MOLECULAR DOCKING OF SELECTED BIOACTIVE COMPOUNDS FROM AZADIRACHTA INDICA FOR THE INHIBITION OF COVID19 PROTEASE

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ABSTRACT

Objective: COVID-19 caused by novel SARS-coronavirus 2 belonging to family Coronaviridae, is a global public health emergency infecting many people all over the world with more than 2,98,000 cases. Hence there is a need for a novel drug that counters SARS-CoV2 is the prime requirement at this time.

Methods: The present study aimed to assess bioactive compounds found in Azadirachta indica as a potential inhibitor of COVID-19 Mpro [6Y2E, 6LU7, and 2GTB] by AutoDock 4.2, with the Lamarckian Genetic Algorithm. COVID-19 Mpro was docked with thirteen bioactive compounds, and docking was analyzed by AutoDock 4.2 and Pymol. Nelfinavir and Saquinavir were used as positive standards for comparison.

Results: Azadirachtin, Azadirachtol, and Salanolide were left out because of the violation of Lipinski’s rule. The binding energies obtained from the docking of 6Y2E with a native ligand, Azadiradione, Betasitosterol, Epiazadiradione, Epoxazadiradione, Kämpferol, Meldenin, Myricetin, Nimbaflavone, Nimbinine, Nimboicoline, Quercitrin, Vepnin, Saquinavir, and Nelfinavir were -7.32, -6.63, -6.69, -7.52, -5.27, -4.54, -6.07, -4.19, -5.62, -5.58, -6.23, -4.71, -3.72, -6.4, -7.14 and -4.67 kcal/mol respectively. The binding energies obtained from the docking of 6LU7 with the native ligand, Azadiradione, Nimbinone, Vepnin, and Saquinavir were -6.14, -6.86, -6.79 and -6.49 kcal/mol correspondingly. The binding energies obtained from the docking of 2GTB with the native ligand, Azadiradione, Epiazadiradione, Epoxyazadiradione, Kämpferol, Meldenin, Myricetin, Nimboicoline, Nimboicoline, Nimbinone, and Nelfinavir were -6.96, -7.13, -6.69, -5.22, -6.44, -5.06, -5.93, -6.66, -5.3, -5.63, -7.11, -6.89 and -5.42 kcal/mol respectively.

Conclusion: Azadiradione, Epiazadiradione, Nimbinone, and Vepnin seemed to have the greatest potential to act as COVID-19 protease inhibitors. However, further research is necessary to explore their prospective medicinal use in vitro and in vivo conditions.

Keywords: COVID-19 Mpro, 6Y2E, 6LU7, AutoDock, Azadirachta indica and Saquinavir

INTRODUCTION

The Coronavirus disease 19 (COVID 19) is a highly transmitted and pathogenic viral infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which started emerging in Wuhan, China and spread around the world in 2019. With an exemption of Antarctica, it is vigorously transmitted from Wuhan city to nearly every country in the world [3]. According to the World Health Organization (WHO) on 24 June 2020 around 91,291,146 cases of persons infected worldwide by COVID-19 out of which 4,73,797 death in 216 countries. Since COVID-19 is quickly spreading, the WHO has declared it as a pandemic disease [8]. Common symptoms of the coronavirus-infected person have respiratory symptoms, fever, cough, shortness of breath, and dyspnea. In severe cases, an infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and even death [5, 6]. Presently there is no specific treatment for COVID 19.

Coronaviruses are enveloped viruses with a positive RNA genome, belonging to the Coronaviridae family, of which many species are present in different animals. The SARS-CoV-2 belongs to the B genus. Coronavirus contains at least four structural proteins: Spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein [7]. Among that Spike helps in host attachment and membrane fusion during the infection of the virus. Therefore, Spike determines to some extent of the host range [5]. The main protease (Mpro)/chymotrypsin-like protease (3CLpro) from COVID-19, which has been successfully crystallized structured, and repositioned in the Protein Data Bank and this protease represents a potential target for the inhibition of Coronavirus replication [8].

