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Original Article

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 around the world, especially in India 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 M^{pr} ^o(6Y2E, 6LU7, and 2GTB) by Autodock 4.2, with the Lamarckian Genetic Algorithm. COVID-19 M^{pr} ^o 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: Azadirachtanin, Azadirachtol, and Salannolide, 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, Beta-sitosterol, Epiazadiradione, Epoxyazadiradione, Kaempferol, Meldenin, Myricetin, Nimbaflavone, Nimbinene, Nimbione, Nimbione, Nimbione, Reta-sitosterol, Epiazadiradione, Epoxyazadiradione, Kaempferol, Meldenin, Myricetin, Nimbaflavone, Nimbinene, Nimbione, Nimbione, Nimbione, Negotive, The binding energies obtained from the docking of 6LU7 with the native ligand, Azadiradione, Nimbione, Vepnin, and Saquinavir were-6.14, 6.48, 6.79 and-6.49 kcal/mol correspondingly. The binding energies obtained from the docking of 2GTB with the native ligand, Azadiradione, Epoxyazadiradione, Kaempferol, Meldenin, Myricetin, Nimbaflavone, Nimbione, Vepnin, and Neadiradione, Epoxyazadiradione, Kaempferol, Meldenin, Myricetin, Nimbaflavone, Nimbione, Vepnin, Saquinavir, and Nelfinavir were-6.96, -7.13, -6.69, -5.22, -6.44, -5.06, -5.33, -5.63, -7.11, -6.89 and -5.42kcal/mol, respectively.

Conclusion: Azadiradione, Epiazadiradione, Nimbione, 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 M^{pro}, 6Y2E, 6LU7 Autodock, *Azadirachta indica* and Saquinavir

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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 [1, 2]. 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 91,29,146 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 [4]. Common symptoms of the corona virus-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 the order Nidovirales, which are divided into four genera, namely α , β , γ , and δ [5]. The SARS-CoV-2 belongs to the β 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 Corona Virus 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 Unani 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, C log*P*>5, 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	
1	Azadinashtanin	C II O	Properties Malagular unsight	Value
1	Azaurachtanin	C32H40O11	Log P	3.82
			H-bond donor	2
			H-bond acceptor	11
			Molar refractivity	148.84
			Number of rotatable bonds	7
2	A 1: 1. 1		Violations	3
Z	Azadirachtoi	C28H36O13	Molecular weight	2 81
			H-bond donor	4
			H-bond acceptor	13
			Molar refractivity	131.30
			Number of rotatable bonds	5
			Violations	3
3	Azadiradione	C ₂₈ H ₃₄ O ₅	Molecular weight	450.57 g/mol
			Log P H bond donor	3.18
			H-bond acceptor	5
			Molar refractivity	125.48
			Number of rotatable bonds	3
			Violations	0
4	Beta-sitosterol	C ₂₉ H ₅₀ O	Molecular weight	414.71 g/mol
			Log P	5.05
			H-bond donor	1
			H-Donu acceptor Molar refractivity	122.22
			Number of rotatable bonds	6
			Violations	2
5	Epiazadiradione	$C_{28}H_{34}O_5$	Molecular weight	450.57 g/mol
			Log P	3.11
			H-bond donor	0
			H-bond acceptor	5
			Molar refractivity	125.48
			Violations	3 0
6	Epoxyazadiradione	C28H34O6	Molecular weight	466.57 g/mol
0	2 pony alla an a dione	020113400	Log P	3.32
			H-bond donor	0
			H-bond acceptor	6
			Molar refractivity	124.96
			Number of rotatable bonds	3
7	Kaampforol	CurtherOr	Violations	U 296.24 g/mol
/	Kaempieroi	C15H10O6	Log P	1 70
			H-bond donor	4
			H-bond acceptor	6
			Molar refractivity	76.01
			Number of rotatable bonds	1
0			Violations	0
8	Meldenin	C28H38O5	Molecular weight	454.60 g/mol
			Log P H-bond donor	5.45 1
			H-bond acceptor	5
			Molar refractivity	126.91
			Number of rotatable bonds	3
			Violations	0
9	Myricetin	$C_{15}H_{10}O_8$	Molecular weight	318.24 g/mol
			Log P	1.08
			n-uona aonor Hebond accontor	b g
			Molar refractivity	80.06
			Number of rotatable bonds	1
			Violations	ō
10	Nimbaflavone	$C_{26}H_{30}O_5$	Molecular weight	422.51 g/mol
			Log P	4.36
			H-bond donor	2
			H-bond acceptor	5
			Mular refractivity	123.48 6
			Violations	0

