In Silico and In Vitro Analyses of Multiple Terpenes Predict Cryptotanshinone as a Potent Inhibitor of the Omicron Variant of SARS-CoV-2

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Table S1 Terpenes with antiviral activities

Source	Class of compounds	Compounds	IC50/ EC50	Target site/virus	References
Stachybotrys nephrospra BCC3900	Spirodihydrobenzofuran terpene	Stachybotrydial (Mer-NF5003F) (1)	4.32 μg/ml	HSV-1	[68]
Stachybotrys species	Sesquiterpene derivative	Stachyflin (2)	0.003 μM 0.2–0.6 μM	A/WSN/33 virus H1 or H2 subtype influenza	[69]
Juniperus oxycedrus L. ssp. oxycedru	monoterpenes	α-Pinene(3) β-myrcene(4)	270μg/ml 200μg/ml	SARS-CoV HSV-1	[70]
Citrus reshni	monoterpene	Limonene (5)	5.9 μg/ml	HSV-1	[71]
Lycopus europaeus	Diterpenoid	Abietane(6)	1.39μM ($3CL^{pro}$)	SARS-CoV	[72]
Perovskia atriplicifolia	terpenoids	Norperovskatone (7) Biperovskatone(8)	0.11 mM(HBsAg)/0.18mM (HBeAg) 0.33mM(HBsAg)/0.12mM (HBeAg)	Hepatitis B Virus	[72]

Cryptomeria japonica	diterpenoids	7-β-hydroxydeoxycryptojaponol(9) cryptojaponol(10)	1.15μM Yea >10μM	SARS-CoV	[72]
Chamaecyparis obtusa var. formosana	diterpenoid (Abietane-type) Diterpenoids (Labdane - type) Sesquiterpenoids Diterpenoids	Ferruginol(11) Pinusolidic acid(12) Dehydroabieta-7-one(13) α-cadinol(14) 6,7-dehydroroyleanone(15)	1.39μM 4.71μM 4.00μM 4.44μM 5.55μM	SARS-CoV	[72]
Illicium jiadifengpi	Diterpenes Sesquiterpenes	Jiadifenoic acid J(16) Jiadifenoic acid K(17) Jiadifenoic acid L(18) Jiadifenoic acid M(19) Jiadifenoic acid N(20) Jiadifenoic acid O(21) Jiadifenoic acid P(22) Sesquicaranoic acid A(23) Sesquicaranoic acid B(24)	151.5±14.8μmol/mL 3.9±0.7μmol/mL 48.1±3.1μmol/mL 11.6±1.6μmol/mL 7.0±0.8μmol/mL 20.6±0.04μmol/mL 22.2±2.4μmol/mL 70.3±6.8μmol/mL >37.0μmol/mL	Coxsackievirus B3	[73]
Illicium majus	Diterpenoids	Majusanic acid E(25) Majusanic acid F(26)	17.4μM/mL 12.8μM/mL	Coxsackievirus B3	[74]

Juniperus formosana	Sesquiterpen 2uoids Diterpenoid	Cedrane-3β,1,2-diol(27) 3β,12-diacetoxyabieta-6,8,11.13-tetraene(28)	>10μM 1.57Mm	SARS-CoV	[72]
Salvia miltiorrhiza	Tanshinones (abietane diterpene)	Tanshinone IIA(29)	89.1 ± 5.2μM ($3CL^{pro}$) 1.6 ± 0.5μM(PL^{pro})	SARSCoV-	[75]
		Methyl tanshinonate (30)	21.1 ± 0.8μM($3CL^{pro}$) 9.2 ± 2.8μM(PL^{pro})		
		Tanshinone IIB(31)	24.8 ± 0.8μM ($3CL^{pro}$) 10.7 ± 1.7μM(PL^{pro})		
		Cryptotanshinone(32)	226.7 ± 6.2μM ($3CL^{pro}$) 0.8 ± 0.2μM(PL^{pro})		
		Rosmariquinone(33)	21.1 ± 0.8μM ($3CL^{pro}$) 30.0 ± 5.5μM(PL^{pro})		
Thymus vulgaris(essential oil)	monoterpenes	p-Cymene(34)	16μg/ml	HSV-1	[76]
Syzygium nervosum (Essential oil)	monoterpenes	α-Terpinene(35) γ-Terpinene(36)	8.5μg/ml 7μg/ml	HSV-1	[76]
	monoterpenoids	α-Terpineol(37)	22μg/ml	HSV-1	[76]

