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In silico screening of some naturally occurring bioactive compounds predicts potential inhibitors against SARS-COV-2 (COVID-19) protease

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The reports pertaining to the mitigation and treatment of the COVID-19 pandemic are still lacking. Compatibility of the natural products and availability of the modern computational techniques motivated us to carry out *In Silico* investigations on some bioactive natural compounds reportedly found in the fruits and leaves of *Anthocephalus Cadamba* commonly known as Kadam or Kadamb Tree, aiming to predict the potential inhibitors against the aforesaid virus. Having modeled the ground state ligand structure of the nine natural compounds applying density functional theory at B3LYP/631+G (d, p) level, we have performed their molecular docking with SARS-COV-2 protease to calculate the binding affinity as well as to screen the binding at S-protein site during ligand-protein interactions. Out of these nine studied naturally occurring compounds; Oleanic Acid has been appeared to be a potential inhibitor for COVID-19 followed by Ursolic Acid, Iso-Vallesiachotamine, Vallesiachotamine, Cadambine, Vincosamide-N-Oxide, Isodihydroamino-cadambine, Pentyle Ester of Chlorogenic Acid and D-Myo-Inositol. Hence, these bioactive natural compounds or their structural analogs may be explored as an anti-COVID-19 drug agents. The solubility and solvent-effect related to the phytochemicals may be the point of concern. *In vivo* investigations on these proposed natural compounds or their structural analogs are invited for designing and developing the potential medicine/vaccine for the treatment of COVID-19 pandemic.

Keywords: DFT, Ligand-protein interaction, Molecular-Docking, Phytochemicals, Potential inhibitors

The severe acute respiratory syndrome coronavirus (SARS-COV-2) identified as COVID-19 in Wuhan City of China[1] spread across the earth as pandemic putting the whole world on high alert[2–5] led to 823626 total cases and 40598 deaths all over the world till 01 April 2020[6]. Available evidence indicates that this virus is transmitted through the respiratory droplets (such as coughing) and by fomites that can propagate through the air at distances of 1 M[7,8] and no evidence is available about its airborne transmission in the current study[9] which establishes the principle of maintaining the distance of more than 1 meter termed as 'social distancing'/physical distancing' along with hand-hygiene for the prevention of COVID-19. A recent study based on the mathematical modeling conducted by the Indian Council of Medical Research (ICMR) has suggested the proper quarantine and isolation as the preventive measures for stopping the outbreak of COVID-19 through community transmission[10]. It appears that in view of this, country-wide lock-down has been declared in India since the midnight of the 23rd March 2020 in continuation to pre-lock down the action of evacuating the possible places expected for people's gathering as well as the social awareness drive already started from the very beginning of February, 2020 which we welcome.

Appreciable contribution in the development of diagnostics, therapeutics, and vaccines for this novel coronavirus has been indicated[11], and based on some clinical investigations, an anti-malarial drug namely chloroquine phosphate has been reported to be having a certain curative effect on the COVID-19[12]. ICMR has also recommended an anti-malarial drug namely Hydroxy-chloroquine for those individuals who are asymptomatic healthcare workers involved in the care of suspected or confirmed cases of COVID-19 and asymptomatic household contacts of laboratory-confirmed cases[13]. The possibility of high risk-factor associated with these suggested drugs has not been ruled out which projected it to be a trial measure. No specific therapy and medicine for the treatment of COVID-19 has ever been reported to the best of our knowledge which inspired us to think on the natural products relying on the concept of particle-antiparticle theory of Nature implying that if there is a novel coronavirus then there must be its anti-virus material in Nature itself and we have obtained motivating

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results in the present research carried out with the help of available computational facility at our home during lock-down period.

We have selected total eleven bioactive natural compounds embedded spontaneously in *Anthocephalus Cadamba* which has been reported to be a miraculous tree having crucial significance in Hindu Mythology containing the largest number of phytochemicals and secondary metabolites having pharmacological and biological properties, however, the solubility and solvent-effect to be the point of concern[14]. The four bio molecules contained in the leaves of the said tree namely 7-hydroxy-6-methoxy coumarian ($C_{10}H_8O_4$), Methyl ester of chlorogenic acid ($C_{17}H_{20}O_9$), Pentyle Ester of Chlorogenic Acid ($C_{21}H_{28}O_9$), D-Myo-Inositol ($C_7H_{14}O_6$); 07 biomolecules contained in fruits of the said tree namely Oleanic Acid ($C_{30}H_{48}O_3$), Ursolic Acid ($C_{30}H_{48}O_3$), Vallesiachotamine ($C_{21}H_{22}N_2O_3$), Iso-Vallesiachotamine ($C_{21}H_{22}N_2O_3$), Cadambine ($C_{27}H_{32}N_2O_{10}$), Vincosamide-N-Oxide ($C_{26}H_{31}N_2O_9$), Isodihydroamino-cadambine ($C_{26}H_{33}N_3O_7$) already reported to be isolated and characterized in Central Drug Research Institute, Lucknow, India[15,16] and the electronic properties of the few of them have already been studied by us computationally[17-21]; were screened as ligands to interact with the targeted SARS-COV-2 protease. The first two of the aforsaid compounds responded negatively but rest nine compounds exhibited the positive results which have been presented in this paper. We expect that the present study will offer a new dimension in developing the drug/vaccine for the COVID-19.