Table 1: Properties of ligand molecules from Azadirachta indica

Sharon

11 Nimbinene C ₂ H ₀ O ² Molecular weight 425.7 g/mol Log P 5.8 H-bond docer 7 H-bond docer 7 Nimber of rotatable bonds 6 Violations 280.37 g/mol Log P 2.24 H-bond acceptor 2.24 Nimber of rotatable bonds 280.37 g/mol Log P 2.24 H-bond docer 1 H-bond acceptor 3 Molecular weight 286.67 g/mol H-bond docer 1 H-bond docer 1 H-bond acceptor 3 Molecular weight 586.7 g/mol Molecular weight 586.7 g/mol H-bond acceptor 10 Molecular weight 586.7 g/mol Molecular weight 10					
12 Nimbione Log P 168 12 Nimbione CuH2:07 125:17 Number of rotatable bonds 6 12 Nimbione 22:17 Number of rotatable bonds 0 12 Nimbione 22:4 13 Nimborine 2:64 14 Nimborine 86:37 g/mol 13 Nimborine 6:07 g/mol 14 Nimborine 6:07 mol 15 Nimborine 6:07 mol 14 Querctrin CuHa:0m 10 14 Querctrin CuHa:0m 10 15 Salannolide CuHa:0m 10 16 H-bond acceptor 10 16 H-bond door 7 17 Nimber of rotatable bonds 6 18 Nearceptor 10 19 H-bond door 7 10 Number of rotatable bonds 5 11 H-bond acceptor 10 Nolar refactivity <td>11</td> <td>Nimbinene</td> <td>C₂₈H₃₄O₇</td> <td>Molecular weight</td> <td>482.57 g/mol</td>	11	Nimbinene	C ₂₈ H ₃₄ O ₇	Molecular weight	482.57 g/mol
12 Nimbione H-bond acceptor 7 12 Nimbione 6 12 Nimbione 28.37 g/mol 12 Nimbione 28.47 cm 13 Nimbione 28.47 cm 13 Nimbione 82.64 13 Nimbione 82.64 13 Nimbione 66.7 g/mol 14 H-bond door 3 15 Nimbori orisatable bonds 66.67 g/mol 16 H-bond door 28.4 17 Maccutar weight 68.67 g/mol 18 Minborinolide 10.4 19 H-bond acceptor 10.4 19 H-bond acceptor 10.4 19 Molar refractivity 10.4 10 Nimber of rotatable bonds 20.4 10 Nimber of rotatable bonds 10.4 10 Nimber of rotatable bonds 20.4 10 Nimber of rotatable bonds 20.4 10 Nimber of rotatable bonds 20.4				Log P	3.68
12 Nimbione $G_{u}H_{2}O_{3}$ Hobor fractacivity Number of rotatable bonds 7 12 Nimbione $G_{u}H_{2}O_{3}$ Molar refractivity Number of rotatable bonds 2.24 14 $Oole = 0$ 2.24 1 10 $P_{u} > 0$ 2.24 1 11 $Oole = 0$ 3 1 1 12 Nimbor onloide $G_{u}H_{u}O_{10}$ 10 2.24 13 Nimbor onloide $Oole = 0$ 3 1 14 Hool onor 1 1 1 14 Quercitrin $G_{u}H_{u}O_{11}$ Mole cular weight 26 2 14 Quercitrin $G_{u}H_{u}O_{11}$ Mole cular weight 100 10 15 Salannolide $G_{u}H_{u}O_{11}$ Mole cular weight 26.27 2 16 Hoon 1 10 1 10 16 Hoon 1 10 1 10 17 Salannolide $G_{u}H_{u}O_{11}$ Molecul				H–bond donor	0
12 Nimbione GaH2:09 Number of rotatable bonds Violations 64 12 Nimbione 263.37 g/mol Log P 263.37 g/mol Log P 263.73 g/mol Log P 13 Nimbocinolide 64 34 13 Nimbocinolide 64 34 14 Nimbocinolide 64 34 14 Nimbocinolide 64 34 14 Quercitrin 64 10 9 14 Quercitrin 62 14.901 10 34 14 Quercitrin 62 14.901 10 9 13 14 Quercitrin 62 14.901 10 10 10 14 Quercitrin 62 14.901 10 10 10 15 Salannolide 62 14.901 10 10 10 16 Vepnin 63 10 10 10 10 16 Vepnin 63 10 10 10 <t< td=""><td></td><td></td><td></td><td>H-bond acceptor</td><td>7</td></t<>				H-bond acceptor	7