Bupleurum falcatum L	terpenoid	Saikosaponin A(38) Saikosaponin B2 (39) Saikosaponin D (40)	8.6 ± 0.3 μmol/L 1.7 ± 0.1 μmol/L 13.2±0.3 μmol/L	HCoV-229E	[77]
Triterygium regelii	Quinone-methide triterpenes	Celastrol(41) Pristimerin(42) Tingenone(43) Iguesterin(44)	0.3 μM 5.5 μM 9.9 μM 2.6 μM	SARS-CoV ($3CL^{pro}$)	[78]
Eucalyptus globulus Labill	Triterpenes	Tereticornate A (45)	0.96 μg/mL	HSV-1	[79]
Brucea javanica		β-Caryophyllene (46)	8 ± 3.4 μM 11 ± 4.9 μM	HSV-1 DENV	[80]
Juniperus formosana	Triterpenoids	Betulonic acid(47)	0.63 μM	SARS-CoV	[72]
Betula pubescens	Pentacyclic triterpenoid	Betulinic acid(48)	10μM($3CL^{pro}$)	SARS-CoV	[72]
Glycyrrhiza uralensis	triterpene glycoside	Glycyrrhizin(49)	365 μM	SARS-CoV	[81]
Ganoderma pfeifferi	triterpenes	Lucialdehyde B (50)	0.075μg/ml	HSV	[82]

Table S2 PubChem ID and binding affinity of terpenes with S1-RBD of SARS CoV-2 Omicron variant.

S.N	**Compounds**	**PubChem ID**	**Binding Affinities (kcal/mol)**
1.	Stachybotrydial	3035835	-7.2
2	Stachyflin	493326	-8.0

3	α-Pinene	6654	-5.4
4	β-myrcene	31253	-5.4
5	limonene	22311	-5.6
6	Abietane	6857485	-7.3
7	Norperovskatone	-	-6.8
8	Biperovskatone	-	-8.8
9	7-β-hydroxydeoxycryptojaponol	14827260	-7.1
10	cryptojaponol	11724205	-6.8
11	Ferruginol	442027	-7.2
12	Pinusolidic acid	25880646	-7.3
13	Dehydroabieta-7-one		-7.5
14	α-cadinol	6431302	-8.7
15	6,7-dehydroroyleanone	2751794	-7.3
16	Jiadifenoic acid J	102369038	-7.1
17	Jiadifenoic acid K	102369039	-7.8
18	Jiadifenoic acid L	102369040	-7.4
19	Jiadifenoic acid M	-	-7.3
20	Jiadifenoic acid N	90468202	-7.3
21	Jiadifenoic acid O	102369041	-7.1
22	Jiadifenoic acid P	90468203	-7.1
23	Sesquicaranoic acid A	102369042	-6.7
24	Sesquicaranoic acid B	102369043	-6.5
25	Majusanic acid E	72705062	-7.2
26	Majusanic acid F	72705063	-7.3
27	Cedrane-3β,1,2-diol	44427462	-6.7
28	3β,12-diacetoxyabieta-6,8,11.13-tetraene	-	-7.4

