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USE OF MOLECULAR DOCKING TO VALIDATE EFFICACY OF BEE POLLEN PHYTOCHEMICALS ON SARS-COV-2 AND PREDICTION OF ADMET/TOXICITY PROPERTIES

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ABSTRACT

SARS-CoV-2 the virus that rooted the outbreak in Wuhan, China continues to spread rapidly across the globe. It belongs to the family Coronaviridae, the same family that caused the SARS pandemic. Such viral outbreaks hindered our societal structure as currently, we do not possess appropriate therapeutic advancements against viral infections. Henceforth, it is necessary to look for and develop plausible therapeutic options that are viable during such viral outbreaks. Bee pollen is one such object of curiosity that has not been well established as therapeutics but shows enormous potential. In this study, we aim to investigate bee pollen phytochemicals specific to the specie *Apis dorsata* against the S-protein of the SARS-CoV-2 as well as the ACE2 receptor complex. A link between these phytochemicals as an inhibitor for the entry of the virus inside the cell will also be established. In order to increase our horizon of study we have performed *in silico* analysis on two sets of protein structures and compared the obtained outcomes.

Keywords: Molecular docking, *Apis dorsata*, bee pollen, SARS-CoV-2, ACE2, ADMET.

INTRODUCTION

COVID-19, a SARSCoV2 infection, causes fever, cough, and shortness of breath **[1, 2]**. Coughing, sneezing, and direct touch with an infected person spread COVID-19 **[2]**. COVID-19 has prompted social isolation and city lockdowns. Even these tight procedures have not been enough in some regions to contain SARS-CoV-2, a

global public health danger **[3]**. SARS-CoV2 has infected almost 20 million individuals, and 800,000 have died of respiratory complications **[4]**. Because of the public health disaster COVID-19 has produced, researchers are looking for therapeutic or preventative medications. Several studies have commended medication repurposing as an effective method in this context. Various research centres have seen promise in hydroxychloroquine/chloroquine, lopinavir/ritonavir, remdesivir, dexamethasone, and ivermectin as COVID-19 therapies **[2, 5]**. mRNA SARS-CoV-2 and adenovirus type-5 vectored vaccines have made breakthroughs in immunotherapy, and their preliminary safety, tolerability, and immunogenicity against COVID-19 are encouraging **[2, 6]**. Isolation and social avoidance are the only ways to prevent the transmission of COVID-19, as no licenced vaccine or antiviral agent has been discovered **[5]**.

Apitherapy-derived pharmaceuticals and nutraceuticals show promise in treating and preventing COVID-19. Honey, propolis, pollen, beeswax, royal jelly, and bee venom exhibit significant antiviral action against the virus **[7]**. Many of these natural compounds have immune system benefits, including allowing immune cells to mature, promoting antibody production, and boosting both innate and adaptive immunological responses.

The binding of viral spike proteins to angiotensin-converting enzyme 2 (ACE2) is a hallmark of infection with SARS-CoV-2, the virus responsible for COVID-19 **[8]**. Proteases like TMPRSS2 have crucial functions in viral infection, and they do so via activating the spike protein [9]. Once within, the coronavirus infection triggers endocytosis and the subsequent elevation of PAK1, a kinase that mediates lung inflammation, lung fibrosis, and other crucial mortality markers. Additionally, elevated PAK1 levels decrease the adaptive immune response, which allows for viral replication **[10, 11]**. Atypical pneumonia, marked by fast respiratory impairment and pulmonary failure, is associated with SARS-CoV-2 infection because of the elevated amounts of chemokines and activated pro-inflammatory cytokines that are produced during infection **[12]**. A major part of the SARS-CoV-2 spectrum has been attributed to immunological/inflammatory events, such as cytokine release syndrome. There is a stronger correlation between these mechanisms and organ dysfunction than between viral load and malfunction **[13]**.

Since honey and propolis have already been shown to have biological effects, we chose to investigate the potential anti-COVID-19 effectiveness of bee pollen. We hope that the results of this study would

shed light on a possible role for bee pollen constituents in anti-COVID-19 therapy.