Methodology

The *In silico* optimized ligand-structure of the aforementioned eleven bioactive natural compounds were obtained as per reported approach of density functional theory at B3LYP/631+G (d, p) level[22-23] implemented through Gaussian 09 program-package[24]. We have used main protease of SARS-COV-2 retrieved from the RCSB protein data bank having PDB ID: 6Y84[25] and PDB ID: 6LU7[26] as potential target protein for the binding of these compounds as ligands. The protein-ligand interaction has been studied by using molecular docking which has been observed to be compatible approach in the recent reports[27,28] and we have implemented molecular docking theory through Autodock 4.2 program package[29-31].The binding of these natural bioactive ligands with the PDB ID: 6CRV which is SARS Spike Glycoprotein for coronavirus SARS-COV emerged in 2002 as a highly transmissible[32] has also been obtained with the help of same docking approach in order to investigate the consistency of the performance of these compounds with the class of viral protein. The binding of these compounds with the PDB ID: 6VXX which is the SARS-COV-2 spike glycoprotein for COVID-19[33] has also been examined to illustrate the potential of these compounds to deactivate this novel coronavirus at the receptor end.

Results

The *In silico* optimized molecular structure of 09 bioactive natural compounds modeled using DFT-B3LY/6-31+G (d,p) level of theory which exhibited the property of inhibitor against the main protease of COVID-19, have been displayed in (Fig. 1). Binding affinity in each cluster rank for the complete cycle of ligand-proteins (PDB ID: 6Y84, 6LU7 and 6CRV) interactions have been depicted in (Table 1) for Oleanic Acid (molecule 1), for Ursolic Acid (molecule 2), for Iso-Vallesiachotamine (molecule 3), for Vallesiachotamine (molecule 4), for Cadambine (molecule 5), for Vincosamide-N-Oxide (molecule 6), for Isodihydroamino-cadambine (molecule 7), for Pentyle Ester of Chlorogenic Acid (molecule 8) and for D-Myo-Inositol (molecule 9). The significant binding of these natural compounds as ligand with the said protease results in the conformational changes in the target protein 6Y84, 6LU7 and 6CRV as displayed in (Fig. 2A) for molecule 1, (Fig. 2B) for molecule 2, (Fig. 2C) for molecule 3 (Fig. 2D) for molecule 4, (Fig. 2E) for molecule 5, (Fig. 2F) for molecule 6, (Fig. 2G) for molecule 7, (Fig. 2H) molecule 8 and (Fig. 2I) for molecule 9.

The significantly negative value of free energy of binding of these molecules as a ligand with SARS-COV-2 spike glycoprotein for COVID-19 (PDB ID: 6VXX) in ligand-protein interaction has also been obtained and the results of best five have been presented in (Table 2).

The significantly negative value of free energy of binding of these molecules with PDB ID: 6VXX (SARS-COV-2 spike glycoprotein for COVID-19) in ligand-protein interaction as depicted in (Table 2) have been obtained and corresponding conformational changes occurred in the target protein are presented in (Figs. 3 & 4).

Similar types of significant binding affinity have been obtained in all the SARS-COV-2 spike glycoprotein –ligand (studied compounds) interactions

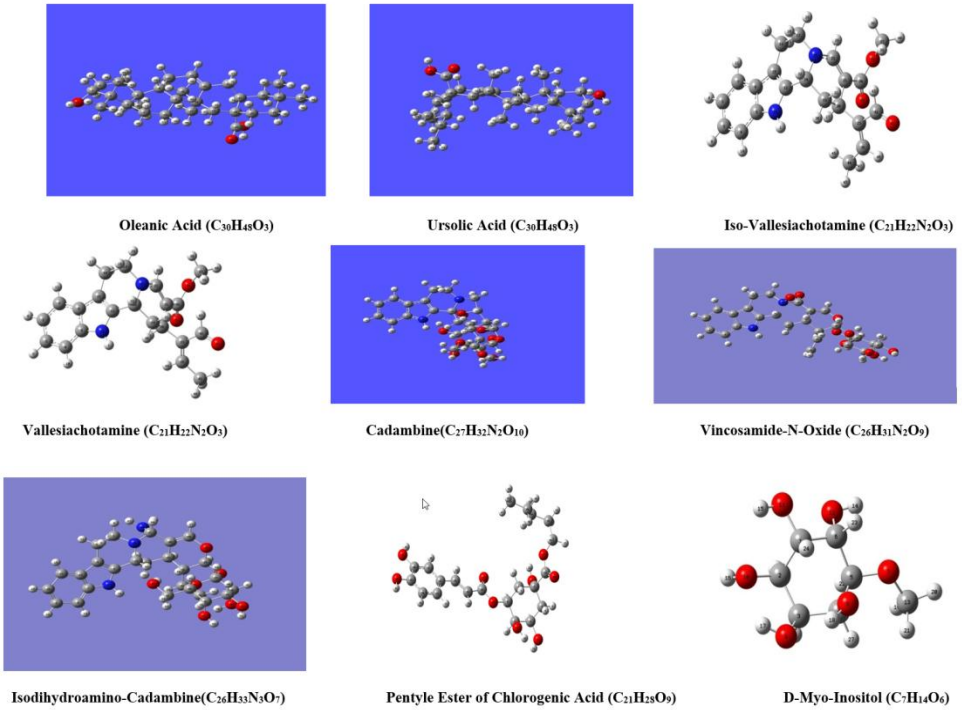
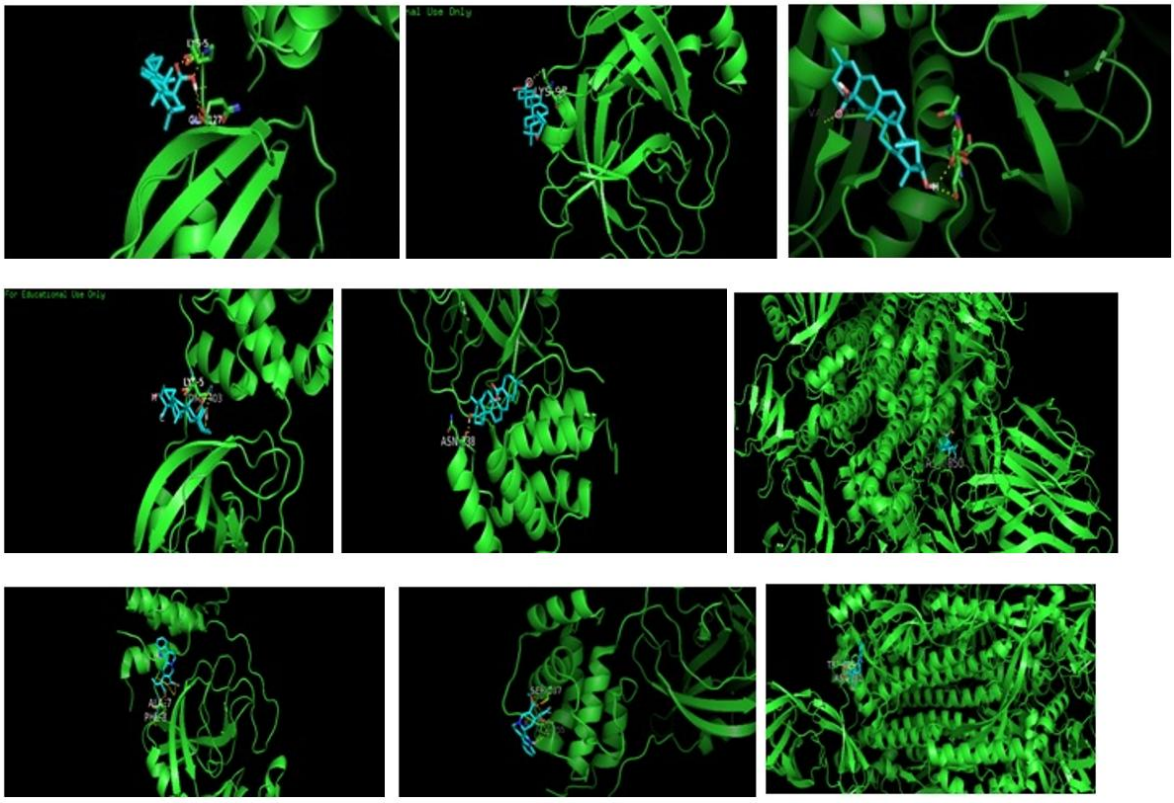