12 Nimbione 6,aH2,O3 Number of rotatable bonds 6 12 Nimbione 2,44 2,44 Log P 2,44 3 H-bond donor 1 3 Nimbocinolide 0,3H3,O1 8,66,7 g/mol 2,64 Number of rotatable bonds 0 0 0 13 Nimbocinolide 0 0 0 0 14 Molocular weight 0 0 0 0 0 14 Quercitrin C ₁₁ H2,O1 Molocular weight 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0				Molar refractivity	128.17
Violations Violations Outcolura weight				Number of rotatable bonds	6
12 Nimbione CgiH2:03 Malecular weight 286.37 g/mol 10 1 1 H-bond door 1 H-bond acceptor 3 13 Nimbocinolide 6.37 Ha:03 13 Nimbocinolide 6.37 Ha:03 14 Nimbocinolide 6.37 Ha:03 15 Quercitrin 6.23 Ha:03 14 Quercitrin 6.21 Ha:03 15 Salannolide 6.21 Ha:03 16 Quercitrin 6.21 Ha:03 17 Quercitrin 6.21 Ha:03 18 Violations 20 19 Number of rotatable bonds 0 10 Number of rotatable bonds 20 11 Hoond door 7 11 Hoond door 7 12 Quercitrin 10 13 Salannolide 2 14 Poind door 7 15 Salannolide CaHa:01 Malecular weight 16 Poind door 1 17 Malecular weight 428.25 g/mol 18 Vepnin CaHa:01 Malecular weight 19 Poind door 1 10 Hoond door 1 </td <td></td> <td></td> <td></td> <td>Violations</td> <td>0</td>				Violations	0
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14 H-bond door 1 13 H-bond acceptor 3 13 Nimbocinolide 6,57 g/mol 13 Minor of rotatable bonds 0 14 Nimbocinolide 6,67 g/mol 14 Nimbocinolide 284 15 Malor refractivity 304 16 Numbor of rotatable bonds 6 17 Malor refractivity 100 donor 18 Molecular weight 483 g/mol 19 H-bond donor 7 10 H-bond acceptor 10 10 Malor refractivity 10 11 H-bond donor 7 12 H-bond donor 7 14 H-bond donor 10 15 Salannolide 6a 16 Molecular weight 6a 17 Molecular weight 36 18 Poind donor 1 19 H-bond donor 1 10 Molecular weight 6a <td></td> <td></td> <td></td> <td>Log P</td> <td>2.24</td>				Log P	2.24
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14 Log P 2.84 H-bond donor 3 Molar refractivity 150.48 Number of rotatable bonds 6 Violations 2 14 Quercitrin 6 14 Quercitrin 6 14 Quercitrin 48.38 g/mol 15 Salannolide 7 14 Molar refractivity 109.00 15 Salannolide 7 16 Molar refractivity 109.00 17 Molar refractivity 109.00 16 Molecular weight 62.871 g/mol 16 Molecular weight 62.871 g/mol 16 Molar refractivity 159.14 17 Molar refractivity 159.14 16 Weight 62.88 g/mol 16 Molar refractivity 159.14 17 Saquinavir CaaHaOs 160 16 Molecular weight 62.88 g/mol 17 Saquinavir GaubaohaOs 160	13	Nimbocinolide	C ₃₂ H ₄₂ O ₁₀	Molecular weight	586.67 g/mol
 H-Bond donor H-Bond denor H-Bond denor H-Bond cecptor Mumber of rotatable bonds Molex refractivity Molecular weight Molecular weight Log P Log P Log P Log P H-Bond donor Totatable bonds H-Bond donor Reserved P Log P H-Bond donor Reserved P H-Bond donor H-Bond donor				Log P	2.84
 H-bond acceptor Number of rotatable bonds Yolations Yolations<				H–bond donor	3