MATERIALS & METHODS

Screening of bee pollen phytochemicals: The phytochemicals were selected indirectly based on the literature review. As the bee, *Apis dorsata* predominantly resides in south-pacific Asia, we focused on literature specific to southern parts of India, particularly Karnataka **[14]**. Primarily, the feeding pattern of the bee was studied followed by the selection of flower species predominantly found to be associated with it. Phytochemicals present in their flower petals, nectar, and pollen, were considered for the study. A list of thirty-two phytochemicals from four distinct flower species was prepared **[15-18]**. This list was further cross-referenced with phytochemical constituents of Apis dorsata obtained from different geographical locations **[18]**.

3D structure retrieval of receptors and ligands: We considered two forms of the receptor structure i.e., SARS-CoV-2 S-protein (Accession code: P0DTC2) and SARS-CoV-2 spike (Accession code: P0DTC2) receptor-binding domain bound with ACE2 (Accession code: Q9BYF1). For each protein, two sets of 3D crystal structures were acquired from PDB (https://www.rcsb.org/). The fetched PDB files (i) PDB ID: 6LZG, 2.50 Å resolution [19], (ii) PDB ID: 6M0J, 2.45 Å resolution **[20]**, (iii) PDB ID: 6VSB, 3.46 Å resolution **[21]**, (iv) PDB ID: 6VXX, 2.80 Å resolution **[22]** further underwent docking preparation on Chimera 1.16. In this procedure, first heteroatoms were removed followed by solvent deletion, replacement of incomplete side chains, and addition of hydrogen and charges **[23]**. Lastly, Gasteiger charges were added **[24]**. Energy minimization of the protein structure was performed on Discovery Studio **[25]**. As for ligands, PubChem database was used to retrieve 3D structures in SDF format **[26]**.

ADMET analysis: PreADMET, Molinspiration cheminformatics (https://www.molinspiration.com) and SwissADME web servers were utilized to predict drug-likeliness, bioactivity, physiochemical descriptors, and pharmacokinetic characteristics of the ligands **[27]**. SMILES format of the ligands was directly retrieved from PubChem and fed into ADME analysis tools. Based on results predicted by the servers a comparative analysis was done to screen the selected ligands. Lastly, toxicity analysis was performed based on results predicted by the Lazar Toxicity Prediction tool **[28]**.

Molecular Docking: Molecular docking was performed using open access software, PyRx. The automated built-in engine AutoDock Vina was used to screen binding affinities of the composed docking library comprised of phytochemicals to the

respective receptor based on the Lamarckian genetic algorithm using the gradient optimization method **[29, 30]**. Energy minimization was performed on the composed docking library. Binding cavities were visualized using Depth web server **[31]**. The four protein structures were subjected to docking against all the ligands, wherein the grid was set around 63 x 74 x 112 Å (6LZG, 6M0J) and 122 x 122 x175 Å (6VXB, 6VXX), exhaustiveness was set to 8. Four independent docking runs were performed. The obtained results were compared, and the interactions were analyzed on Discovery Studio 2021.

RESULTS AND DISCUSSION

Screening of bee pollen phytochemicals: It was elucidated based on the literature review that bee products obtained from *Apis dorsata* are commonly unifloral in nature. In this study, our approach was to identify plants that are predominantly preferred by this species in Karnataka, India. We discovered *Acacia catechu*, *Azadirachta indica*, *Bauhinia purpurea*, and *Eucalyptus globulus* to be present predominantly in the selected geographical area which in turn influences the feeding habits of the bees. Structures of phytochemicals screened for further analysis are listed below **(Table 1)**.