Azadirachta indica (Neem) is a member of the Meliaceae family and its role in health-promoting outcome is recognized because of its antioxidant property and various other antimicrobial properties. It has been widely used in Chinese, Ayurvedic, and lhani medicines worldwide, especially in the Indian subcontinent in the treatment and prevention of various diseases [9]. Azadirachta indica has more than 140 chemically active compounds isolated from its different parts, including i.e. seeds, roots, flowers, fruits leaves, and bark and are used traditionally for curing many diseases. These active compounds have been identified as anti-mutagenic, anti-inflammatory, anti-oxidant, anti-ulcer, anti-hyperglycaemic, immune-modulator, anti-carcinogenic, and anti-viral drugs [10]. Since COVID-19 is the highest outbreak in almost all nations, novel methodologies of drug strategy and discovery can be exploited as a promising tool for finding various therapeutic drug agents.

In the present study, we observe the binding affinity of 16 ligands, which are present in Azadirachta indica for COVID-19 6LU7, 6Y2E proteases, and 2GTB. The main purpose of this study is to make use of recognized natural products to be used to strengthen defences against coronavirus by developing drugs based on natural products.

MATERIALS AND METHODS

Macromolecule (protein) and ligand

The COVID-19 Mpro (PDB ID: 6LU7, 6Y2E, 2GTB) structures of the main protease were obtained from the Protein Data Bank (https://www.rcsb.org/) in pdb format. The 6LU7 protein contains two chains, A and B, which form a homodimer. Chain A alone was taken for macromolecule preparation. Drug-like properties were calculated using Lipinski’s rule of five, which suggested that molecules with poor permeation and oral absorption have molecular weights >500, ClogP >5, more than 5 Hydrogen-bond donors, more than 10 acceptor groups, more than 10 rotatable bonds, and Molar refractivity was not in the range between 40 and 130 [8, 11, 12]. Adherence to Lipinski’s rule of five was calculated using SWISSADME (http://www.swissadme.ch/).
| S. No. | Name of the ligand         | Molecular formula | Lipinski's rule of five                                      | Properties                      | Value                  |
|-------|-----------------------------|-------------------|-------------------------------------------------------------|---------------------------------|------------------------|
|       |                             |                   |                                                             | Molecular weight                | 600.65 g/mol           |
| 1     | Azadirachtanin              | \(\text{C}_{32}\text{H}_{40}\text{O}_{11}\)       |                                                             | Log P                           | 3.82                   |
|       |                             |                   |                                                             | H–bond donor                     | 2                      |
|       |                             |                   |                                                             | H–bond acceptor                  | 11                     |
|       |                             |                   |                                                             | Molar refractivity               | 148.84                 |
|       |                             |                   |                                                             | Number of rotatable bonds        | 7                      |
|       |                             |                   |                                                             | Violations                       | 3                      |
| 2     | Azadirachtol                | \(\text{C}_{32}\text{H}_{40}\text{O}_{12}\)       |                                                             | Molecular weight                | 580.58 g/mol           |
|       |                             |                   |                                                             | Log P                           | 2.81                   |
|       |                             |                   |                                                             | H–bond donor                     | 4                      |
|       |                             |                   |                                                             | H–bond acceptor                  | 13                     |
|       |                             |                   |                                                             | Molar refractivity               | 131.30                 |
|       |                             |                   |                                                             | Number of rotatable bonds        | 5                      |
|       |                             |                   |                                                             | Violations                       | 3                      |
| 3     | Azadiradione                | \(\text{C}_{32}\text{H}_{34}\text{O}_{6}\)        |                                                             | Molecular weight                | 450.57 g/mol           |
|       |                             |                   |                                                             | Log P                           | 3.18                   |
|       |                             |                   |                                                             | H–bond donor                     | 0                      |
|       |                             |                   |                                                             | H–bond acceptor                  | 5                      |
|       |                             |                   |                                                             | Molar refractivity               | 125.48                 |
|       |                             |                   |                                                             | Number of rotatable bonds        | 3                      |
|       |                             |                   |                                                             | Violations                       | 3                      |
| 4     | Beta-sitosterol             | \(\text{C}_{32}\text{H}_{50}\text{O}_{6}\)        |                                                             | Molecular weight                | 414.71 g/mol           |
|       |                             |                   |                                                             | Log P                           | 5.05                   |
|       |                             |                   |                                                             | H–bond donor                     | 1                      |
|       |                             |                   |                                                             | H–bond acceptor                  | 1                      |
|       |                             |                   |                                                             | Molar refractivity               | 133.23                 |
|       |                             |                   |                                                             | Number of rotatable bonds        | 6                      |
|       |                             |                   |                                                             | Violations                       | 2                      |
| 5     | Epiazadiradione             | \(\text{C}_{32}\text{H}_{34}\text{O}_{6}\)        |                                                             | Molecular weight                | 450.57 g/mol           |
|       |                             |                   |                                                             | Log P                           | 3.11                   |