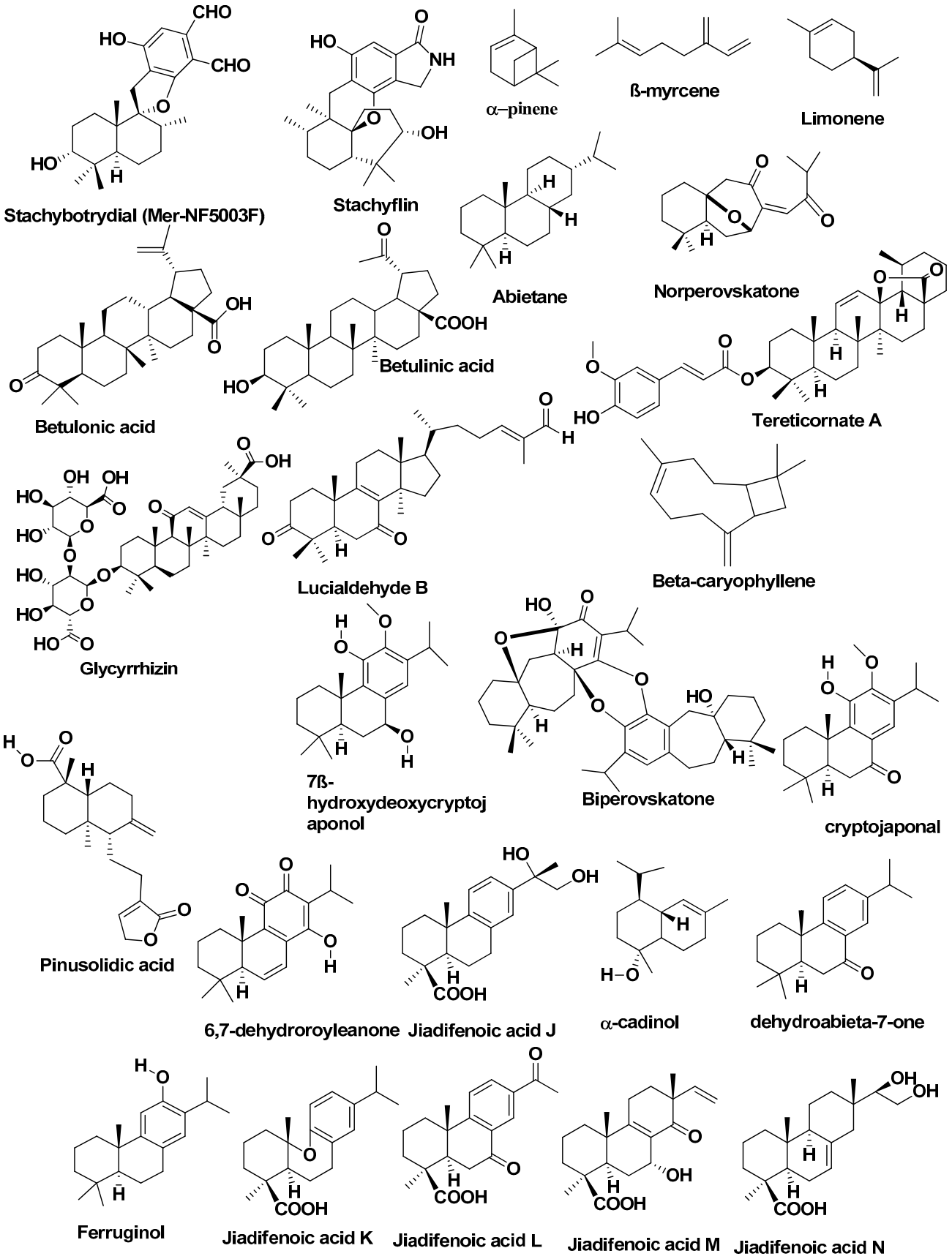
29	Tanshinone IIA	164676	-7.6
30	Methyl tanshinonate	14610613	-6.8
31	Tanshinone IIB	9926694	-7.4
32	Cryptotanshinone	160254	-7.6
33	Rosmariquinone	160142	-7.2
34	p-Cymene	7463	-5.6
35	α-Terpinene	7462	-5.6
36	γ-Terpinene	7461	-5.5
37	α-Terpineol	17100	-5.3
38	Saikosaponin A	167928	-8.7
39	Saikosaponin B2	21637642	-9.3
40	Saikosaponin D	107793	-9.1
41	Celastrol	122724	-7.5
42	Pristimerin	159516	-7.3
43	Tingenone	101520	-8.1
44	Iguesterin	46881919	-7.7
45	Tereticornate A	129316505	-9.2
46	Beta-caryophyllene	20831623	-5.9
47	Betulonic acid	122844	-7.4
48	Betulinic acid	64971	-7.2
49	Glycyrrhizin	14982	-9.1
50	Lucialdehyde B	10343868	-7.3

