocotrienol	-7.2
16	Cycloart-23-methyl-7,20,22-triene-3beta,25-diol	-7.2
17	Cycloeucalenol	-7.2
18	Nigellamine C	-7.2
19	Avenasterol-7-ene	-7.0
20	Campesterol	-6.8
21	Gramisterol	-6.8
22	24-Methylene-cycloartanol	-6.7

Table S3. Binding energy scores of secondary metabolites in *N. sativa* to M^{pro}. Binding energy scores were obtained from molecular docking

No.	Ligand	Affinity/kcal.mol^{-1}
23	Avenasterol-5-ene	-6.7
24	Tirucallol	-6.7
25	Nigellicine	-6.6
26	Cycloartenol	-6.6
27	*β*-sitosterol	-6.6
28	Citrostadienol	-6.5
29	*α*-Tocopherol	-6.4
30	Obtusifoliol	-6.3
31	Stigmastanol	-6.2
32	Lophenol	-6.1
33	Nigeglanine	-5.9
34	Nigellicimine-N-oxide	-5.6
35	Nigellicimine	-5.5
36	*γ*-Tocopherol	-5.4
37	Apiole	-5.4
38	Dillapiol	-5.4
39	Longifolene	-5.3
40	Myristicin	-5.2
41	*α*-Longipinene	-5.1
42	Thymoquinone	-4.9
43	Dihydrocarvone	-4.9
44	Carvone	-4.8
45	Carvacrol	-4.8
46	*p*-Cymene-8-ol	-4.8
47	*α*-Phellandrene	-4.7
48	*p*-Cymene	-4.6
49	*α*-Terpineol	-4.6
50	Thymol	-4.6
51	*γ*-Terpinene	-4.6
52	Sabinene	-4.6
53	Limonene	-4.5
54	Trans-anethole	-4.4
55	Anisaldehyde	-4.4
56	Estragole	-4.4
57	*α*-Pinene	-4.4
58	Fenchone	-4.3
59	*α*-Thujene	-4.3
60	*β*-Pinene	-4.2
61	Myrcene	-4.0

Table S4. $\Delta G°_{MMGBSA}$ median values of ligands bound to M^{pro}

No	Ligand	$\Delta G°_{MMGBSA}$/kcal.mol^{-1}		
		Q1	Median	Q3
1	N3	-64.6	-61.7	-60.1
2	Leupeptin	-43.96	-41.8	-40.2
3	Nigellamine A2	-47.6	-43.9	-42.3
4	Nigellamine A3	-45.7	-36.2	-32.7
5	Melanthigenin	-34.0	-32.8	-31.7
6	Nigellidine-4-O-sulfite	-29.0	-26.5	-24.6
7	Taraxerol	-18.8	-16.1	-14.7
8	Nigellidine	-16.8	-12.9	-10.6