|       |                             |                   |                                                             | H–bond donor                     | 0                      |
|       |                             |                   |                                                             | H–bond acceptor                  | 5                      |
|       |                             |                   |                                                             | Molar refractivity               | 125.48                 |
|       |                             |                   |                                                             | Number of rotatable bonds        | 3                      |
|       |                             |                   |                                                             | Violations                       | 3                      |
| 6     | Epoxyazadiradione           | \(\text{C}_{32}\text{H}_{34}\text{O}_{6}\)        |                                                             | Molecular weight                | 466.57 g/mol           |
|       |                             |                   |                                                             | Log P                           | 3.32                   |
|       |                             |                   |                                                             | H–bond donor                     | 0                      |
|       |                             |                   |                                                             | H–bond acceptor                  | 6                      |
|       |                             |                   |                                                             | Molar refractivity               | 124.96                 |
|       |                             |                   |                                                             | Number of rotatable bonds        | 3                      |
|       |                             |                   |                                                             | Violations                       | 0                      |
| 7     | Kaempferol                  | \(\text{C}_{32}\text{H}_{10}\text{O}_{6}\)        |                                                             | Molecular weight                | 286.24 g/mol           |
|       |                             |                   |                                                             | Log P                           | 1.70                   |
|       |                             |                   |                                                             | H–bond donor                     | 4                      |
|       |                             |                   |                                                             | H–bond acceptor                  | 6                      |
|       |                             |                   |                                                             | Molar refractivity               | 76.01                  |
|       |                             |                   |                                                             | Number of rotatable bonds        | 1                      |
|       |                             |                   |                                                             | Violations                       | 0                      |
| 8     | Meldenin                   | \(\text{C}_{32}\text{H}_{10}\text{O}_{6}\)        |                                                             | Molecular weight                | 454.60 g/mol           |
|       |                             |                   |                                                             | Log P                           | 3.43                   |
|       |                             |                   |                                                             | H–bond donor                     | 1                      |
|       |                             |                   |                                                             | H–bond acceptor                  | 5                      |
|       |                             |                   |                                                             | Molar refractivity               | 126.91                 |
|       |                             |                   |                                                             | Number of rotatable bonds        | 3                      |
|       |                             |                   |                                                             | Violations                       | 0                      |
| 9     | Myricetin                  | \(\text{C}_{15}\text{H}_{10}\text{O}_{5}\)        |                                                             | Molecular weight                | 318.24 g/mol           |
|       |                             |                   |                                                             | Log P                           | 1.08                   |
|       |                             |                   |                                                             | H–bond donor                     | 6                      |
|       |                             |                   |                                                             | H–bond acceptor                  | 8                      |
|       |                             |                   |                                                             | Molar refractivity               | 80.06                  |
|       |                             |                   |                                                             | Number of rotatable bonds        | 1                      |
|       |                             |                   |                                                             | Violations                       | 0                      |
| 10    | Nimbaflavone                | \(\text{C}_{15}\text{H}_{10}\text{O}_{5}\)        |                                                             | Molecular weight                | 422.51 g/mol           |
|       |                             |                   |                                                             | Log P                           | 4.36                   |
|       |                             |                   |                                                             | H–bond donor                     | 2                      |
|       |                             |                   |                                                             | H–bond acceptor                  | 5                      |
|       |                             |                   |                                                             | Molar refractivity               | 123.48                 |
|       |                             |                   |                                                             | Number of rotatable bonds        | 6                      |
|       |                             |                   |                                                             | Violations                       | 0                      |
The protease files were prepared for COVID-19 6LU7 protease, 6Y2E protease, and 2GTB separately using Autodock 4.2. The macromolecule was prepared using ‘X’ chain of the protease, the water molecule was deleted, polar hydrogen atoms and charges were added. The file was saved in the PDBQT format for further analysis. The grid box was used to obtain the X, Y, and Z coordinates. The ligand files in the mol2 format were converted to the PDBQT format by Open Babel after detecting the torsion root.