Fig. 1 — The optimized molecular structure of studied Bioactive Natural Compounds *reported to be found in fruits (molecule no 1 to 7) and leaves (molecule no 8 to 9) of *Anthocephalus cadamba*[14-16]. Blue balls: N-atoms, black balls: C-atoms, white balls: H-atoms, red balls: O-atoms



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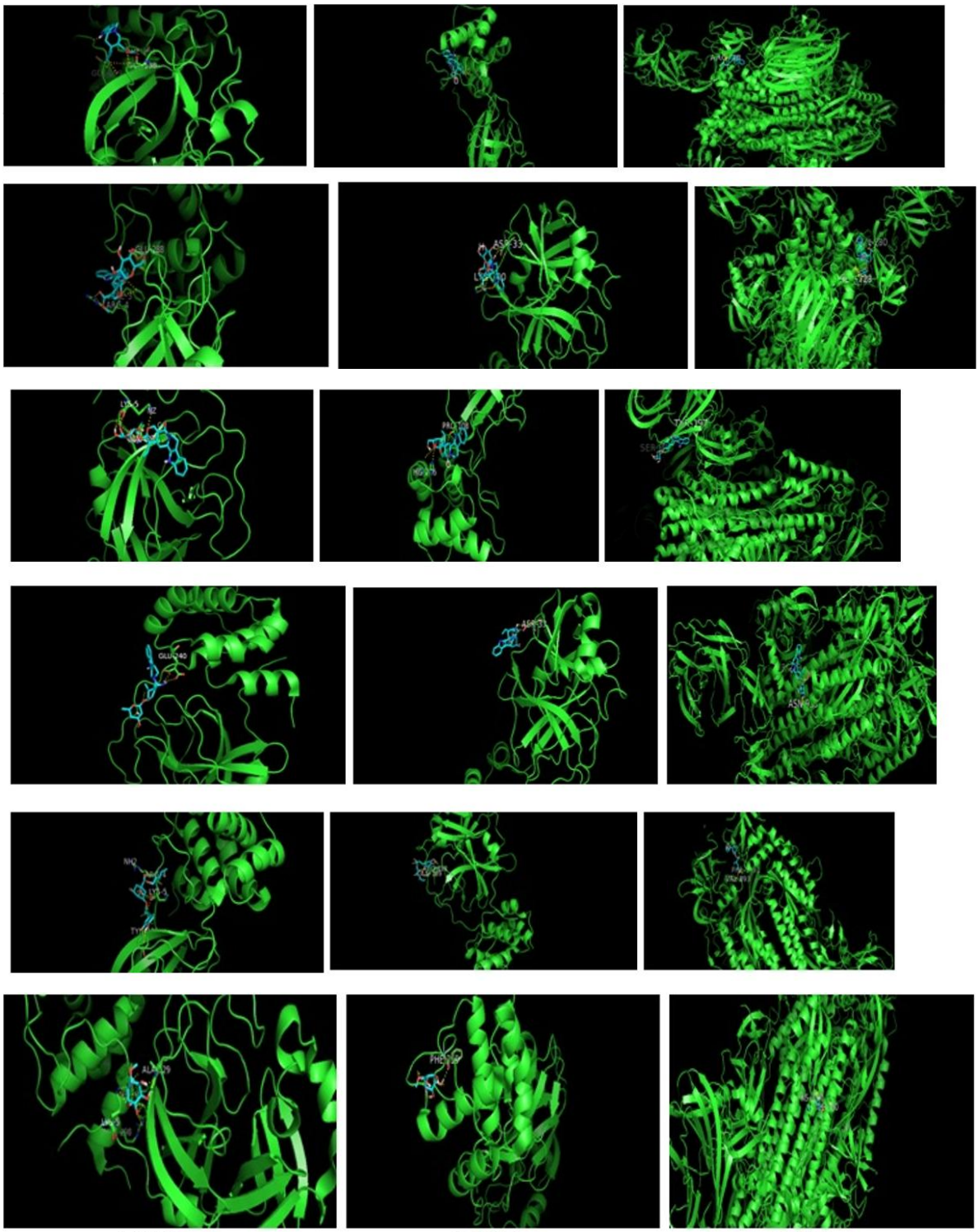