Table 1: Phytochemicals isolated from bee pollen obtained from respective sources

Sl.no.	PubChem ID	Phytochemical	Specie name
1	442154	Afzelechin	*Acacia catechu* (Black cutch)
2	5280863	Kaempferol	
3	443639	Epiafzelechin	
4	11033582	Mesquitol	
5	11461162	Ophioglonin	
6	122850	Aromadendrin	
7	129754	Naheedin	*Azadirachta indica* (Neem)
8	12308714	Azadiradione	
9	259846	Lupeol	
10	108058	Nimbin	
11	94162	Sugiol	
12	5281426	Umbelliferone	*Bauhinia purpurea* (Butterfly tree)
13	1794427	Chlorogenic acid	
14	5281855	Ellagic acid	
15	16043	Tert-butylhydroquinone	
16	73160	Catechin	*Eucalyptus globulus* (Gum tree)
17	5281672	Myricetin	
18	5280343	Quercetin	
19	5280443	Apigenin	
20	5280445	Luteolin	
21	5280805	Rutin	
22	5281654	Isorhamnetin	
23	68071	Pinocembrin	
24	65084	Gallocatechin	
25	445858	Ferulic acid	
26	444539	Cinnamic acid	
27	182232	Epicatechin	*Common among multiple species
28	72281	Hesperetin	
29	439246	Naringenin	
30	689043	Caffeic acid	
31	10742	Syringic acid	
32	637542	p-coumaric acid	

Table 2: Predictions obtained from SwissADME web server for screened ligands

PHYTOCHEMICAL	MR	TPSA	Consensus Log P	ESOL Class	GI absorption	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Lipinski #violations	Ghose #violations	Veber #violations	Egan #violations	Muegge #violations	Bioavailability Score	Synthetic Accessibility
Afzelechin	72.31	90.15	1.17	Soluble	High	Yes	No	No	No	No	No	0	0	0	0	0	0.55	3.39
Kaempferol	76.01	111.13	1.58	Soluble	High	No	Yes	No	No	Yes	Yes	0	0	0	0	0	0.55	3.14
Epiafzelechin	72.31	90.15	1.17	Soluble	High	Yes	No	No	No	No	No	0	0	0	0	0	0.55	3.39
Mesquitol	74.33	110.38	1.12	Soluble	High	Yes	No	No	No	No	No	0	0	0	0	0	0.55	3.49
Ophioglonin	80.55	120.36	1.52	Soluble	High	No	Yes	No	No	Yes	Yes	0	0	0	0	0	0.55	3.37
Aromadendrin	72.73	107.22	0.99	Soluble	High	No	No	No	No	No	No	0	0	0	0	0	0.55	3.42
Naheedin	148.27	93.06	4.55	Poorly soluble	High	Yes	No	No	No	No	No	1	3	0	0	1	0.55	6.97
Azadiradione	125.48	73.58	4.34	Moderately soluble	High	Yes	No	No	No	No	No	0	0	0	0	0	0.55	5.89