Using the protease, PDBQT file, ligand, PDBQT file and the X, Y, and Z coordinates, binding affinity was calculated using Lamarckian genetic algorithms by AutoDock 4.2. The conformations were played ranked by energy. The hydrogen bonds were viewed and the complex was saved in pdbqt format and then converted to pdb format. The 3D structure of the protease-ligand complex interaction was visualized using PyMOL, and the 2D structure of the molecular interaction was visualized using the Biovia Discovery Studio 2620 Client.

### Molecular docking

The protease files were prepared for COVID-19 6LU7 protease, 6Y2E protease, and 2GTB separately using Autodock 4.2. The ligands present in the study were Azadirachtin, Azadirachtol, Azadiradione, Betasitosterol, Epiazadiradione, Epopiazadiradione, Kaempferol, Meldenin, Myricetin, Nimbaflavone, Nimbinene, Nimbine, Nimbocinolide, Quercitrin, Salannolide, and Vepnin. The three-dimensional structures of the selected ligands from Azadirachta indica were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in sdf format. The properties of ligand molecules chosen for the study were listed in table 1. Anti-HIV drugs, Saquinavir, and Nelfinavir were used as positive control.

### Table 1

| PMI | Name      | Molecular weight | Molar refractivity | Log P | Number of rotatable bonds | Molar ref | H-bond donor | H-bond acceptor | Number of rotatable bonds | Violations | Violations | Violations | Violations | Violations |
|-----|-----------|------------------|--------------------|-------|---------------------------|-----------|---------------|-------------------|---------------------------|------------|------------|------------|------------|------------|
| 11  | Nimbinene | C₂₀H₂₇O₂         | 482.57 g/mol       | 3.68  | 0                         | 7         | 1             | 0                 | 6                         | 0          | 0          | 0          | 0          |
| 12  | Nimbione  | C₂₀H₂₇O₂         | 286.37 g/mol       | 2.24  | 0                         | 7         | 1             | 0                 | 6                         | 0          | 0          | 0          | 0          |
| 13  | Nimbocinolide | C₂₀H₂₇O₂ | 586.67 g/mol       | 2.84  | 3                         | 10        | 3             | 0                 | 6                         | 2          | 2          | 2          | 2          |
| 14  | Quercitrin| C₂₀H₂₇O₂         | 448.38 g/mol       | 1.60  | 7                         | 11        | 1             | 0                 | 3                         | 2          | 2          | 2          | 2          |
| 15  | Salannolide | C₂₀H₂₇O₂       | 628.71 g/mol       | 3.65  | 1                         | 11        | 1             | 11                | 9                         | 3          | 3          | 3          | 3          |
| 16  | Vepnin    | C₂₀H₂₇O₂         | 452.58 g/mol       | 3.50  | 0                         | 5         | 0             | 5                 | 3                         | 0          | 0          | 0          | 0          |
| 17  | Saquinavir| C₂₀H₂₇N₂O₂       | 670.84 g/mol       | 3.66  | 5                         | 0         | 5             | 7                 | 3                         | 2          | 2          | 2          | 2          |
| 18  | Nelfinavir| C₂₀H₂₇N₂O₂       | 567.78 g/mol       | 3.87  | 4                         | 5         | 4             | 5                 | 12                        | 2          | 2          | 2          | 2          |
RESULTS AND DISCUSSION

Coronaviruses (CoVs) represented a major group of viruses, mostly affecting human beings through zoonotic transmission [13]. In the past two decades, this was the third occurrence of the emergence of a new coronavirus, after severe acute respiratory syndrome (SARS) in 2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 [14, 15].