Table S3 Drug-like properties of cryptotanshinone and luteolin.

S.N.	Compounds	TPSA (Å^2)	Log P	Lipinski Rule of Five
1	Cryptotanshinone	43.37	3.43	Yes; 0 violation
2	Luteolin	111.13	1.86	Yes; 0 violation

Table S4 ADMET profiles of cryptotanshinone and luteolin.

S. N.	Compounds	Water Solubility (log mol/litre)	Caco-2 Permeability (log Papp 10^{-6} cm/s)	Intestinal absorption (%absorbed)	BBB Permeation (log BB)	CYP Inhibition	Total Clearance (log ml/min/kg)	Ames Toxicity	Hepatotoxicity	Toxicity class
1	Cryptotanshinone	-3.79 (moderately soluble)	1.327	97.189	0.284	Yes (CYP1A2, CYP2C19, CYP3A4)	0.845	No	No	VI
2	Luteolin	-3.71 (soluble)	0.096	81.13	-0.907	Yes (CYP1A2, CYP2C9)	0.495	No	No	V


Stachybotrydial (Mer-NF5003F)
Stachyflin
α–pinene
ß-myrcene
Limonene
Abietane
Norperovskatone
Betulonic acid
Betulinic acid
Tereticornate A
Lucialdehyde B
Beta-caryophyllene
Glycyrrhizin
7ß-hydroxydeoxycryptojaponol
Biperovskatone
cryptojaponal
Pinusolidic acid
6,7-dehydroroyleanone
Jiadifenoic acid J
α-cadinol
dehydroabieta-7-one
Ferruginol
Jiadifenoic acid K
Jiadifenoic acid L
Jiadifenoic acid M
Jiadifenoic acid N