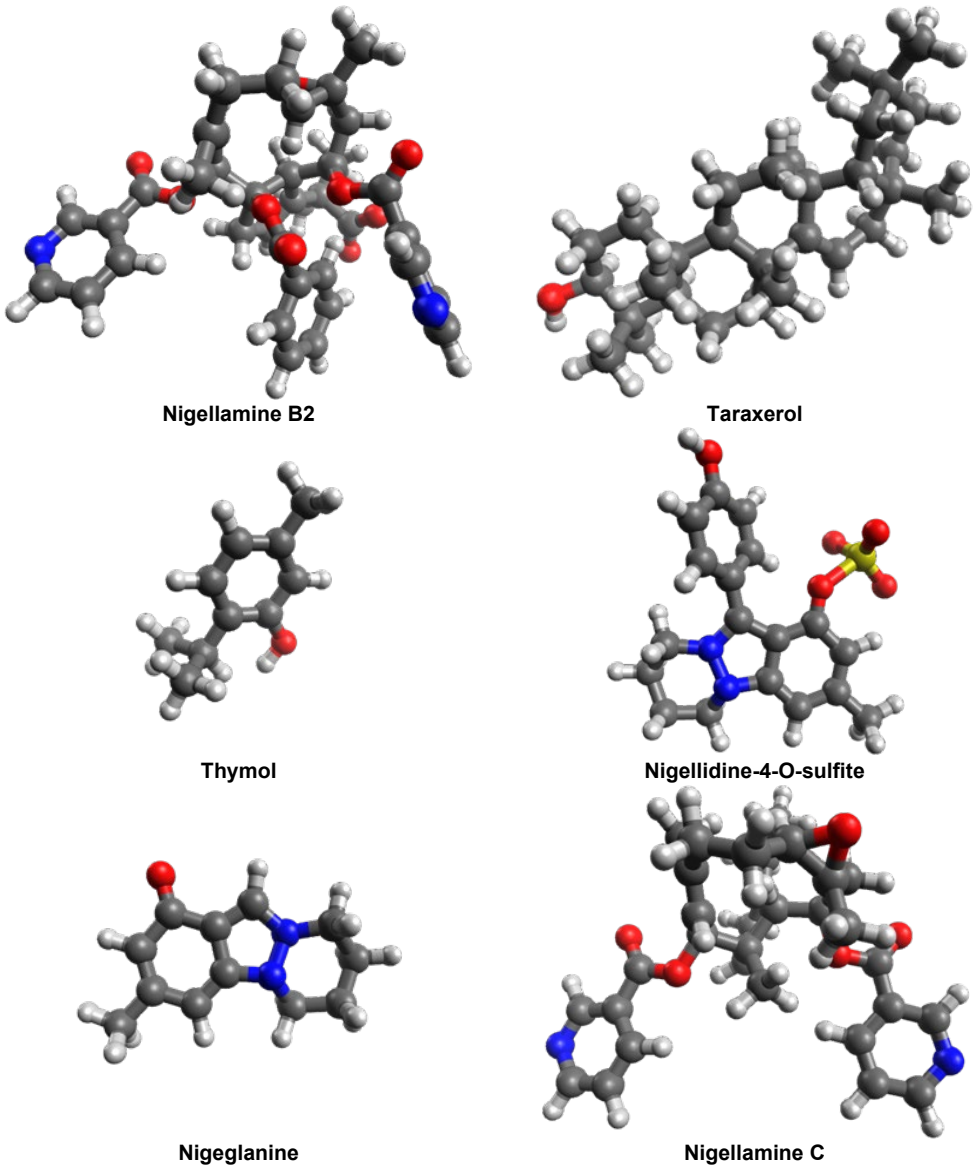


Fig S1. Optimized geometry of the representative secondary metabolites in *N. sativa*. Geometry optimization was performed by using a semiempirical method of PM6

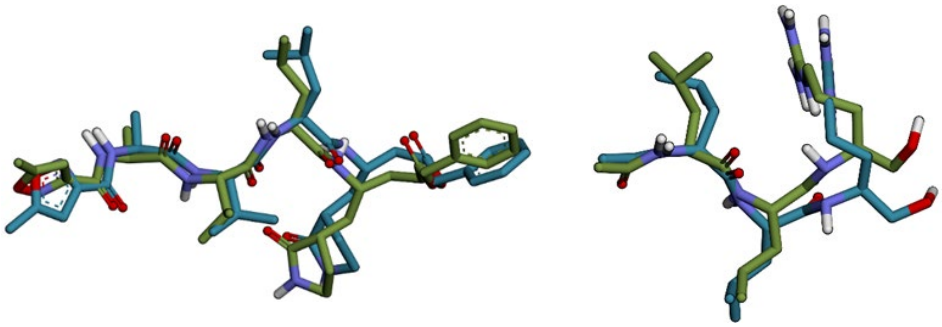


Fig S2. Overlaying between crystal and re-docking structures of N3 (a) and leupeptin (b). The RMSD value on the heavy atoms of crystal and redocking structures of N3 is 1.50 Å and 1.47 Å for that of leupeptin. The blue color denotes crystal structures, whereas the green colour is redocking structures

