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In silico screening of *Vigna radiata* and *Vigna mungo* phytochemicals for their binding affinity to SARS-CoV-2 (COVID-19) main protease (3CL^{pro})

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Abstract

The seeds of *Vigna radiata* (green gram) are a favorite dish in Bangladesh and India and also considered an important nutritional and medicinal food in Ayurveda. According to Ayurveda, the pulse can pacify kapha and pitta but can aggravate vata. The seeds of another pulse, *Vigna mungo* (black gram) are also widely consumed in India and Bangladesh, more so by the low income groups of population. According to Ayurveda, this pulse is good for pacifying vata. A new coronavirus infection COVID-19 or SARS-CoV-2 have become a pandemic and as of June 28, 2020, have infected 10,080,224 persons and caused 501,262 deaths throughout the world. The statistics for USA, India and Bangladesh are, respectively, 2,596,537 infections and 128,152 deaths, 529,577 infections and 16,103 deaths, and 133,978 infections and 1,695 deaths. The respective populations for these three countries are 328.2 million (2019 figure), 1.353 billion (2018 figure), and 161.4 million (2018 figure). Thus despite the higher poverty level and population density, India and Bangladesh have managed to keep COVID infection levels much lower than an advanced country like USA. Although there can be a number of factors behind low COVID levels in India and Bangladesh, dietary habits may be one of them. The two pulses are taken on a daily basis and more often more than one times per day in India and Bangladesh by all sections of the population. *In silico* screening of the major phytochemicals of the two pulses revealed that a number of phytochemicals present in the pulses have high binding affinities for the C-3 like main protease of COVID-19, which is vital for viral replication. Although a definite conclusion cannot be reached without doing anti-viral tests, it is plausible that inhibition of the protease by the phytochemicals present in the two pulses may contribute to the low incidences of COVID-19 infections in India and Bangladesh.

Keywords: *Vigna radiata*, *Vigna mungo*, COVID-19, phytochemicals, *in silico*

Introduction

Human coronaviruses are associated with multiple forms of respiratory diseases including cold, pneumonia, and bronchitis [1]. Coronaviruses are so named because the viruses contain spike (S) protein on their surfaces resembling a corona. Seven known HCoVs have been identified so far, namely HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2 or COVID-19, which emerged in December 2019 in Wuhan, China. Four HCoVs (HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1) are common and globally circulated in the human population and contribute to approximately one-third of common cold infections in humans every year [2], which usually does not get complicated and passes off in a week or so with mild symptoms of cough, cold and fever and easily dismissed as 'viral flu'.

Coronaviruses attach to specific receptors on the human cell through their spike (S) protein. For SARS-CoV and SARS-CoV-2, the receptor is the angiotensin-converting enzyme (ACE)-2. Following receptor binding, nucleocapsid is released into the cytoplasm of the host cell [3]. The genome of SARS and SARS-CoV-2 encodes two large polyproteins, pp1a and 5 pp1ab. These polyproteins are cleaved and transformed in mature non-structural proteins (NSPs) by the two proteases 3CL^{pro} (3C-like protease or chymotrypsin-like protease) and PL^{pro} (Papain Like Protease) encoded by the open reading frame 1.

The 3C-like proteases of SARS and SARS-CoV-2 differ by only 12 amino acids; the substrate binding pockets exhibit a strikingly high level of alignment of the key residues involved in substrate binding, including the CYS145-HIS41 dyad, and HIS163/HIS172/GLU166 [4]. Since the 3CL^{pro} is necessary for viral replication, researchers believe that it can be a key target for development of therapeutics. However, as of now, there have been no discoveries of drugs or vaccines against SARS or SARS-CoV-2.

In the absence of proper anti-COVID therapeutics, COVID-19 or SARS-CoV-2 has become a pandemic and as of June 28, 2020, have infected 10,080,224 persons and caused 501,262 deaths throughout the world. The statistics for USA, India and Bangladesh are, respectively, 2,596,537 infections and 128,152 deaths, 529,577 infections and 16,103 deaths, and 133,978 infections and 1,695 deaths. The respective populations for these three countries are 328.2 million (2019 figure), 1.353 billion (2018 figure), and 161.4 million (2018 figure). Thus while India has about 4 times the population of USA, and Bangladesh about half the population of USA, the infection statistics show that India has around 20% and Bangladesh about 5% of the number of people infected compared to USA. Thus despite the higher poverty level and population density (making social distancing almost impossible), India and Bangladesh have managed to keep COVID infection levels much lower than an advanced country like USA. Although there can be a number of factors behind low COVID levels in India and Bangladesh, dietary habits may be one of them.