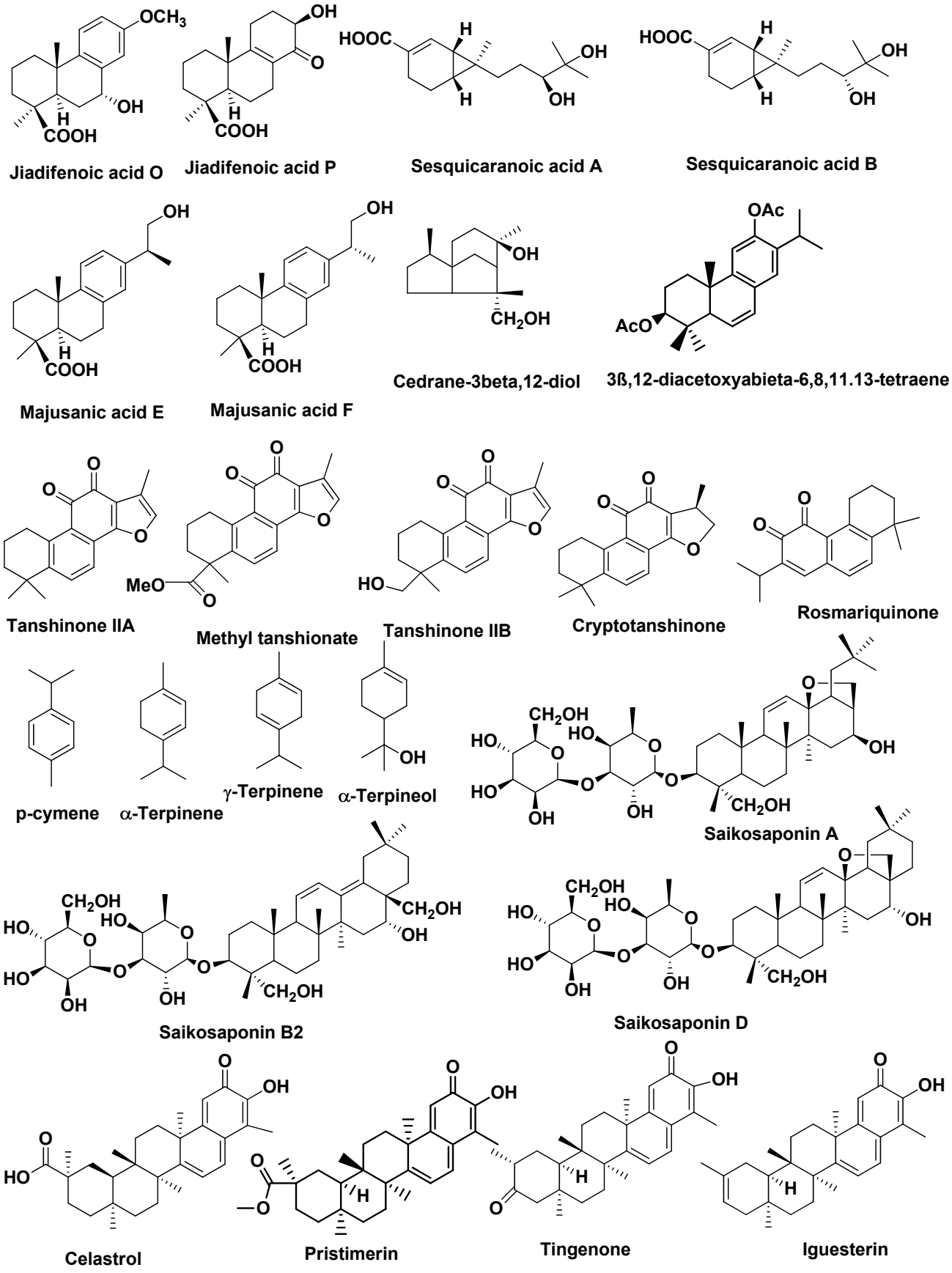


Figure S1: Structures of selected terpenes.