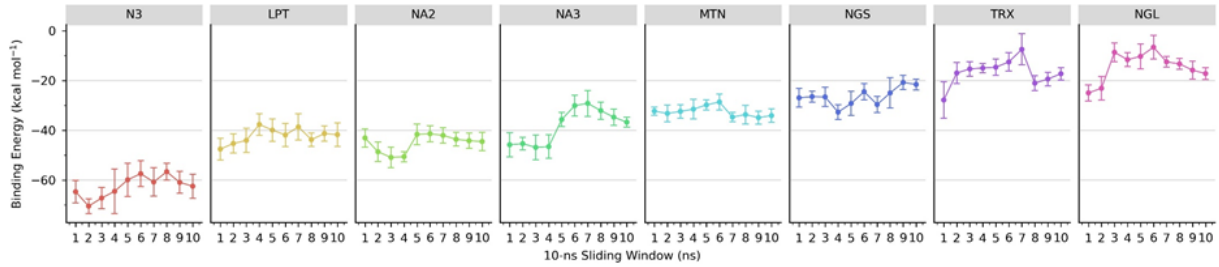


Fig S3. Trajectory of $\Delta G°_{MMGBSA}$ values of several ligands binding to M^{pro}. Each data point was generated from every 10 ns MD trajectory. The ligands include N3 inhibitor, leupeptin (LPT), and secondary metabolites in *N. sativa* seeds. They are nigellamine A2 (NA2), nigellamine A3 (NA3), melanthigenin (MTN), nigellidine-4-O-sulfite (NGS), taraxerol (TRX), and nigellidine (NGL)

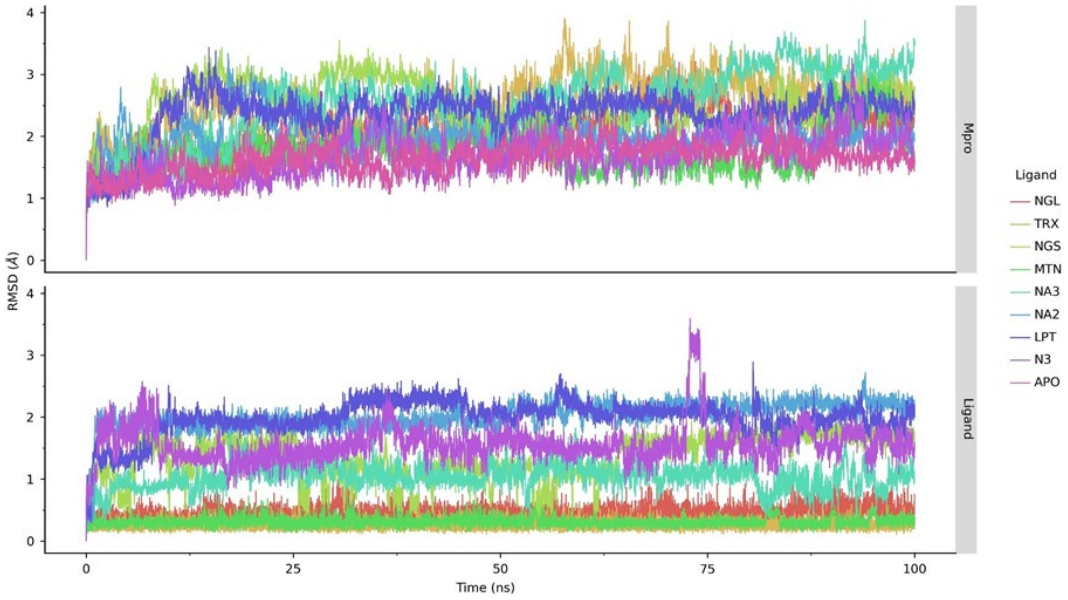


Fig S4. RMSD plots of M^{pro} in apo and ligand-bound forms and the ligands. The top panels are RMSD plots of the protein, whereas the lower panels are RMSD plots of ligands. The ligands are N3 inhibitor, leupeptin (LPT), and secondary metabolites in *N. sativa* seeds, including nigellamine A2 (NA2), nigellamine A3 (NA3), melanthigenin (MTN), nigellidine-4-O-sulfite (NGS), taraxerol (TRX), and nigellidine (NGL)