2GTB was the main protease found in the coronavirus associated with the severe acute respiratory syndrome (SARS), which could be retrieved in PDB and was recommended to be a potential drug target for 2019-nCoV and it shared 96% similarity with that of SARS [16]. 6Y2E and 6LU7 were the main protease found in Coronavirus and their structures were available in PDB. Ligands and anti-HIV compounds had been selected based on adherence to Lipinski’s rule of five. The selected ligands that had 2 or less than 2 violations of Lipinski's rule were used in molecular docking experiments with the target proteins (6LU7 protease, 6Y2E protease, and 2GTB). Azadirachtin, Azadiractol, and Salannolide were omitted because of the violation of Lipinski’s rule. The drug scanning outcomes (table 1) showed that 13 bioactive compounds and 2 positive controls used in this study were accepted by Lipinski’s rule of five. Table 2, 3, and 4 showed the molecular docking analysis results of bioactive compounds against 6Y2E, 6LU7, and 2GTB, respectively including binding energy/Gibbs Energy, inhibition constant, intermolecular energy, torsional energy and internal energy. Fig. 1, 2, and 3 showed 3D visualization of binding sites of various bioactive compounds from Azadirachta indica to the active sites of coronavirus protease 6Y2E, 6LU7, and 2GTB.

Table 2: Molecular docking analysis of bioactive molecules from Azadirachta indica against 6Y2E

| S. No. | Name of the ligand | Binding energy (Kcal/mol) | Inhibition constant | Intermolecular energy | Torsional energy | Internal energy |
|-------|-------------------|--------------------------|---------------------|-----------------------|-----------------|----------------|
| 1     | Azadiradione      | -7.32                    | 4.34                | -8.21                 | 0.89            | -1.17          |
| 2     | Beta-sitosterol   | -6.63                    | 13.76               | -8.72                 | 2.09            | -1.13          |
| 3     | Epiazadiradione   | -7.52                    | 3.06                | -8.42                 | 0.89            | -1.3           |
| 4     | Epoxyazadiradione | -5.27                    | 136.58              | -6.17                 | 0.89            | -1.22          |
| 5     | Kaempferol        | -4.54                    | 472.42              | -6.03                 | 1.49            | -1.36          |
| 6     | Meldenin          | -6.07                    | 35.26               | -7.27                 | 1.19            | -1.51          |
| 7     | Myricetin         | -4.19                    | 851.61              | -6.28                 | 2.09            | -2.03          |
| 8     | Nimbavine         | -5.02                    | 208.13              | -7.41                 | 2.39            | -2.54          |
| 9     | Nimbione          | -5.58                    | 80.63               | -7.37                 | 1.79            | -2.09          |
| 10    | Nimbione          | -6.23                    | 27.13               | -6.53                 | 0.3             | 0.05           |
| 11    | Nimbocinolide     | -4.71                    | 351.27              | -7.4                  | 2.68            | -4.3           |
| 12    | Quercitrin        | -3.72                    | 1890                | -6.7                  | 2.98            | -4.03          |
| 13    | Vepnin            | -6.4                     | 20.19               | -7.3                  | 0.89            | -1.04          |
| 14    | Saquinavir        | -7.14                    | 5.87                | -10.72                | 3.58            | 39.33          |
| 15    | Nelfinavir        | -4.67                    | 374.41              | -8.25                 | 3.58            | -2.94          |

Out of 13 compounds evaluated from Azadirachta indica, the binding energies lesser than the upper threshold (≤-6Kcal/mol) were generally regarded as a cut-off in ligand binding studies [17, 18]. The binding affinity for protease 6Y2E ranged between 3.72 (Quercitrin) to -7.52 (Epiazadiradione). The binding affinities for protease 6Y2E were -7.32, -6.63, -6.07, -6.23, and -6.4 for Azadiradione, Beta-sitosterol, Meldenin, Nimbione, and Vepnin respectively. The binding energy of Saquinavir was -6.89. When comparing the values of binding energies, Epiazadiradione and Azadiradione had more binding energy than Saquinavir.