Recent attention of scientists has focused on plant-based compounds (phytochemicals) in their effort to find a suitable therapeutic against COVID-19. Among the suitable targets of COVID-19 is 3CL^{pro}, and as a first step *in silico* screening is going on with the binding of a number of phytochemicals against the protease. Plants have been chosen because not only many important allopathic drugs have been discovered from plants (vincristine, vinblastine, quinine, artemisinin, reserpine, to name only a few) [5], but also synthetic drugs have not proved to be of much effect against COVID-19. A number of Traditional Chinese Medicines (TCMs) containing plants or plant parts traditionally used for respiratory illnesses have been tried against COVID-19 (which primarily causes respiratory disorders) with varying degrees of success [6]. Ayurvedic 'rasayana' (prevention of diseases) botanicals like the plant *Withania somnifera* have been suggested for COVID prophylaxis [7].

COVID-19 causes disorders in a number of organs in the body and possibly imbalances all three 'doshas', namely pitta, kapha, and vata. As such, if pacification of these three doshas is due to dietary elements in India and Bangladesh, those dietary elements must have two things in common – they should be common dietary elements in both countries, and

they should singly or in combination pacify any or all of the aggravated doshas. The seeds of *Vigna radiata* (L.) R. Wilczek (green gram) are a favorite dish in Bangladesh and India and also considered an important nutritional and medicinal food in Ayurveda. According to Ayurveda, the pulse can pacify kapha and pitta but can aggravate vata. The seeds of another pulse, *Vigna mungo* (L.) Hepper (black gram) are also widely consumed in India and Bangladesh, more so by the low income groups of population. According to Ayurveda, this pulse is good for pacifying vata. From that view point, we targeted these pulses as the two pulses can pacify doshas and are commonly consumed once or more daily in practically every household of the two countries. The objective of this study was to perform *in silico* screening of the phytochemicals present in the pulses and determine whether they can bind to 3CL^{pro} thereby inhibiting the protease and subsequent viral replication.

Methods
Three-dimensional structure of COVID-19 major protease (3C-like protease)
The pdb file (6LU7) of the main protease of SARS-CoV-2 3C-like protease or SARS-CoV-2 3CL^{pro} published before [8] was used in the present study following removal of inhibitor N3. The active residues of SARS-CoV-2 3C-like protease are His41 and Cys145. Monomeric form of protein was used for molecular docking. Binding of some selected phytochemicalss to SARS-CoV-2 3CL^{pro} have been shown to illustrate the binding site of the phytochemicals to the protease and the amino acids involved in the binding.

Compounds used in docking studies
We have studied various classes of phytochemicals known to occur in *Vigna radiata* and *Vigna mungo*. Ligand molecules were downloaded from Pubchem [9] in sdf format. Optimization was done with the force field type MMFF94 using Openbable softwares and saved as pdbqt format. The names of the compounds present in *Vigna radiata and Vigna mungo* were obtained from published reports [10, 11].

Ligand molecular docking studies
Molecular docking (blind) was conducted using AutoDock Vina [12]. We report ΔG values as an average of six values from the docking program. We show the pose of phytochemicals bound to SARS-CoV-2 main protease in our figures as obtained from PyMOL and displayed in Discovery Studio [13].