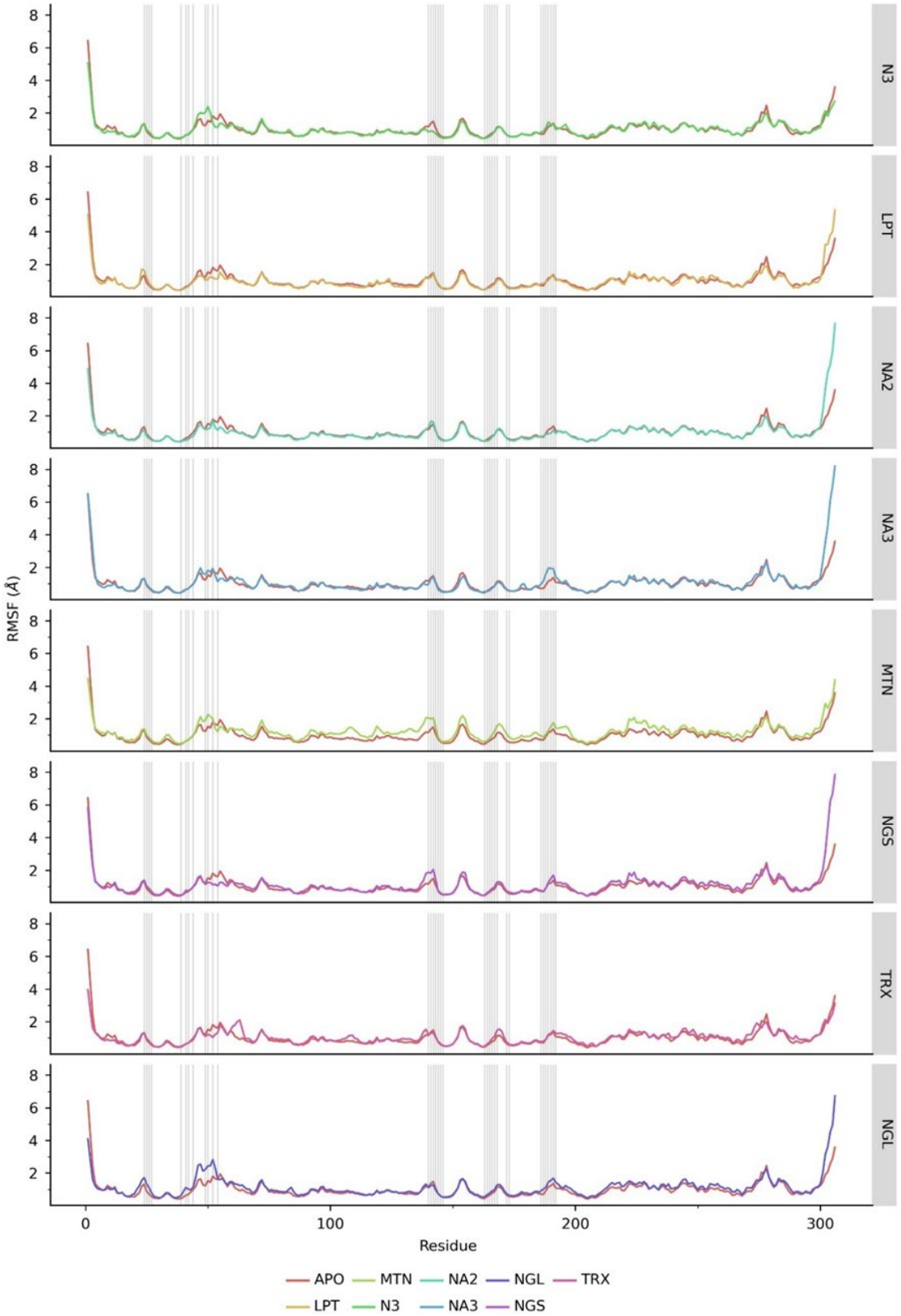


Fig S5. RMSF plots of M^{pro} in apo and ligand-bound forms. The ligands are N3 inhibitor, leupeptin (LPT), and secondary metabolites in *N. sativa* seeds, including nigellamine, A2 (NA2), and A3 (NA3)