14 Quercitrin C ₂ H ₂₀ 01 Molector fortable bonds Violations 2 14 Quercitrin C ₂ H ₂₀ 01 Molecular weight Molecular weight 48.38 g/mol 14 Quercitrin C ₂ H ₂₀ 01 Molecular weight 48.38 g/mol 14 Quercitrin C ₂ H ₂₀ 01 10 9 15 Salannolide C ₂ H ₄₀ 01 Molecular weight 09.00 15 Salannolide C ₂ H ₄₀ 01 Molecular weight 8 0 16 Holond donor Holond donor 1 1 1 16 Wolecular weight Gal 8.71 g/mol 1 1 16 Wolecular weight Gal 8.71 g/mol 1 1 16 Wolecular weight Gal 8.71 g/mol 1 1 16 Wolecular weight Gal 8.70 g/mol 1 1 17 Saquinavir C ₂₈ H ₈₀ Nofs Molecular weight 1 1 18 Moleinavir Gal 8.50 Mol Gal 8.50 Mol 1 1 <td< td=""><td></td><td></td><td></td><td>H-bond acceptor</td><td>10</td></td<>				H-bond acceptor	10
 14 Quercitrin 14 Quercitrin 12:H2001 14:Molecular weight 15:Molecular weight 14:Molecular weight 15:Molecular weight 14:Molecular weight 14:Molecul				Molar refractivity	150.48
14 Quercitrin C2:H2001 Violations 2 14 Quercitrin C2:H2001 Molecular weight 484.38 g/mol 160 100 1 160 1 100 17 Salannolide C3:H4:001 100 15 Salannolide C3:H4:001 Molecular weight 20.71 15 Salannolide C3:H4:001 Molecular weight 20.71 16 Wolecular weight 20.71 3.65 17 Salannolide C3:H4:001 H-bond acceptor 11 18 Vepnin C2:H3:05 Molecular weight 20.71 17 Saquinavir C3:H3:05 Molecular weight 20.71 18 Vepnin C3:H3:05 Molecular weight 20.71 17 Saquinavir C3:H3:05 Molecular weight 20.71 18 Nelfinavir C3:H3:05 Molecular weight 20.72 19 H-bond acceptor 10 10 10 Malecular weight 20.71 20.71 10 Molecular weight 60.71 20.72 10 Molecular weight 60.71 20.72 10 Molecular weight 70.72 20				Number of rotatable bonds	6
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 15 Salannolide 15 Salannolide C34H4011 Molocular weight H-bond acceptor Molecular weight C34H4011 Molecular weight C34H4011 Molecular weight C34H4011 C34H4011 Molecular weight C34H4011 C34H4011 Molecular weight C34H4011 C34H40111 C34H40111 C34H40111 C34H40111 C34H40111 C34	14	Quercitrin	$C_{21}H_{20}O_{11}$	Molecular weight	448.38 g/mol
 H-bond donor H-bond acceptor H-bond acceptor H-bond acceptor Homer of rotatable bonds Violations 283 Salannolide C₃₄H₄₄O₁₁ Mole cular weight Log P H-bond donor H-bond donor				Log P	1.60
15 Salannolide G ₃ H ₄₄ O ₁₁ H-bond acceptor 10 15 Salannolide G ₃ H ₄₄ O ₁₁ Moler of rotatable bonds 28 15 Salannolide G ₃ H ₄₄ O ₁₁ Mole cular weight 628.71 g/mol 16 Upper double weight 10 10 10 17 Vepnin C ₂₈ H ₃₆ O ₅ Mole cular weight 52.58 g/mol 16 Vepnin C ₂₈ H ₃₆ O ₅ Mole cular weight 52.58 g/mol 16 Vepnin C ₂₈ H ₃₆ O ₅ Mole cular weight 52.58 g/mol 17 Vepnin C ₂₈ H ₃₆ O ₅ Mole cular weight 52.58 g/mol 17 Vepnin C ₂₈ H ₃₆ O ₅ Mole cular weight 52.58 g/mol 17 Saquinavir C ₃₈ H ₃₀ N ₀ O ₅ Molecular weight 60.000 10 18 Melfinavir C ₃₈ H ₃₀ N ₀ O ₅ Molecular weight 60.000 10 18 Melfinavir C ₃₈ H ₃₀ N ₀ O ₅ Molecular weight 60.000 10 19 Molecular weight G ₁₀ C				H–bond donor	7
 15 Salannolide Gashave Ga				H-bond acceptor	11
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Number of rotatable bonds12Violations2				Molar refractivity	166.17
Violations 2				Number of rotatable bonds	12
				Violations	2