Fig. 2 — Conformational changes observed due to the binding of (A) ligand-1 (Oleanic Acid); (B) ligand-2 (Ursolic Acid); (C) ligand-3 (Iso-Vallesiachotamine); (D) ligand-4 (Vallesiachotamine); (E) ligand-5 (Cadambine); (F) ligand-6 (Vincosamide-N-Oxide); (G) ligand-7 (Isodihydroamino-cadambine); (H) ligand-8 (Pentyle Ester of Chlorogenic Acid); (I) ligand-9 (D-Myo-Inositol) with the COVID-19 protease and S-protein receptor

Table 1 — Binding Affinity of natural compounds with the target proteins

Name of Natural Compound	Cluster Rank	SARS-COV-2 protease (PDB ID: 6Y84)		SARS-COV-2 protease (PDB ID: 6LU7)		SARS-COV Spike Glycoprotein (PDB ID: 6CRV)	
		Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant
	1	−11.28 kcal/mol	5.38 nM	−6.11 kcal/mol	33.45 μM	−5.90 kcal/mol	47.30 μM
	2	−9.78 kcal/mol	67.90 nM	−5.83 kcal/mol	52.86 μM	−5.73 kcal/mol	63.60 μM
	3	−9.60 kcal/mol	91.21 nM	−5.70 kcal/mol	66.07 μM	−5.41 kcal/mol	107.78 μM
	4	−9.10 kcal/mol	213.24 nM	−5.59 kcal/mol	79.53 μM	−4.91 kcal/mol	249.99 μM
Oleanic	5	---	---	−5.57 kcal/mol	82.47 μM	−4.39 kcal/mol	607.04 μM
Acid	6	---	---	−5.44 kcal/mol	102.54 μM	−4.28 kcal/mol	723.95 μM
($C_{30}H_{48}O_3$)	7	---	---	−5.08 kcal/mol	189.25 μM	−4.25 kcal/mol	768.82 μM
	8	---	---	−4.89 kcal/mol	258.48 μM	---	---
	9	---	---	-4.77 kcal/mol	318.64 μM	---	---
	1	−10.94 kcal/mol	9.63 nM	−6.40 kcal/mol	20.38 μM	−6.96 kcal/mol	7.88 μM
	2	−10.30 kcal/mol	28.35 nM	−6.34 kcal/mol	22.70 μM	−6.81 kcal/mol	10.26 μM
	3	−9.74 kcal/mol	72.73 nM	−6.00 kcal/mol	40.32 μM	−6.76 kcal/mol	11.17 μM
Ursolic	4	−9.72 kcal/mol	74.54 nM	−5.89 kcal/mol	47.97 μM	−6.61 kcal/mol	14.24 μM
Acid	5	−9.36 kcal/mol	137.19 nM	−5.34 kcal/mol	122.36 μM	−6.30 kcal/mol	24.24 μM
($C_{30}H_{48}O_3$)	6	−8.90 kcal/mol	299.83 nM	−4.96 kcal/mol	232.96 μM	−6.19 kcal/mol	29.17 μM
	7	---	---	---	---	−6.14 kcal/mol	31.59 μM
	8	---	---	---	---	−6.08 kcal/mol	34.90 μM
	9	---	---	---	---	−5.91 kcal/mol	46.80 μM
	10	---	---	---	---	−5.86 kcal/mol	50.93 μM
	1	−9.55 kcal/mol	99.42 nM	−6.00 kcal/mol	39.82 μM	−5.16 kcal/mol	165.89 μM
	2	−7.93 kcal/mol	1.53 μM	−5.71 kcal/mol	65.58 μM	−5.00 kcal/mol	214.52 μM
	3	−7.63 kcal/mol	2.54 μM	−5.70 kcal/mol	66.53 μM	−4.94 kcal/mol	237.57 μM
	4	−7.61 kcal/mol	2.63 μM	−5.35 kcal/mol	20.56 μM	−4.81 kcal/mol	296.22 μM
Iso-	5	−7.45 kcal/mol	3.43 μM	−5.11 kcal/mol	178.30 μM	−4.75 kcal/mol	332.16 μM
Vallesiachotamine	6	−6.51 kcal/mol	16.94 μM	−4.86 kcal/mol	275.30 μM	−4.70 kcal/mol	357.55 μM
($C_{21}H_{22}N_2O_3$)	7	---	---	---	---	−4.68 kcal/mol	370.48 μM
	8	---	---	---	---	−4.45 kcal/mol	548.28 μM
	9	---	---	---	---	−4.36 kcal/mol	640.89 μM
	10	---	---	---	---	−4.03 kcal/mol	1.11 mM
	1	−9.50 kcal/mol	109.66 nM	−4.98 kcal/mol	224.33 μM	−6.35 kcal/mol	22.14 μM
	2	−9.32 kcal/mol	148.51 nM	−4.80 kcal/mol	302.86 μM	−6.04 kcal/mol	37.63 μM
	3	−8.77 kcal/mol	371.00 nM	−4.43 kcal/mol	564.68 μM	−5.52 kcal/mol	89.42 μM
	4	−8.33 kcal/mol	784.52 nM	−4.31 kcal/mol	693.59 μM	−5.05 kcal/mol	197.72 μM
Vallesiachotamine	5	−6.91 kcal/mol	8.64 μM	−4.30 kcal/mol	699.66 μM	−4.88 kcal/mol	262.69 μM
($C_{21}H_{22}N_2O_3$)	6	−6.87 kcal/mol	9.28 μM	−4.28 kcal/mol	723.92 μM	−4.77 kcal/mol	320.94 μM
	7	−6.62 kcal/mol	13.94 μM	−3.94 kcal/mol	1.30 mM	−4.36 kcal/mol	633.00 μM
	8	---	---	−3.73 kcal/mol	1.84 mM	−4.25 kcal/mol	769.77 μM
	9	---	---	−2.84 kcal/mol	8.22 mM	−4.02 kcal/mol	1.12 mM
	10	---	---	---	---	−3.90 kcal/mol	1.39 mM
	1	−8.85 kcal/mol	323.97 nM	−6.93 kcal/mol	13.86 μM	−5.35 kcal/mol	118.85 μM
	2	−7.57 kcal/mol	2.81 μM	−4.80 kcal/mol	301.24 μM	−4.83 kcal/mol	287.35 μM
	3	−7.46 kcal/mol	3.39 μM	−4.44 kcal/mol	553.34 μM	−4.65 kcal/mol	391.37 μM
	4	−7.18 kcal/mol	5.45 μM	−4.26 kcal/mol	756.51 μM	−4.56 kcal/mol	451.55 μM
Cadambine	5	−6.79 kcal/mol	10.57 μM	−4.24 kcal/mol	779.20 μM	−4.33 kcal/mol	673.01 μM
($C_{27}H_{32}N_2O_{10}$)	6	−6.22 kcal/mol	27.54 μM	−4.05 kcal/mol	1.07 mM	−4.24 kcal/mol	783.98 μM
	7	−6.15 kcal/mol	30.85 μM	−4.03 kcal/mol	1.11 mM	−3.78 kcal/mol	1.71 mM
	8	−5.85 kcal/mol	51.89 μM	−3.72 kcal/mol	1.88 mM	−3.50 kcal/mol	2.70 mM
	9	−5.72 kcal/mol	64.44 μM	−3.33 kcal/mol	3.60 mM	−3.06 kcal/mol	5.75 mM
	10	−5.45 kcal/mol	100.34 μM	−3.31 kcal/mol	3.77 mM	−2.88 kcal/mol	7.78 mM