Lupeol	135.14	20.23	7.26	Poorly soluble	Low	No	No	No	No	No	No	1	3	0	1	2	0.55	5.49
Nimbin	138.81	118.34	3.24	Moderately soluble	High	No	No	No	No	No	No	1	3	0	0	0	0.55	6.54
Sugiol	92.06	37.3	4.64	Moderately soluble	High	No	No	Yes	Yes	No	No	0	0	0	0	1	0.55	3.45
Umbelliferone	44.51	50.44	1.51	Soluble	High	No	Yes	No	No	No	No	0	1	0	0	1	0.55	2.56
Chlorogenic acid	83.5	164.75	-0.38	Very soluble	Low	No	No	No	No	No	No	1	1	1	1	2	0.11	4.16
Ellagic acid	75.31	141.34	1	Soluble	High	No	Yes	No	No	No	No	0	0	1	1	0	0.55	3.17
Tert-butylhydroquinone	49.76	40.46	2.24	Soluble	High	No	Yes	No	No	No	No	0	0	0	0	1	0.55	1.03
Catechin	74.33	110.38	0.85	Soluble	High	Yes	No	No	No	No	No	0	0	0	0	0	0.55	3.5
Myricetin	80.06	151.59	0.79	Soluble	Low	No	Yes	No	No	No	Yes	1	0	1	1	2	0.55	3.27
Quercetin	78.03	131.36	1.23	Soluble	High	No	Yes	No	No	Yes	Yes	0	0	0	0	0	0.55	3.23
Apigenin	73.99	90.9	2.11	Soluble	High	No	Yes	No	No	Yes	Yes	0	0	0	0	0	0.55	2.96
Luteolin	76.01	111.13	1.73	Soluble	High	No	Yes	No	No	Yes	Yes	0	0	0	0	0	0.55	3.02
Rutin	141.38	269.43	-1.29	Soluble	Low	Yes	No	No	No	No	No	3	4	1	1	4	0.17	6.52
Isorhamnetin	82.5	120.36	1.65	Soluble	High	No	Yes	No	No	Yes	Yes	0	0	0	0	0	0.55	3.26
Pinocembrin	69.55	66.76	2.26	Soluble	High	No	Yes	Yes	No	No	No	0	0	0	0	0	0.55	2.96
Gallocatechin	76.36	130.61	0.42	Soluble	High	No	No	No	No	No	No	1	0	0	0	1	0.55	3.53
Ferulic acid	51.63	66.76	1.36	Soluble	High	No	No	No	No	No	No	0	0	0	0	1	0.85	1.93
Cinnamic acid	43.11	37.3	1.79	Soluble	High	No	No	No	No	No	No	0	2	0	0	1	0.85	1.67
Epicatechin	74.33	110.38	0.85	Soluble	High	Yes	No	No	No	No	No	0	0	0	0	0	0.55	3.5
Hesperetin	78.06	96.22	1.91	Soluble	High	Yes	Yes	No	No	No	Yes	0	0	0	0	0	0.55	3.22
Naringenin	71.57	86.99	1.84	Soluble	High	Yes	Yes	No	No	No	Yes	0	0	0	0	0	0.55	3.01
Caffeic acid	47.16	77.76	0.93	Very soluble	High	No	No	No	No	No	No	0	0	0	0	1	0.56	1.81
Syringic acid	48.41	75.99	0.99	Very soluble	High	No	No	No	No	No	No	0	0	0	0	1	0.56	1.7
P-coumaric acid	45.13	57.53	1.26	Soluble	High	No	No	No	No	No	No	0	0	0	0	1	0.85	1.61