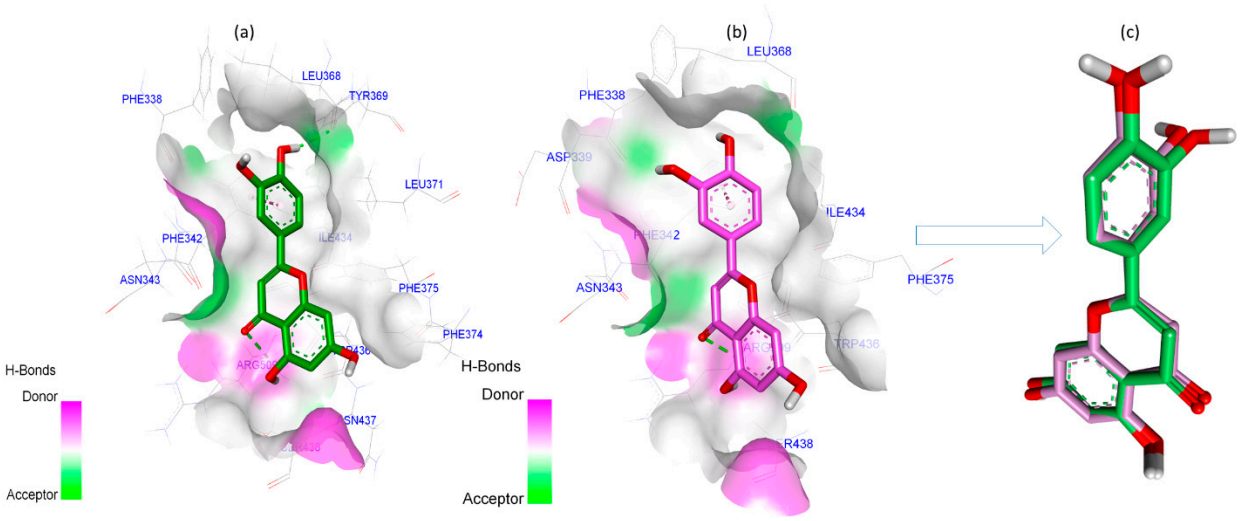


Figure S2: Validation of docking protocol. (a) First docked luteolin (green) (b) second docked luteolin (magenta) and (c) superimposition of two docked luteolin (RMSD = 0.621 Å)

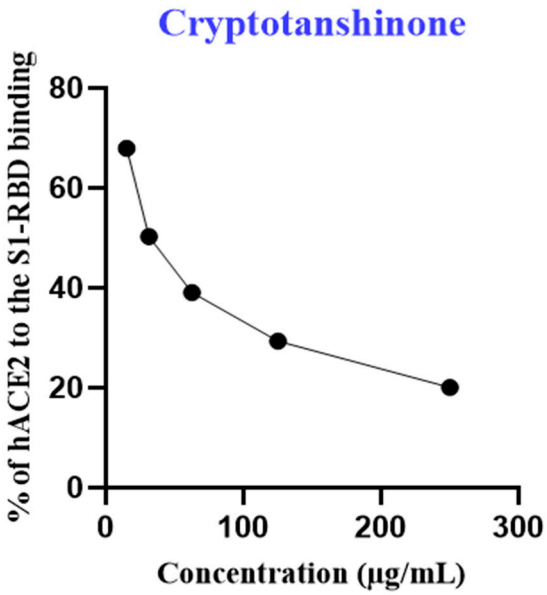


Figure S3: Binding curve of hACE2 receptor to S1-RBD of SARS-CoV-2 Omicron variant in the presence of different concentration of cryptotanshinone as determined by ELISA.

References

68. Sawadjoon, S.; Kittakoop, P.; Isaka, M.; Kirtikara, K.; Madla, S.; Thebtaranonth, Y. Antiviral and Antiplasmodial Spirodihydrobenzofuran Terpenes from the Fungus Stachybotrys nephrospora. *Planta Med* **2004**, *70*, 1085–1087, doi:10.1055/s-2004-832652.
69. Yoshimoto, J.; Kakui, M.; Iwasaki, H.; Fujiwara, T.; Sugimoto, H.; Hattori, N. Identification of a Novel HA Conformational Change Inhibitor of Human Influenza Virus. *Arch. Virol.* **1999**, *144*, 865–878, doi:10.1007/s007050050552.
70. Phytochemical Analysis and in Vitro Antiviral Activities of the Essential Oils of Seven Lebanon Species - Loizzo - 2008 - Chemistry & Biodiversity - Wiley Online Library Available online: https://onlinelibrary.wiley.com/doi/10.1002/cbdv.200890045 (accessed on 15 January 2024).