(*Contd*.)

Table 1 — Binding Affinity of natural compounds with the target proteins (*Contd*.)

Name of Natural Compound	Cluster Rank	SARS-COV-2 protease (PDB ID: 6Y84)		SARS-COV-2 protease (PDB ID: 6LU7)		SARS-COV Spike Glycoprotein (PDB ID: 6CRV)	
		Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant	Free Energy of Binding	Inhibition Constant
	1	−8.81 kcal/mol	348.65 nM	−5.81 kcal/mol	55.52 μM	−4.59 kcal/mol	434.05 μM
	2	−8.77 kcal/mol	370.30 nM	−4.78 kcal/mol	312.98 μM	−4.47 kcal/mol	532.26 μM
	3	−8.41 kcal/mol	686.48 nM	−4.49 kcal/mol	511.38 μM	−4.28 kcal/mol	730.11 μM
	4	−8.08 kcal/mol	1.19 μM	−3.93 kcal/mol	1.31 mM	−4.05 kcal/mol	1.07 mM
Vincosamide-	5	−7.92 kcal/mol	1.58 μM	−3.89 kcal/mol	1.40 mM	−4.00 kcal/mol	1.17 mM
N-Oxide	6	−7.70 kcal/mol	2.26 μM	−3.75 kcal/mol	1.79 mM	−3.49 kcal/mol	2.78M
($C_{26}H_{31}N_2O_9$)	7	−7.22 kcal/mol	5.08 μM	−3.48 kcal/mol	2.83 mM	−3.44 kcal/mol	3.02 mM
	8	−6.26 kcal/mol	25.76 μM	−3.41 kcal/mol	3.15 mM	−3.38 kcal/mol	3.31 mM
	9	−5.08 kcal/mol	187.55 μM	−3.33 kcal/mol	3.64 mM	−3.29 kcal/mol	3.91 mM
	10	---------	---------	−3.03 kcal/mol	5.96 mM	−3.04 kcal/mol	5.93 mM
	1	−8.57 kcal/mol	520.33 nM	−4.85 kcal/mol	277.88 μM	−4.10 kcal/mol	922.19 μM
	2	−7.88 kcal/mol	1.67 μM	−4.10 kcal/mol	990.69 μM	−3.90 kcal/mol	1.39 mM
	3	−6.49 kcal/mol	17.43 μM	−4.08 kcal/mol	1.02 mM	−3.53 kcal/mol	2.60 mM
	4	−5.27 kcal/mol	137.23 μM	−3.09 kcal/mol	5.42 mM	−3.44 kcal/mol	2.99 mM
Isodihydroamino-	5	−5.11 kcal/mol	181.03 μM	−2.92 kcal/mol	7.26 mM	−3.31 kcal/mol	3.73 mM
cadambine	6	−4.76 kcal/mol	324.79 μM	−2.58 kcal/mol	12.88 mM	−3.00 kcal/mol	6.28 mM
($C_{26}H_{33}N_3O_7$)	7	−4.21 kcal/mol	816.29 μM	−2.53 kcal/mol	14.04 mM	−2.97 kcal/mol	6.63 mM
	8	−3.90 kcal/mol	1.39 mM	−2.51 kcal/mol	14.54 mM	−2.88 kcal/mol	7.71 mM
	9	---------	---------	−1.04 kcal/mol	172.81 mM	−2.84 kcal/mol	8.27 mM
	10	---------	---------	---------	---------	−2.69 kcal/mol	10.75 mM
	1	−5.61 kcal/mol	77.71 μM	−1.95 kcal/mol	37.57 mM	−2.35 kcal/mol	19.10 mM
	2	−5.10 kcal/mol	182.54 μM	−1.52 kcal/mol	76.24 mM	−1.77 kcal/mol	50.49 mM