Table 3: LazarTox prediction for toxicity of screened ligands

S.NO.	PHYTOCHEMICAL	ALGAE AT	AMES TEST	CARCINO MOUSE	CARCINO RAT	DAPHNIA AT	HERG INHIBITION	MEDAKA AT	MINNOW AT	TA100 10RLI	TA100 NA	TA1535 10RLI	TA1535 NA
1	Afzelechin	0.036335	Mutagen	Negative	Negative	0.18317	Medium_risk	0.051697	0.032932	Negative	Positive	Negative	Negative
2	Kaempferol	0.048322	Mutagen	Negative	Positive	0.196882	Medium_risk	0.064254	0.029489	Negative	Positive	Negative	Negative
3	Epiafzelechin	0.036335	Mutagen	Negative	Negative	0.18317	Medium_risk	0.051697	0.032932	Negative	Positive	Negative	Negative
4	Mesquitol	0.028838	Mutagen	Negative	Positive	0.197951	Medium_risk	0.061858	0.037898	Negative	Negative	Negative	Negative
5	Ophioglonin	0.05064	Mutagen	Negative	Negative	0.259064	Low_risk	0.113591	0.056697	Negative	Positive	Negative	Negative
6	Aromadendrin	0.052098	Mutagen	Negative	Positive	0.255214	Medium_risk	0.102155	0.053138	Negative	Positive	Negative	Negative
7	Naheedin	0.00571	Non-mutagen	Positive	Positive	0.033665	Ambiguous	0.002315	0.001614	Negative	Negative	Negative	Negative
8	Azadiradione	0.02925	Non-mutagen	Positive	Positive	0.050855	Medium_risk	0.004999	0.0041	Negative	Negative	Negative	Negative
9	Lupeol	0.00133	Mutagen	Negative	Positive	0.003051	Low_risk	1.89E-05	1.60E-06	Negative	Negative	Negative	Negative
10	Nimbin	0.031927	Mutagen	Negative	Positive	0.090015	Medium_risk	0.016877	0.021039	Negative	Negative	Negative	Negative
11	Sugiol	0.009915	Non-mutagen	Negative	Negative	0.018556	Low_risk	0.000579	0.000806	Negative	Negative	Negative	Negative
12	Umbelliferone	0.130271	Mutagen	Positive	Positive	0.406501	Medium_risk	0.205774	0.090854	Positive	Positive	Positive	Positive
13	Chlorogenic acid	0.041505	Mutagen	Positive	Negative	1.3461	Medium_risk	2.63779	2.19597	Negative	Negative	Negative	Positive
14	Ellagic acid	0.043818	Mutagen	Negative	Positive	0.15038	Low_risk	0.039986	0.021897	Negative	Positive	Negative	Negative
15	Tert-butylhydroquinone	0.029372	Mutagen	Negative	Negative	0.205975	Low_risk	0.051648	0.040018	Negative	Positive	Positive	Negative
16	Catechin	0.028731	Mutagen	Negative	Negative	0.196185	Medium_risk	0.060826	0.036149	Negative	Positive	Negative	Negative
17	Myricetin	0.029142	Mutagen	Negative	Positive	0.23573	Low_risk	0.096131	0.039996	Negative	Positive	Negative	Negative
18	Quercetin	0.037814	Mutagen	Negative	Positive	0.214345	Medium_risk	0.077881	0.033503	Negative	Positive	Negative	Negative
19	Apigenin	0.052748	Mutagen	Negative	Positive	0.130131	Medium_risk	0.028058	0.015273	Positive	Positive	Negative	Negative
20	Luteolin	0.041631	Mutagen	Negative	Positive	0.139325	Medium_risk	0.032988	0.016905	Negative	Positive	Negative	Negative
21	Rutin	0.006959	Non-mutagen	Negative	Negative	2.55255	Ambiguous	12.3433	5.4421	Negative	Negative	Negative	Negative
22	Isorhamnetin	0.036171	Mutagen	Negative	Positive	0.190058	Medium_risk	0.061367	0.033279	Negative	Positive	Negative	Negative
23	Pinocembrin	0.061787	Mutagen	Negative	Negative	0.144608	Medium_risk	0.032239	0.021757	Positive	Positive	Positive	Negative
24	Gallocatechin	0.022363	Mutagen	Negative	Negative	0.212479	Low_risk	0.073013	0.041758	Negative	Positive	Negative	Negative
25	Ferulic acid	0.084839	Mutagen	Negative	Positive	0.39795	Medium_risk	0.201675	0.127863	Negative	Positive	Negative	Positive
26	Cinnamic acid	0.124309	Mutagen	Negative	Negative	0.401241	Medium_risk	0.192287	0.104523	Positive	Negative	Negative	Negative
27	Epicatechin	0.028731	Mutagen	Negative	Negative	0.196185	Medium_risk	0.060826	0.036149	Negative	Positive	Negative	Negative
28	Hesperetin	0.035876	Mutagen	Negative	Positive	0.148029	Medium_risk	0.035662	0.023743	Negative	Positive	Negative	Negative
29	Naringenin	0.04756	Mutagen	Negative	Negative	0.155909	Medium_risk	0.038448	0.022097	Positive	Positive	Negative	Negative
30	Caffeic acid	0.084998	Mutagen	Negative	Positive	0.422898	Medium_risk	0.22974	0.110442	Negative	Positive	Negative	Positive
31	Syringic acid	0.076215	Mutagen	Negative	Positive	0.660163	Low_risk	0.523704	0.298169	Negative	Negative	Positive	Negative
32	P-coumaric acid	0.10446	Mutagen	Positive	Positive	0.403109	Medium_risk	0.202018	0.103074	Positive	Negative	Negative	Positive