The ligands for which the active compound present in *Azadirachta indica* were downloaded from Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/phytochem/search/list). The ligands present in the study were Azadirachtanin, Azadirachtol, Azadiradione, Betasitosterol, Epiazadiradione, Epoxyazadiradione, Kaempferol, Meldenin, Myricetin, Nimbaflavone, Nimbinene, Nimbione, 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.

Molecular docking

The protease files were prepared for, COVID-19 6LU7 protease, 6Y2E protease, and 2GTB separately using Autodock 4.2. The

macromolecule was prepared using 'A' 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. Mol 2 format was 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 2020 Client.

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 (MERSCoV) 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 2019nCov 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). Azadirachtanin, Azadirachtol, 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 2 GTB.

Fable 2: Molecular docking	analysis of bioactive	molecules from Az	zadirachta indica	against 6Y2E
				0

S. No.	Name of the ligand	Binding energy	Inhibition	Intermolecular energy	Torsional	Internal
		(Kcal/mol)	constant		energy	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	Nimbaflavone	-5.02	208.13	-7.41	2.39	-2.54
9	Nimbinene	-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.



Fig. 1: 3D Visualization of docking analysis of 6Y2E protease binding with 1. Azadiradione 2. Beta-sitosterol 3. Epiazadiradione 4. Meldenin 5. Nimbione 6. Vepnin 7. Saquinavir. The yellow dots represent hydrogen bonds

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.

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S. No.	Name of the ligand	Binding	Inhibition	Intermolecular energy	Torsional	Internal
	-	energy	constant		energy	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	1100	-6.42	2.39	-1.94
9	Nimbinene	-5.2	155.19	-6.99	1.79	-2.02
10	Nimbione	-6.48	17.84	-6.78	0.3	0.04
11	Nimbocinolide	-4.99	220.62	-7.67	2.68	-4.79
12	Quercitrin	-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

S. No.	Name of the ligand	Binding energy	Inhibition	Intermolecular	Torsional energy	Internal
	-	(Kcal/mol)	constant	energy		energy
1	Azadiradione	-6.96	7.88	-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	-1.09
5	Kaempferol	-5.22	149.3	-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	Nimbione	-6.66	13.06	-6.96	0.3	0.04
11	Nimbocinolide	-5.3	129.63	-7.99	2.68	-4.28
12	Quercitrin	-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-6Kcal/mol were selected. The binding affinity for protease 2GTB ranged between-4.68 (Nimbinene) to-7.13 (Epiazadiradione). The binding affinities for protease 2GTB 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 6V2E, 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 2 GTB.

Azadirachtin and Epoxyazadiradione had significantly higher or similar binding efficacy against their SARS-CoV-2 targets 6VSB (surface spike glycoprotein) 6Y84 (main Protease) and 6M71 (RNA dependent RNA polymerase) when compared to Lopinavir/Ritonavir and Remdesivir [19]. Shanmuga Subramanian in 2020 showed that the binding energies obtained from the docking of 6LU7 with Meliacinanhydride, Nimocinol, Isomeldenin. Nimbandiol, Nimbolide. Zafaral. Nimbin. Nimbinene. Desacetylnimbin 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].



Fig. 4: 2D Visualization of molecular interaction with A) 6Y2E B) 6LU7 C) 2GTB with A1. Azadiradione, A2. Beta-sitosterol, A3. Epiazadiradione, A4. Meldenin, A5. Nimbione, A6. Vepnin, A7. Saquinavir, B1. Azadiradione, B2. Nimbione, B3. Vepnin, C1. Azadiradione, C2. Beta-sitosterol, C3. Epiazadiradione, C4. Epoxyazadiradione, C5. Meldenin, C6. Nimbione, C7. Vepnin, C8. Saquinavir

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, Nimbione, 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.

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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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