Phytochemicals
The structures of the various phytochemicals screened are shown in Figures 1 and 2.
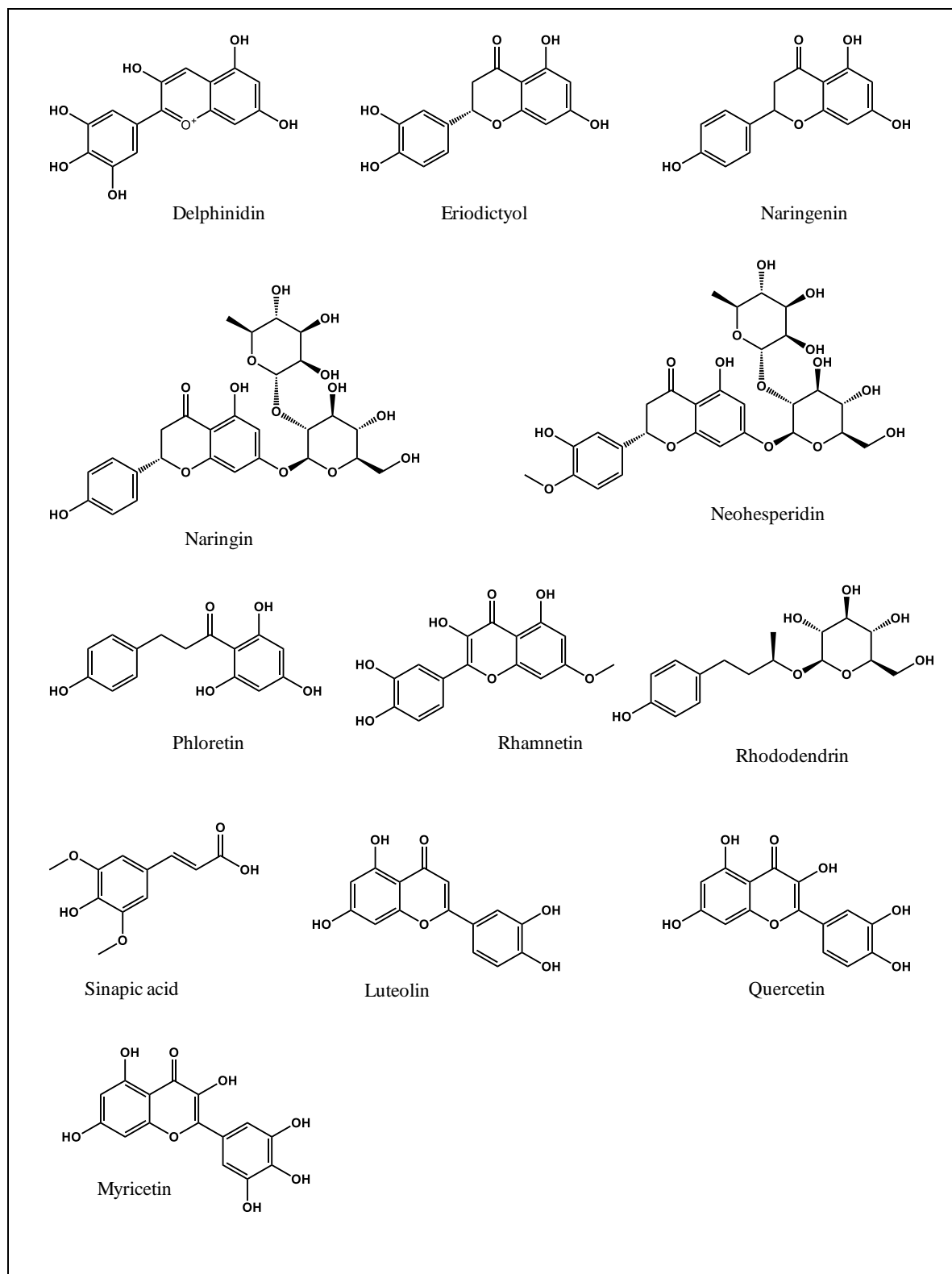
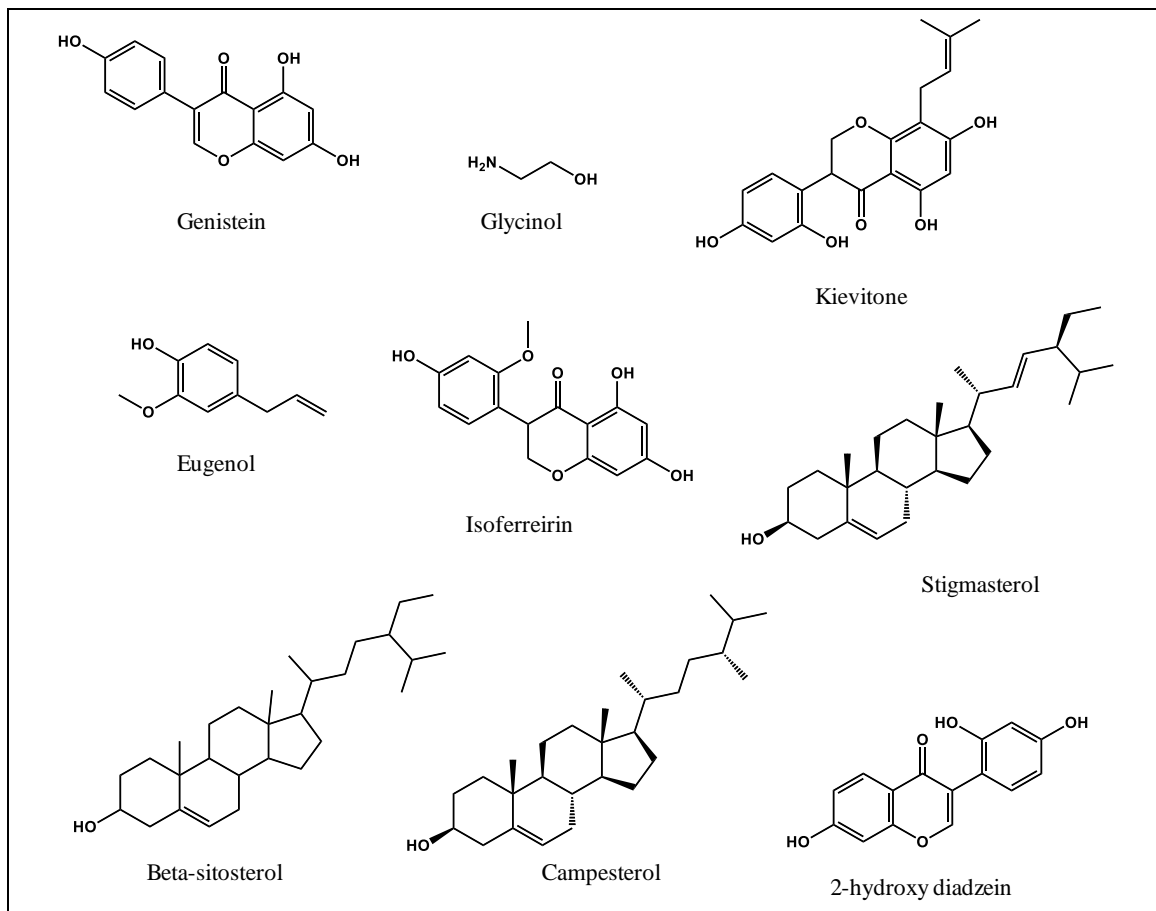**Fig 1:** Structure of phytochemicals screened from *Vigna radiata*.