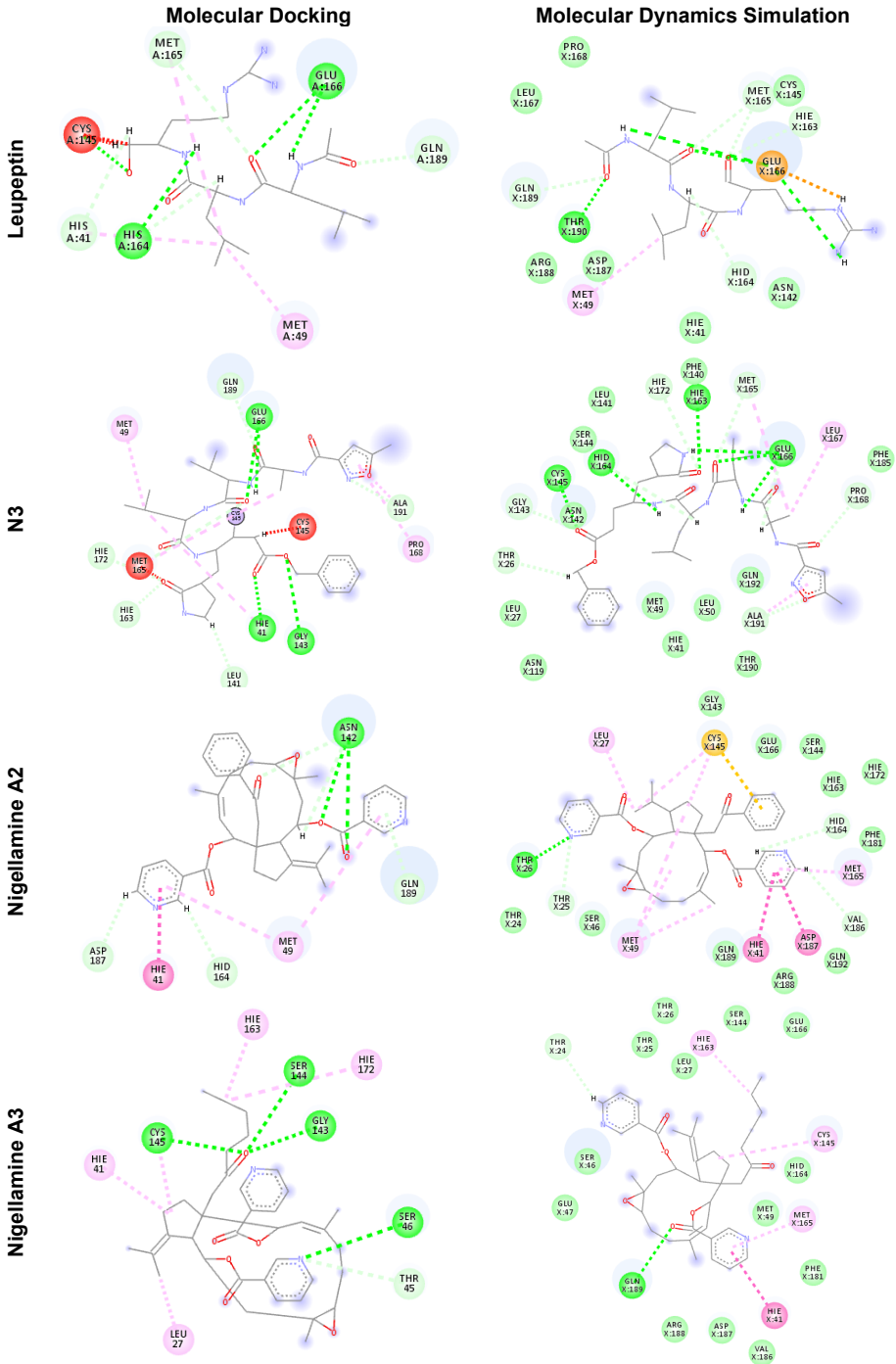


Fig S6. Non-bonded interactions between ligands and the binding site residues of M^{pro} before and after MD simulations. For MD simulations, the non-bonded interactions were extracted from the last frame of the MD trajectories

Note: Significance Test on $\Delta G°_{MMGBSA}$ Values

Below are the results of significance tests on $\Delta G°_{MMGBSA}$ values of several ligands to M^{pro}. These ligands are N3, leupeptin (LPT), nigellamine A2 (NA2), nigellamine A3 (NA3), and melanthigenin (MTN). Based on the Shapiro test,

binding energy data is normally distributed (p-value = 9.34×10^{-2}; α = 5%). Nonetheless, according to the Bartlett test, the data lack variance homogeneity (p-value = 2.33×10^{-3}). Therefore, we performed a non-parametric significance test, the Kruskal-Wallis rank-sum test.

Kruskal-Wallis rank sum test

data: deltaG by Ligand
Kruskal-Wallis chi-squared = 19.278, df = 3, p-value = 0.0002395

Since the Kruskal-Wallis rank-sum test showed the significant difference of $\Delta G°_{MMGBSA}$ values among ligands (p-value = 2.40×10^{-4}), we conducted Dunn's multiple comparison test with the Bonferroni method as the post hoc test. The result suggests that the binding energy values leupeptin, nigellamine A2, and nigellamine A3 to M^{pro} are not significantly different.

Comparison of $\Delta G°_{MMGBSA}$ by group
(Bonferroni)

Col Mean- / Row Mean	LPT	MTN	NA2
MTN	-3.194259		
	0.0042*		
NA2	0.994619	4.188879	
	0.9598	0.0001*	
NA3	-1.013746	2.180512	-2.008366
	0.9321	0.0877	0.1338

alpha = 0.05
Reject Ho if p <= alpha/2

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