	3	−5.06 kcal/mol	194.58 μM	−1.46 kcal/mol	85.21 mM	−1.66 kcal/mol	60.43 mM
	4	−5.05 kcal/mol	198.85 μM	−1.03 kcal/mol	176.88 mM	−1.64 kcal/mol	62.51 mM
Chlorogenic acid	5	−4.18 kcal/mol	869.07 μM	−0.86 kcal/mol	233.66 mM	−1.37 kcal/mol	99.72 mM
($C_{21}H_{28}O_9$)	6	−3.40 kcal/mol	3.21 mM	−0.61 kcal/mol	356.69 mM	−1.23 kcal/mol	126.32 mM
	7	−3.30 kcal/mol	3.80 mM	−0.42 kcal/mol	490.95 mM	−1.03 kcal/mol	176.85 mM
	8	−3.15 kcal/mol	4.87 mM	−0.37 kcal/mol	531.76 mM	−0.20 kcal/mol	713.11 mM
	9	−3.03 kcal/mol	6.04 mM	−0.06 kcal/mol	903.01 mM	−0.10 kcal/mol	840.36 mM
	10	−2.86 kcal/mol	8.01 mM	+0.05 kcal/mol	----------	−0.00 kcal/mol	991.66 mM
	1	−5.51 kcal/mol	90.67 μM	−2.22 kcal/mol	23.63 mM	−3.11 kcal/mol	5.22 mM
	2	−5.06 kcal/mol	195.69 μM	−2.05 kcal/mol	31.17 mM	−2.77 kcal/mol	9.34 mM
	3	−4.30 kcal/mol	702.05 μM	−1.86 kcal/mol	43.37 mM	−2.64 kcal/mol	11.57 mM
	4	−3.56 kcal/mol	2.47 μM	−1.74 kcal/mol	53.02 mM	−2.53 kcal/mol	13.88 mM
D-Myo-Inositol	5	−3.04 kcal/mol	5.86 mM	−1.72 kcal/mol	54.66 mM	−2.32 kcal/mol	20.09 mM
($C_7H_{14}O_6$)	6	−3.00 kcal/mol	6.30 mM	−1.53 kcal/mol	75.47 mM	−2.30 kcal/mol	20.46 mM
	7	−2.93 kcal/mol	7.09 mM	−1.38 kcal/mol	98.07 mM	−2.11 kcal/mol	28.50 mM
	8	---------	---------	−1.10 kcal/mol	154.91 mM	−1.98 kcal/mol	35.50 mM
	9	---------	---------	−1.07 kcal/mol	164.03 mM	−1.94 kcal/mol	37.54 mM
	10	---------	---------	---------	---------	−1.56 kcal/mol	71.41 mM

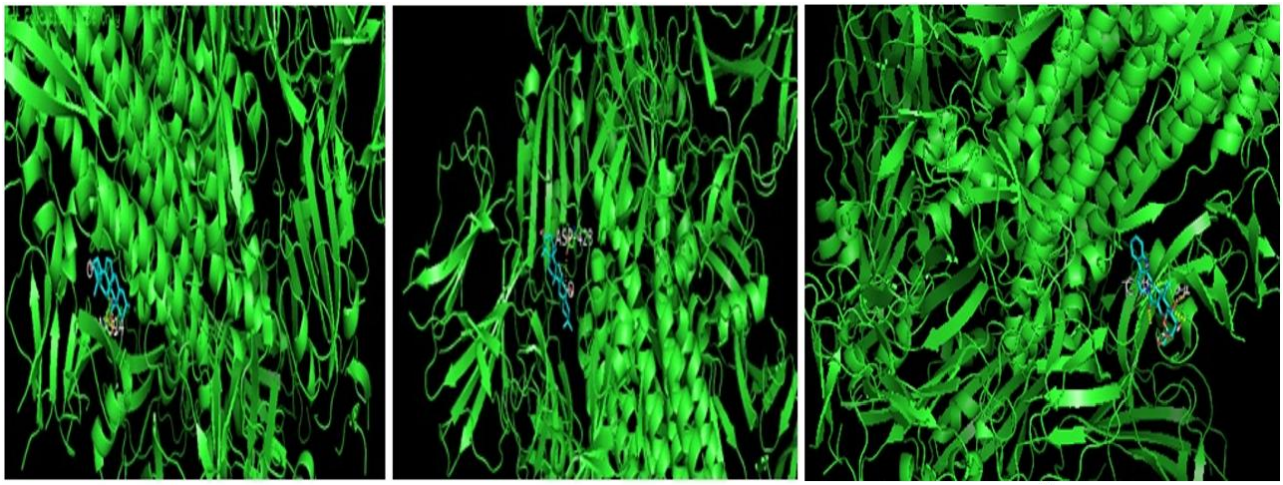
Table 2 — Binding affinity of a studied molecule with SARS-COV-2 spike glycoprotein

Cluster Rank►	1	2	3	4	5	6	7	8	9	10
Ligand-Protein Interaction▼	B.E. I.C.	B.E. I.C.	B.E. I.C.	B.E. I.C.	B.E. I.C.	B.E. I.C.	B.E. I.C.	. B.E. I.C.	B.E. I.C.	. B.E. I.C.
Oleanic Acid ($C_{30}H_{48}O_3$) -6VXX	−6.76 kcal/mol 11.05 μM	−6.54 kcal/mol 16.17 μM	−6.41 kcal/mol 19.85 μM	−6.41 kcal/mol 19.90 μM	−6.12 kcal/mol 32.71 μM	−6.04 kcal/mol 37.45 μM	−5.80 kcal/mol 56.45 μM	−5.70 kcal/mol 66.58 μM	−5.45 kcal/mol 101.12 μM	--------- ---------

(*Contd*.)