Fig 2: Structure of phytochemicals screened from *Vigna mungo*.

Results and Discussion

The binding energies (ΔG expressed in kcal/mol) of various phytochemicals of *Vigna radiata* screened in the present study are shown in Table 1. A value less than -7.0 was considered good affinity; value of -7.5 or less was considered as strong affinity with the phytochemical possessing the potential of becoming a therapeutic, or at least a lead compound worthy of more studies. The structures of the various phytochemicals are shown in Figure 1 (*Vigna radiata*) and Figure 2 (*Vigna mungo*).

Table 1: Binding affinities of *Vigna radiata* phytochemicals to SARS-CoV-2 3C-like protease.

Phytochemicals	Binding energy (ΔG = kcal/mol)
Delphinidin	-7.4
Eriodictyol	-7.4
Naringenin	-7.3
Naringin	-7.8
Neohesperidin	-8.3
Phloretin	-6.8
Rhamnetin	-7.6
Rhododendrin	-7.1
Sinapic acid	-5.7
Luteolin	-7.6
Quercetin	-7.4
Myricetin	-7.5

The binding of naringin to SARS-CoV-2 3C-like protease is shown in Figure 3. If the key residues involved in substrate binding, including the Cys145-His41 dyad, and His163/His172/Glu166 are taken into account, naringin binds to Cys145 in the active site of the protease along with binding to flanking amino acids Gly143 and His163. This multiple binding possibly contributes to the high binding affinity of this flavanone-7-*O*-glycoside. The main protease monomer contains three domains; Domain 1 (residues 8-101) and domain 2 (residues 102-184) are made of antiparallel beta-barrel structures in a chymotrypsin-like fold, and which is responsible for catalysis [14]. Most interactions of the phytochemicals of *Vigna radiata* and *Vigna mungo* appear to be to the domain 2 of the catalytic site.