Among the thirteen compounds evaluated from Azadirachta indica, the binding energies more than the upper threshold limit, of -6Kcal/mol were only three. The binding affinity for protease 6LU7 ranged between -2.84 (Quercitrin) to -6.79 (Vepnin). The binding affinities for protease 6LU7 were -6.14, and -6.48 for Azadiradione, and Nimbione respectively. The binding energy of Saquinavir was -6.49. The binding affinities of these three compounds were reasonably compared well with that of Saquinavir.
Table 3: Molecular docking analysis of bioactive molecules from Azadirachta indica against 6LU7

| S. No. | Name of the ligand      | Binding energy | Inhibition constant | Intermolecular energy | Torsional energy | Internal energy |
|-------|-------------------------|----------------|---------------------|-----------------------|-----------------|----------------|
| 1     | Azadiradione            | -6.14          | 31.51               | -7.04                 | 0.89            | -1.27          |
| 2     | Beta-sitosterol         | -4.94          | 238.75              | -7.03                 | 2.09            | -1.65          |
| 3     | Epiazadiradione         | -5.92          | 46.01               | -6.81                 | 0.89            | -1.34          |
| 4     | Epoxyazadiradione       | -5.51          | 90.85               | -6.41                 | 0.89            | -1.15          |
| 5     | Kaempferol              | -5.27          | 137.8               | -6.76                 | 1.49            | -1.4           |
| 6     | Meldenin                | -5.05          | 197.21              | -6.25                 | 1.19            | -2.12          |
| 7     | Myricetin               | -5.08          | 189.67              | -7.17                 | 2.09            | -2.17          |
| 8     | Nimbaflavone            | -4.04          | 110.0               | -6.42                 | 2.39            | -1.94          |
| 9     | Nimbinene               | -5.2           | 155.19              | -6.99                 | 1.79            | -2.02          |
| 10    | Nimbine                 | -6.48          | 17.84               | -6.78                 | 0.3             | 0.04           |
| 11    | Nimboicinide            | -6.49          | 120.62              | -7.67                 | 2.68            | -4.79          |
| 12    | Quercitin               | -2.84          | 8.32                | -5.82                 | 2.98            | -4.81          |
| 13    | Vepnin                  | -6.79          | 10.49               | -7.69                 | 0.89            | -0.85          |
| 14    | Saquinavir              | -6.49          | 17.36               | -10.67                | 3.58            | 38.13          |
| 15    | Nelfinavir              | -3.22          | 4.36                | -6.8                  | 3.58            | -3.48          |

Fig. 2: 3D Visualization of docking analysis of 6LU7 protease binding with 1. Azadiradione 2. Nimbione 3. Vepnin 4. Saquinavir. The yellow dots represent hydrogen bonds

Table 4: Molecular docking analysis of bioactive molecules from Azadirachta indica against 2GTB

| S. No. | Name of the ligand      | Binding energy (Kcal/mol) | Inhibition constant | Intermolecular energy | Torsional energy | Internal energy |
|-------|-------------------------|---------------------------|---------------------|-----------------------|-----------------|----------------|
| 1     | Azadiradione            | -6.96                     | 7.89                | -7.86                 | 0.89            | -1.2           |
| 2     | Beta-sitosterol         | -6.63                     | 22.94               | -8.42                 | 2.09            | -1.24          |
| 3     | Epiazadiradione         | -7.13                     | 5.9                 | -8.03                 | 0.89            | -1.31          |
| 4     | Epoxyazadiradione       | -6.69                     | 12.44               | -7.59                 | 0.89            | -1.89          |
| 5     | Kaempferol              | -5.22                     | 14.93               | -6.71                 | 1.49            | -1.38          |
| 6     | Meldenin                | -6.44                     | 19.0                | -7.63                 | 1.19            | -1.68          |
| 7     | Myricetin               | -5.06                     | 194.42              | -7.15                 | 2.09            | -2.02          |
| 8     | Nimbaflavone            | -5.93                     | 44.69               | -8.32                 | 2.39            | -1.96          |
| 9     | Nimbinene               | -4.68                     | 370.49              | -6.47                 | 1.79            | -2.04          |
| 10    | Nimbine                 | -6.66                     | 13.06               | -6.96                 | 0.3             | 0.04           |
| 11    | Nimboicinolide          | -5.3                      | 129.63              | -7.99                 | 2.68            | -4.28          |
| 12    | Quercitin               | -5.63                     | 75.17               | -8.61                 | 2.98            | -4.09          |
| 13    | Vepnin                  | -7.11                     | 6.15                | -8.0                  | 0.89            | -0.86          |
| 14    | Saquinavir              | -6.89                     | 8.92                | -10.47                | 3.58            | 38.02          |
| 15    | Nelfinavir              | -5.42                     | 106.48              | -9.0                  | 3.58            | -3.49          |