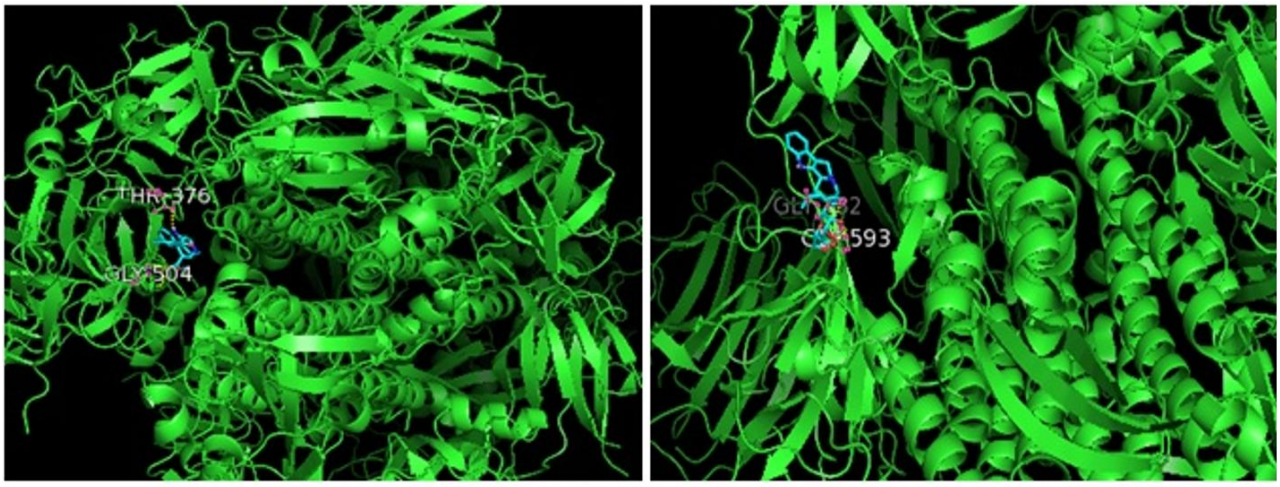
Table 2 — Binding affinity of a studied molecule with SARS-COV-2 spike glycoprotein

Cluster Rank►	1	2	3	4	5	6	7	8	9	10
Ursolic Acid ($C_{30}H_{48}O_3$) -6VXX	−7.15 kcal/mol 5.73 μM	−7.07 kcal/mol 6.53 μM	−6.74 kcal/mol 11.56 μM	−6.72 kcal/mol 11.78 μM	−6.48 kcal/mol 17.64 μM	−6.39 kcal/mol 20.63 μM	−6.29 kcal/mo 24.67 μM	−6.22 kcal/mol 27.76 μM	−6.20 kcal/mol 28.40 μM	−5.81 kcal/mol 55.41 μM
VincosamieN-Oxide ($C_{26}H_{31}N_2O_9$) -6VXX	−4.63 kcal/mol 400.48 μM	−3.96 kcal/mol 1.25 mM	−3.91 kcal/mol 1.36 mM	−3.90 kcal/mol 1.39 mM	−3.62 kcal/mol 2.22 mM	−3.48 kcal/mol 2.81 mM	−3.20 kcal/mol 4.51 mM	−3.18 kcal/mol 4.70 mM	−3.13 kcal/mol 5.09 mM	−2.74 kcal/mol 9.79 mM
Iso-Vallesiachot-amine ($C_{21}H_{22}N_2O_3$) -6VXX	−5.18 kcal/mol 160.30 μM	−5.12 kcal/mol 177.97 μM	−5.01 kcal/mol 211.92 μM	−4.76 kcal/mol 322.60 μM	−4.72 kcal/mol 344.73 μM	−4.55 kcal/mol 462.95 μM	−4.32 kcal/mol 676.74 μM	−4.25 kcal/mol 762.33 μM	−3.58 kcal/mol 2.37 mM	−3.40 kcal/mol 3.20 mM
Isodihydroamino-cadambine ($C_{26}H_{33}N_3O_7$) -6VXX	−5.36 kcal/mol 118.39 μM	−3.88 kcal/mol 1.44 mM	−3.56 kcal/mol 2.46 mM	−3.48 kcal/mol 2.80 mM	−3.35 kcal/mol 3.49 mM	−3.22 kcal/mol 4.39 mM	−2.88 kcal/mol 7.78 mM	−2.65 kcal/mol 11.39 mM	−2.62 kcal/mol 12.08 mM	−2.53 kcal/mol 14.00 mM



A B C

Fig. 3 — (A) Oleanic Acid - 6VXX interaction; (B) Ursolic Acid - 6VXX interaction, & (C) Vincosamide-N-Oxid- 6VXX interaction



A B

Fig. 4 — (A) Iso-Vallesiachotamine - 6VXX interaction; & (B) Isodihydroamino-cadambine - 6VXX interaction

Table 3 — Final free energy of binding and nucleic acid residue sites in the studied ligand-protein interactions