The binding of neohesperidin to SARS-CoV-2 3C-like protease is shown in Figure 4. Unlike naringenin, neohesperidin does not bind to Cys145, but binds to Thr25, His41, Leu141, Asn142, Gly143, Glu166, Pro168, and Gln189. The binding to His41 and Glu166 also shows that this flavanone glycoside also binds at the active site of the protease. One important aspect evident from the binding studies of naringin, naringenin and neohesperidin is that presence of glycosidic residues increases docking affinity, like naringin and neohesperidin (with two glycosidic residues) shows higher binding affinity than naringenin (no glycosidic moiety attached, see Figure 1 for details). The importance of the flavonoid group is shown by the low docking affinity of phloretin versus that of rhamnetin and luteolin, the latter two containing flavonoid moieties versus phloretin, which has none (see Figure 1).

The binding energies (ΔG expressed in kcal/mol) of various phytochemicals of *Vigna mungo* screened in the present study are shown in Table 2.

Table 2: Binding affinities of *Vigna mungo* phytochemicals to SARS-CoV-2 3C-like protease.

Phytochemicals	Binding energy (ΔG = kcal/mol)
Genistein	-6.9
Kievitone	-7.9
Eugenol	-6.0
Glycinol	-7.5

Isoferreirin	-7.4
Stigmasterol	-7.9
Beta-sitosterol	-7.3
Campesterol	-7.5
2-hydroxy diadzein	-6.6

The binding of kievitone to SARS-CoV-2 3C-like protease is shown in Figure 5. A strong bonding affinity can be seen because of the compound's interaction with Cys145, Met165, Glu166, Pro168, and Gln189. The binding of this compound is in the active site of the protease, thus raising the possibility that kievitone can be an inhibitor of the protease and so possess therapeutic values.

Interestingly, the three plant sterols, stigmasterol, beta-sitosterol, and campesterol gave high binding energies of -7.9, -7.3, and -7.5, respectively. The binding of campesterol to the protease is shown in Figure 6. The phytosterol interacts with His41, Met49, Cys145, His 163, and Met165 of the protease. A double bond linking C22 and C23 in stigmasterol may account for the higher binding affinity of stigmasterol compared to campesterol and beta-sitosterol (see Figure 2). The binding of stigmasterol to 3C-like protease is shown in

Figure 7. Like campesterol, stigmasterol binds to both His 41 and Cys145, but unlike campesterol, stigmasterol interacts with 18 other amino acids.

Although *Vigna radiata* and *Vigna mungo* are consumed on a regular basis even 2-3 times daily by the mainstream population of India and Bangladesh, it cannot be concluded that mere consumption of these two pulses (along with the lentil, *Lens culinaris*) accounts for the low COVID incidences in the two countries. However, the population density and lack of hygienic facilities and non-maintenance of social distancing should have resulted in much more COVID infections. The notable thing about the two pulses is presence of a number of compounds (including flavonoids and their derivatives) and phytosterols, which in binding studies indicate that they bind to or close to the active site of the 3C-like protease of COVID-19 and can possibly act as an inhibitor to the protease. Flavonoids like herbacetin, rhoifolin, and pectolinarin have been reported to bind to the catalytic site and inhibit the C3-like protease [15].The same thing can happen with the flavonoids present in the two pulses.
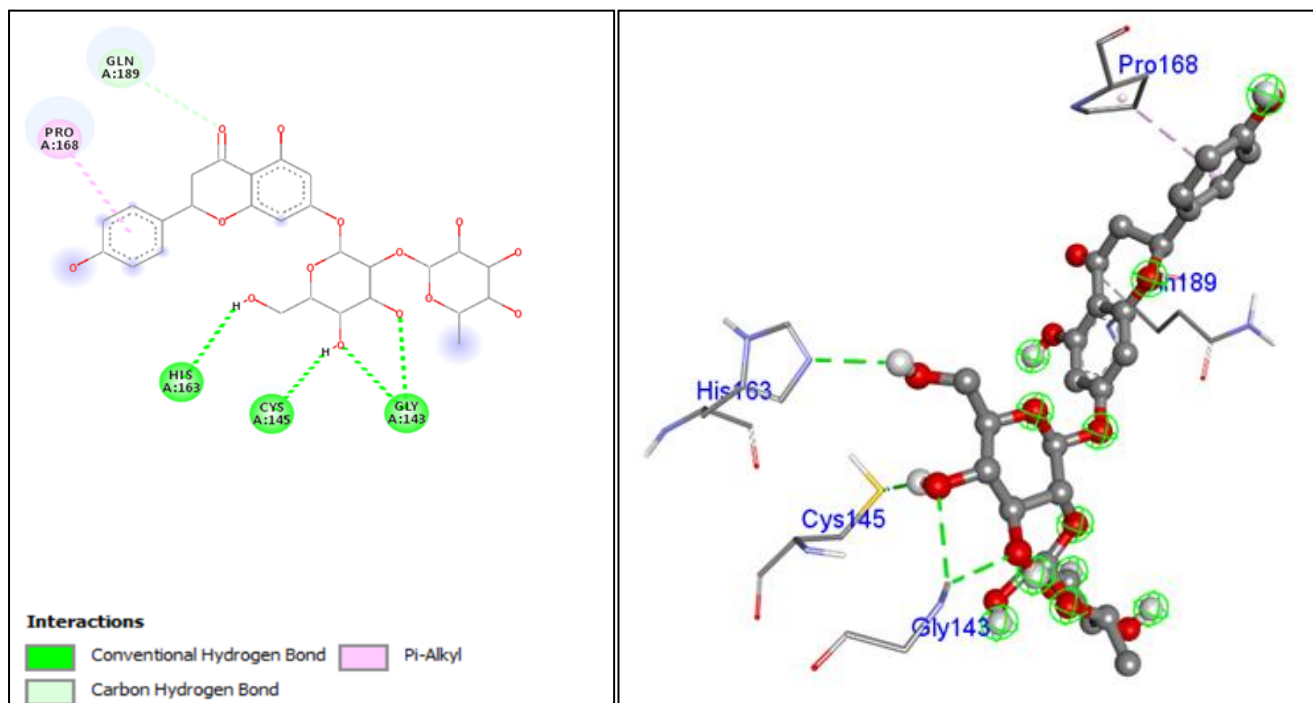