Fig. 3: 3D Visualization of docking analysis of 2GTB protease binding with 1. Azadiradione 2. Beta-sitosterol 3. Epiazadiradione 4. Epoxyazadiradione 5. Meldenin 6. Nimbione 7. Vepnin 8. Saquinavir. The yellow dots represent hydrogen bonds
Out of 13 compounds evaluated from *Azadirachta indica* the binding energies more than the upper threshold limit, of -6 Kcal/mol were selected. The binding affinity for protease 2G7B ranged between -4.68 (Nimbine) to -7.13 (Epiazadiradione). The binding affinities for protease 2G7B were -6.96, -7.13, -6.44, -6.66, -5.63, -7.11, -6.89 for Azadiradione, Beta-sitosterol, Epoxyazadiradione, Meldenin, Nimbione, and Vepnin respectively. The binding energy of Saquinavir was -6.89. The binding affinities of these compounds were reasonably compared well with that of Saquinavir. Nelfinavir showed binding energies lesser than the threshold limit with all the 3 proteases.

The binding affinities for protease 2G7B were -6.96, -7.13, -6.69, -6.44, -6.66, -5.63, -7.11, -6.89 for Azadiradione, Beta-sitosterol, Epoxyazadiradione, Meldenin, Nimbione, and Vepnin respectively. The binding energy of Saquinavir was -6.89. The binding affinities of these compounds were reasonably compared well with that of Saquinavir. Nelfinavir showed binding energies lesser than the threshold limit with all the 3 proteases.

Fig. 4 showed 2D visualization of binding sites of various bioactive compounds from *Azadirachta indica* to the active sites of coronavirus proteases 6Y2E, 6LU7, and 2GTB. The 3D and 2D visualization results clearly indicated that the ligand molecules bind to the active site of the coronavirus proteases and therefore, it could be expected to inhibit the enzyme activity and stopped the replication of the virus. Bioactive compounds (ligands) Azadiradione, Beta-sitosterol, Epoxyazadiradione, Meldenin, Nimbione, and Vepnin responded well against the Coronavirus proteases 6Y2E, 6LU7 and 2GTB.

Azadirachtin and Epoxyazadiradione had significantly higher or similar binding efficacy against their SARS-CoV-2 targets 6NSB (surface spike glycoprotein) 6Y84 (main Protease) and 6M71 (RNA dependent RNA polymerase) when compared to Lopinavir/Ritonavir and Remdesivir [19]. Shanmuga Subramanian et al. in 2020 showed that the binding energies obtained from the docking of 6LU7 with Melianianhydride, Nimocinol, Isomezelnin, Nimbolide, Zafaral, Nimbandiol, Nimbin, Nimbione, Desacetyl Nimbin were -14.3, -12.4, -12.3, -12.2, -11.9, -11.8, -11.7, -11.7, -11.4 kcal/mol respectively. These compounds showed better binding energies when compared with Remdesivir and HydroxyCholoroquine [20]. Apart from these bioactive compounds, neem leaves contain other compounds like Zinc, Vitamin A, Vitamin B1, Vitamin B2, Vitamin B6, Vitamin C, Vitamin E, etc. which may boost immunity [21].
CONCLUSION
The use of natural products for the disease cure and prevention is increasing all over the world because of its lesser side effects. *Azadirachta indica* is a common tree that will be present in all the houses and its leaves, bark, trunk; seeds had been already known for its antiviral and antibacterial properties around the world. The present study also proved that the bioactive compounds like Azadiradione, Beta-sitosterol, Epoxyazadiradione, Meldenin, Nimbinone, and Vepnin from *Azadirachta indica* had antiviral property against Coronavirus. However, further in vitro and in vivo tests are required to assess the compounds from the tree as clinical drugs.

FUNDING
Nil

AUTHORS CONTRIBUTIONS
All the authors have contributed equally.

CONFLICTS OF INTERESTS
Author declares that there are no conflicts of interest.

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