3D structure retrieval of receptors and ligands: In this study, we aim to provide a comparative analysis and discover plausible inhibitors that might either arrest the entry of SARS-CoV-2 in the cell or inhibit ACE2 receptor binding. Here the analysis was performed on complete spike glycoprotein of SARS-CoV-2 as well as on RBD of S-protein complexed with ACE2. There are 2 PDB files considered for each of the selected macromolecules, namely PDB ID: 6LZG, PDB ID: 6M0J, and PDB ID: 6VSB, PDB ID: 6VXX **(Figure 1)**.

We considered multiple structures of the same protein as there are minute differences between them i.e., the A chain length of ACE2 is 596 & 603 whereas the E chain length of S-protein S1 is 209 & 229 for 6LZG and 6M0J, respectively. Similarly, the S-protein sequence length is 1281 and 1288 for 6VXX and 6VSB, respectively.

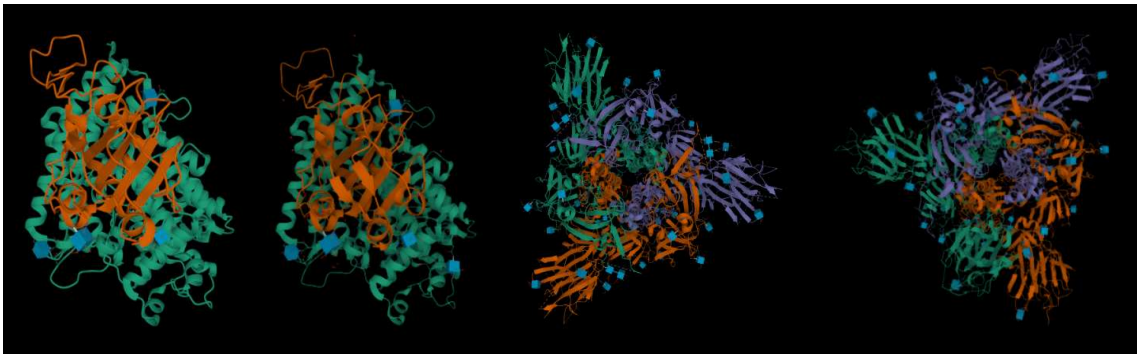

Figure 1: Structures of proteins retrieved from PDB, starting from left to right 6LZG, 6M0J, 6VXX, and 6VSB

ADMET analysis: Predictions for ADME parameters of the screened ligands obtained via *in silico* tools were utilized to investigate their drug-likeliness. As the therapeutic substance is naturally obtained, we aimed to discover its properties as a possible oral drug candidate. There are several independent rule-based filters developed to screen biochemical libraries for compatible pharmacokinetic profiles. SwissADME provides a total of five such rule-based filters **[32]**. Among thirty-two screened ligands rutin was predicted as least drug-like with four Ghose and Muegge violations, three Lipinski's violations and one Veber and Egan violations **(Table 2)**. Out of the rest, sixteen ligands were predicted to have zero violations across all five filters, while the remaining had at least one violation. The bioavailability of all the ligands was considerable.

Lipophilicity is another property that determines the pharmacokinetic behaviour of a bioactive within a system. There are many computational models to predict lipophilicity, SwissADME provides an arithmetic mean of predictions obtained from five such models. In the case of our phytochemicals, only rutin and lupeol had lipophilicity out of the range.

Pgp and Cyp proteins are two main candidates responsible for the metabolism and clearance of xenobiotics from the system, their inhibition or inactivation could possibly lead to severe side effects as well as drug toxicity as it directly correlates to their bioavailability. Among all the ligands from our library, none inhibited all the Cyp-isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) i.e., at least two of the proteins were not inhibited. The results for the bioavailability of the ligands have been depicted in the boiled egg **(Figure 2)**.