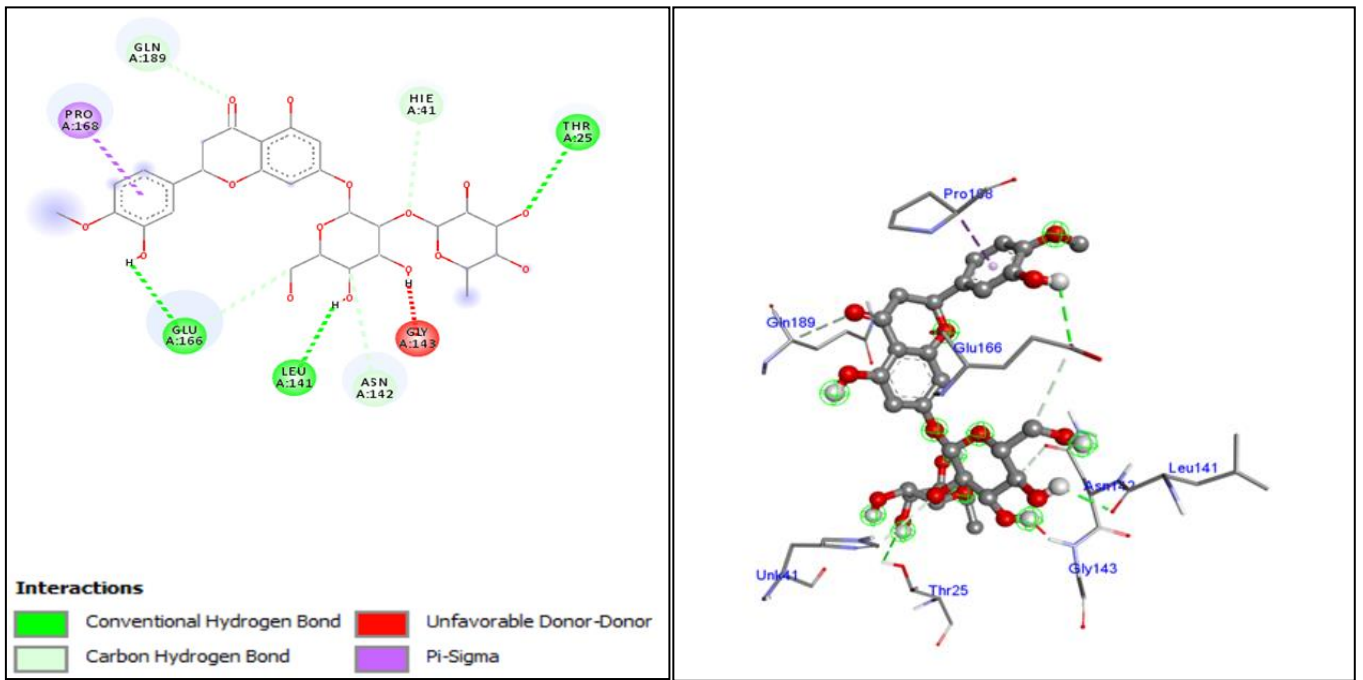**Fig 3:** Binding of naringin to SARS-CoV-2 3C-like protease.