S No.	Name of Ligands	PDB ID: 6Y84		PDB ID: 6LU7		PDB ID: 6CRV		PDB ID: 6VXX	
		Final free energy of binding at inhibition constant	Nucleic acid residue site of binding	Final free energy of binding at inhibition constant	Nucleic acid residue site of binding	Final free energy of binding at inhibition constant	Nucleic acid residue site of binding	Final free energy of binding at inhibition constant	Nucleic acid residue site of binding
1	Oleanic Acid ($C_{30}H_{48}O_3$)	−9.10 kcal/mol at 213.24 nM	GLY-127, LYS-5	−4.77 kcal/mol at 318.64 μM	LYS-97	−4.25 kcal/mol at 768.82 μM	VAL-147	−5.45 kcal/mol at 101.12 μM	ASP-428
2	Ursolic Acid ($C_{30}H_{48}O_3$)	−8.90 kcal/mol at 299.83 nM	LYS-5, DMY-403	−4.96 kcal/mol at 232.96 μM	ASN-238	−5.86 kcal/mol at 50.93 μM	ASP-850	−5.81 kcal/mol at 55.41 μM	GLY-594
3	Iso-Vallesiachotamine ($C_{21}H_{22}N_2O_3$)	−6.51 kcal/mol at 16.94 μM	ALA-7, PHE-8	−4.86 kcal/mol at 275.30 μM	SER-267	−4.03 kcal/mol at 1.11 mM	TRP-619, ASN-281	−3.40 kcal/mol at 3.20 mM	THR-376, GLY-504
4	Vallesiachotamine ($C_{21}H_{22}N_2O_3$)	−6.62 kcal/mol at 13.94 μM	GLY-138, GLN-299	−2.84 kcal/mol at 8.22 mM	ILE-281, ASN-238	−3.90 kcal/mol at 1.39 mM	ARG-118	NA	NA
5	Cadambine ($C_{27}H_{32}N_2O_{10}$)	−5.45 kcal/mol at 100.34 μM	GLU-288, ARG-4	−3.31 kcal/mol at 3.77 mM	LYS-100, ASP-33	−2.88 kcal/mol at 7.78 mM	SEN-728, GLN-280	NA	NA
6	Vincosamide-N-Oxide ($C_{26}H_{31}N_2O_9$)	−5.08 kcal/mol at 187.55 μM	LYS-4	−3.03 kcal/mol at 5.96 mM	PRO-108, HIS-246	−3.04 kcal/mol at 5.93 mM	TYR-197, SER-516	−2.74 kcal/mol at 9.79 mM	ASP-294, NAG-1309, VAL-595
7	Isodihydroamino-cadambine ($C_{26}H_{33}N_3O_7$)	−3.90 kcal/mol at 1.39 mM	GLU-240	−1.04 kcal/mol at 172.81 mM	ASR-330	−2.69 kcal/mol at 10.75 mM	ASN-955	−2.53 kcal/mol at 14.00 mM	GLY-282
8	Pentyle ester of chlorogenic acid ($C_{21}H_{28}O_9$)	−2.86 kcal/mol at 8.01 mM	ARG-4, LYS-5	Nil	Nil	Nil	NA	NA	NA
9	D-Myo-Inositol ($C_7H_{14}O_6$)	−2.93 kcal/mol at 7.09 mM	ALA-229, LYS-5	−1.07 kcal/mol at 164.03 mM	PHE-219	−1.56 kcal/mol at 71.41 mM	NA	NA	NA

NA: This protease has been left in the random selection to perform Molecular docking and does not implicate that data are not available

whose results are available on demand but not depicted due to space limitations.

Discussion

We observe the complete cycle for the binding of all these compounds with the said protease in maximum ten cluster run and the final free energy of binding is significant for all the ligand-protein interactions. The final free energy of binding and nucleic acid residue sites of binding which induce the conformational changes in the target protein and may result in the deactivation of the SARS-COV-2 virus has been presented in (Table 3).

The significantly negative value of the final free energy of binding obtained through the molecular docking of these compounds with the SARS-COV-2 protease and the observed nucleic acid residues sites of binding reveal that these molecules may be explored as potential inhibitor against COVID-19.

The insignificant result for the binding of molecule no.8 (pentyl ester of chlorogenic acid) with 6LU7 and 6CRV receptors have been obtained whereas the same molecule exhibits a significant final free energy of binding with 6Y84 protease of COVID-19 as obvious from the (Table 1). Since these compounds are naturally occurring, hence they do not cost for their synthesis and bears less or nil side-effect which enables them to be explored as user-friendly drug candidate for SARS-COV-2 virus. However, the solubility and the effect of the solvent which are usually associated with the phytochemicals may be the point of attention while using these compounds as drug candidate. The other chemical structure may also be derived from these bioactive natural compounds to further design potential drug agent for COVID-19. The binding of randomly selected five molecules with the SARS-COV-2 spike glycoprotein as depicted in the (Table 3), reveals that these molecules or their structural-derivatives may stop the replication of this virus at the receiver end in the human body.

Conclusion

We have carried out a schematic *In silico* investigation applying density functional theory and molecular docking approach on the nine naturally

occurring bioactive compounds listed in (Table 2) spontaneously found in the leaves and fruits of *Anthocephalus cadamba*. On the basis of the binding affinity, we have found these compounds as potential inhibitors against the COVID-19 having final free energy of binding in the order of molecule 1>2>3>4>5>6>7>8>9. These compounds have emerged as potential inhibitors against the SARS-COV-2 protease in our investigation; hence, an *in vivo* study is invited on these compounds for developing user-friendly drug for COVID-19. The pre-print of this research is announced by the archive of Cornell University, USA[34]. The further study on derived structures of these compounds is going on in our laboratory. Reports on some aspects of SARS-CoV-2 are available in the recent literatures[35-36].

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Conflict of interest

All authors declare no conflict of interest.

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