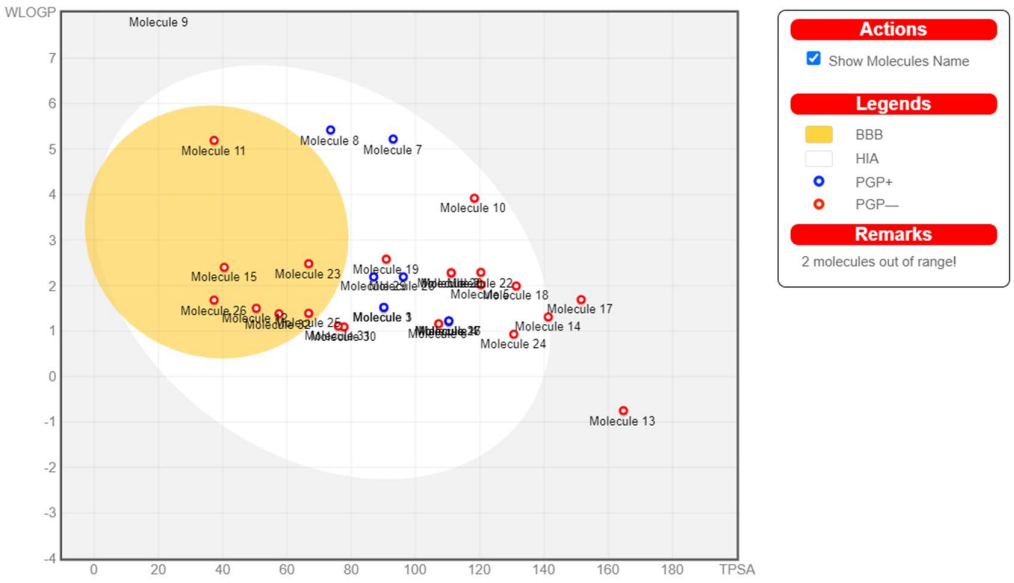

Figure 2: Results from SwissADME in boiled egg format

The toxicity predictions obtained from PreADMET were further examined and compared to predictions obtained from LazarTox, an *in silico* prediction tool. According to PreADM*Et al*l the ligands are predicted as mutagen against the AMES test except for naheedin, aradiradione, sugiol, and rutin. In addition, umbelliferone was not a suitable candidate as a therapeutic molecule since it was predicted to be a mutagen (AMES test), a carcinogen (mouse and rat model), and mutagen against all four strains of *Salmonella* species **(Table 3)**. Whilst according to LazarTox predictions, it was discovered that the toxicity profile of p-coumaric acid, ferulic acid, and quercetin

was not ideal. Furthermore, for nine of the ligands namely naheedin, aradiradione, nimbin, sugiol, chlorogenic acid, ellagic acid, myricetin, rutin, and gallocatechin at least 50 % of the predictions could not be provided due to insufficient dataset. Besides these, only six ligands namely hesperetin, epicatechin, catechin, mesquitol, epiafzelechin, and afzelechin were safe based on at least five parameters. However, toxicity results cannot be taken further without confirming via *in vitro* or *in vivo* analysis.

Molecular Docking: The spike protein on the virus giving it a crown-like appearance has been as established as the segment responsible for the internalization of the virus. The spike protein is a trimer consisting of S1 and S2 subunits. Among these subunits, S1 consists of RBD that binds to the ACE2 receptor while the S2 domain aids in internalization. This study screened potential therapeutics in bee pollen which might have inhibitory effects on the RBD-receptor binding phenomenon or internalization of the viral particle. As the complete structure of the S1 RBD-ACE2 complex and S-protein is not available online, the docking was performed on multiple structures to obtain a holistic result. Based on the binding affinity predicted based on the algorithm given by Trott and Olsen, it was found that rutin had the maximum binding affinity for three of the selected structures, while luteolin showed the best binding affinity **(Table 4)**. The best docking conformation binding affinity of the ligands is depicted in the table. Whilst the average docking score in the order of highest to lowest is as follows:

Rutin > ophioglonin > luteolin > ellagic acid > lupeol > gallocatechin > mesquitol > sugiol > pinocembrin > naringenin > Catechin > aromadendrin > myricetin > isorhamnetin > afzelechin > apigenin > quercetin > azadiradione > hesperetin > epicatechin > kaempferol > epiafzelechin > naheedin > nimbin > chlorogenic acid > caffeic acid > umbelliferone > cinnamic acid > p-coumaric acid > ferulic acid > tert-butylhydroquinone > syringic acid.