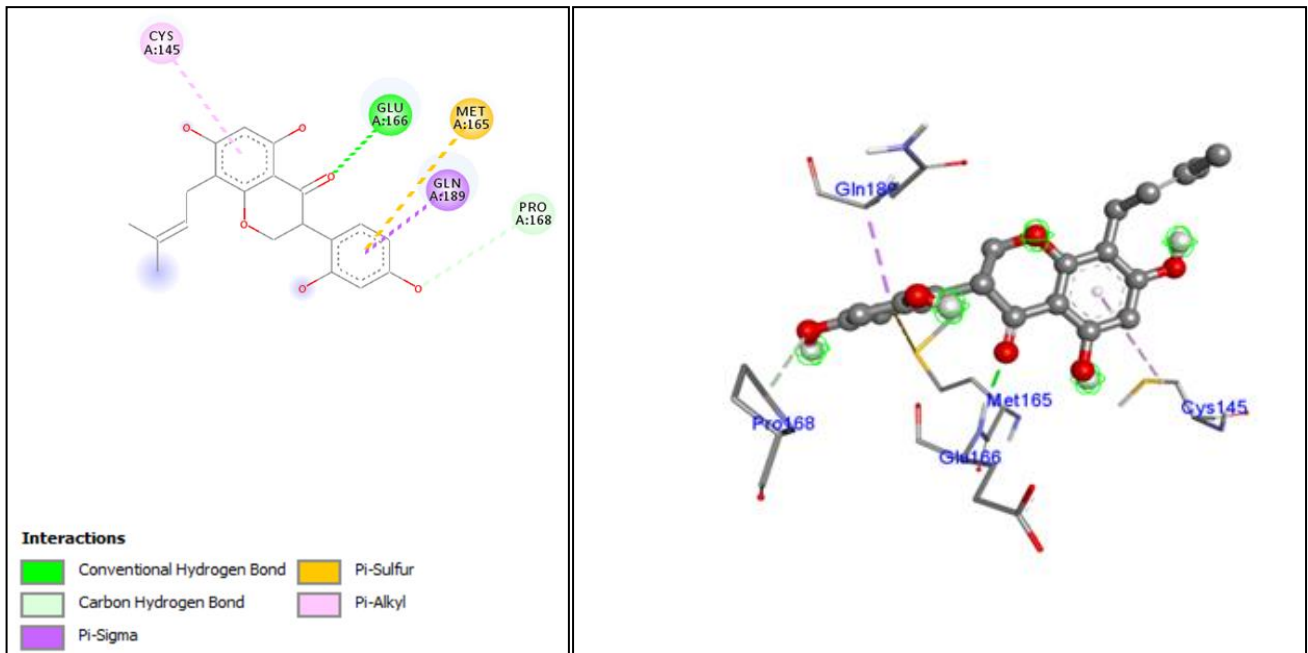**Fig 4:** Binding of neohesperidin to SARS-CoV-2 3C-like protease.