71. Nagoor Meeran, M.F.; Seenipandi, A.; Javed, H.; Sharma, C.; Hashiesh, H.M.; Goyal, S.N.; Jha, N.K.; Ojha, S. Can Limonene Be a Possible Candidate for Evaluation as an Agent or Adjuvant against Infection, Immunity, and Inflammation in COVID-19? *Heliyon* **2021**, *7*, e05703, doi:10.1016/j.heliyon.2020.e05703.
72. Wen, C.-C.; Kuo, Y.-H.; Jan, J.-T.; Liang, P.-H.; Wang, S.-Y.; Liu, H.-G.; Lee, C.-K.; Chang, S.-T.; Kuo, C.-J.; Lee, S.-S.; et al. Specific Plant Terpenoids and Lignoids Possess Potent Antiviral Activities against Severe Acute Respiratory Syndrome Coronavirus. *J. Med. Chem.* **2007**, *50*, 4087–4095, doi:10.1021/jm070295s.
73. Zhang, G.-J.; Li, Y.-H.; Jiang, J.-D.; Yu, S.-S.; Wang, X.-J.; Zhuang, P.-Y.; Zhang, Y.; Qu, J.; Ma, S.-G.; Li, Y.; et al. Diterpenes and Sesquiterpenes with Anti-Coxsackie Virus B3 Activity from the Stems of Illicium Jiadifengpi. *Tetrahedron* **2014**, *70*, 4494–4499, doi:10.1016/j.tet.2014.05.006.
74. Wang, Y.-D.; Zhang, G.-J.; Qu, J.; Li, Y.-H.; Jiang, J.-D.; Liu, Y.-B.; Ma, S.-G.; Li, Y.; Lv, H.-N.; Yu, S.-S. Diterpenoids and Sesquiterpenoids from the Roots of Illicium Majus. *J. Nat. Prod.* **2013**, *76*, 1976–1983, doi:10.1021/np400638r.
75. Park, J.-Y.; Kim, J.H.; Kim, Y.M.; Jeong, H.J.; Kim, D.W.; Park, K.H.; Kwon, H.-J.; Park, S.-J.; Lee, W.S.; Ryu, Y.B. Tanshinones as Selective and Slow-Binding Inhibitors for SARS-CoV Cysteine Proteases. *Bioorganic & Medicinal Chemistry* **2012**, *20*, 5928–5935, doi:10.1016/j.bmc.2012.07.038.
76. Astani, A.; Reichling, J.; Schnitzler, P. Comparative Study on the Antiviral Activity of Selected Monoterpenes Derived from Essential Oils. *Phytotherapy Research* **2010**, *24*, 673–679, doi:10.1002/ptr.2955.
77. Cheng, P.-W.; Ng, L.-T.; Chiang, L.-C.; Lin, C.-C. Antiviral Effects of Saikosaponins on Human Coronavirus 229e in Vitro. *Clinical and Experimental Pharmacology and Physiology* **2006**, *33*, 612–616, doi:https://doi.org/10.1111/j.1440-1681.2006.04415.x.
78. Ryu, Y.B.; Park, S.-J.; Kim, Y.M.; Lee, J.-Y.; Seo, W.D.; Chang, J.S.; Park, K.H.; Rho, M.-C.; Lee, W.S. SARS-CoV 3CLpro Inhibitory Effects of Quinone-Methide Triterpenes from Tripterygium Regelii. *Bioorganic & Medicinal Chemistry Letters* **2010**, *20*, 1873–1876, doi:10.1016/j.bmcl.2010.01.152.
79. Viruses | Free Full-Text | Anti-Infectivity against Herpes Simplex Virus and Selected Microbes and Anti-Inflammatory Activities of Compounds Isolated from Eucalyptus Globulus Labill. Available online: https://www.mdpi.com/1999-4915/10/7/360 (accessed on 15 January 2024).
80. Evaluation of in Vitro Antiviral Activity of Essential Oil Compounds Against Dengue Virus | Pharmacognosy Journal Available online: https://phcogj.com/article/470 (accessed on 15 January 2024).
81. Hoever, G.; Baltina, L.; Michaelis, M.; Kondratenko, R.; Baltina, L.; Tolstikov, G.A.; Doerr, H.W.; Cinatl, J. Antiviral Activity of Glycyrrhizic Acid Derivatives against SARS−Coronavirus. *J. Med. Chem.* **2005**, *48*, 1256–1259, doi:10.1021/jm0493008.
82. Niedermeyer, T.H.J.; Lindequist, U.; Mentel, R.; Gördes, D.; Schmidt, E.; Thurow, K.; Lalk, M. Antiviral Terpenoid Constituents of Ganoderma Pfeifferi. *J. Nat. Prod.* **2005**, *68*, 1728–1731, doi:10.1021/np0501886.