Fig 5: Binding of kievitone to SARS-CoV-2 3C-like protease.
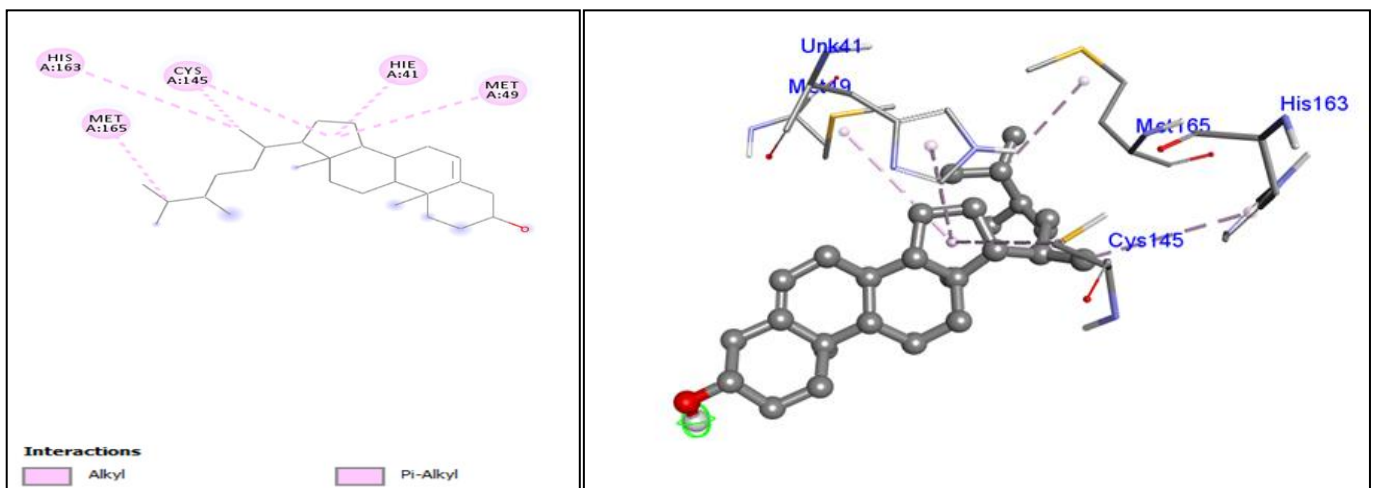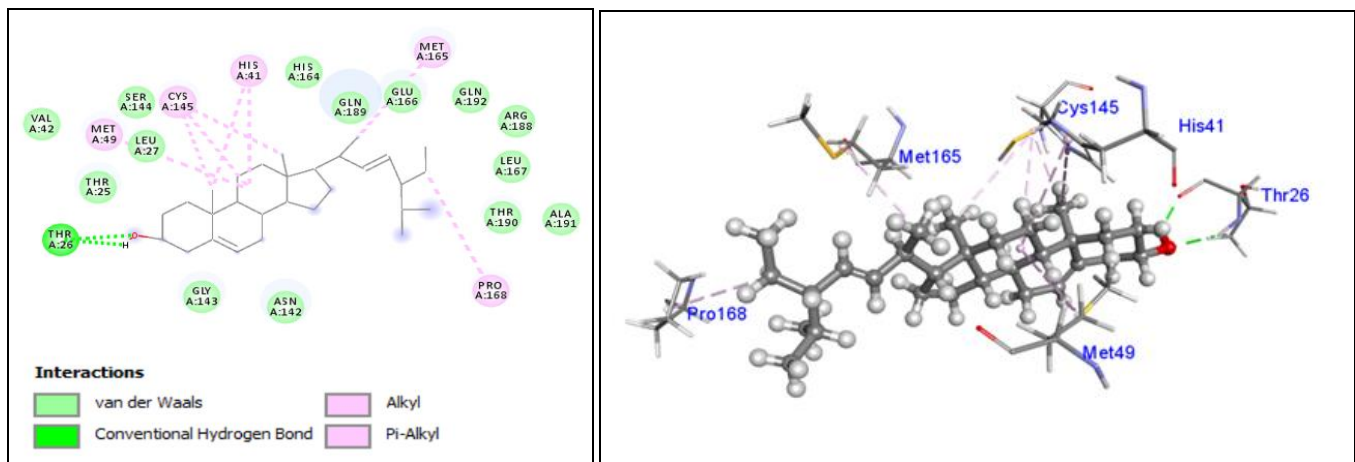

Fig 6; Binding of campesterol to SARS-CoV-2 3C-like protease.

Fig 7: Binding of stigmasterol to SARS-CoV-2 3C-like protease.

Conclusion

Various phytoconstituents of the two pulses *Vigna radiata* and *Vigna mungo* have high binding affinities to the C3-like protease of COVID-19 and can possibly be future therapeutics against this coronavirus if *in silico* studies are confirmed with antiviral activity studies. It remains a possibility that consumption of these two pulses on a daily basis may contribute to the lower incidences of COVID-19 cases in India and Bangladesh compared to say a country like USA.

Acknowledgements

Conflicts of interest

The authors declare that they have no conflicts of interest.

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