Table 4: Binding affinity of best-docked conformation of phytochemicals against selected protein structures

S.no.	PHYTOCHEMICALS	Binding Affinity			
		6M0J	6LZG	6VSB	6VXX
1	Afzelechin	-7.6	-9.2	-8.2	-8.2
2	Kaempferol	-8	-7.8	-8.7	-7.7
3	Epiafzelechin	-7.7	-7.8	-8.3	-8.2
4	Mesquitol	-7.9	-9.1	-8.5	-8.5
5	Ophioglonin	-8.6	-8.7	-9.1	-9.4
6	Aromadendrin	-7.5	-9.2	-8.7	-7.9
7	Naheedin	-8.3	-8.1	-7.9	-7.3
8	Azadiradione	-8.2	-8.5	-8	-7.9
9	Lupeol	-8.3	-8.4	-8.8	-9.1
10	Nimbin	-7.7	-7.5	-7.8	-8.1
11	Sugiol	-8.4	-8.3	-8.2	-9.1

12	Umbelliferone	-6.6	-7.6	-6.4	-6.6
13	Chlorogenic acid	-7.5	-6.8	-7.9	-8.2
14	Ellagic acid	-8.4	-8.7	-8.6	-9.2
15	Tert-butylhydroquinone	-6	-6.9	-6.2	-6.1
16	Catechin	-8.6	-8.9	-8.3	-7.6
17	Myricetin	-8.5	-8.3	-8.5	-8
18	Quercetin	-7.9	-7.8	-8.4	-8.6
19	Apigenin	-7.6	-9.3	-8.1	-8
20	Luteolin	-8.3	-9.8	-8.6	-8.8
21	Rutin	-8.7	-9.3	-9.9	-9.7
22	Isorhamnetin	-8	-8.4	-8.8	-8.1
23	Pinocembrin	-7.5	-9.2	-8.9	-7.9
24	Gallocatechin	-8.1	-9.1	-8.5	-8.7
25	Ferulic acid	-6.7	-7.5	-6.4	-6
26	Cinnamic acid	-7.2	-7.1	-6.3	-6.4
27	Epicatechin	-7.3	-8.5	-8.8	-7.7
28	Hesperetin	-8	-8.3	-8.2	-7.9
29	Naringenin	-7.9	-9.1	-8.8	-7.7
30	Caffeic acid	-6.9	-7.2	-6.8	-6.6
31	Syringic acid	-6	-5.8	-5.8	-5.6
32	P-coumaric acid	-7.2	-7.2	-6.4	-6.1

It has been observed that these ligands show a higher binding affinity at the pocket of the ACE2 receptor, while some conformations did fit in the RBD of S-protein and the interface however their binding affinities were low. Consequently, such bindings would destabilize the interactions between RBD and ACE2 indirectly inhibiting the internalization of the virus. As for the complete S-protein, it has been elucidated that the ligands are interacting with either of the subunits while certain ligand conformations interact with all three chains. Epiafzelechin, mesquitol, ophioglonin, azadiradione, sugiol, tert-butylhydroquinone, catechin, myricetin, quercetin, apigenin, luteolin, rutin, gallocatechin, ferulic acid, hesperetin, syringic acid showed best binding affinity towards S2 of the spike that is involved in cleavage during internalization into the cell. It was also evident that binding affinities of ligands in the S2 subunit were comparatively stronger than the ones binding at other sites. These possible interactions could hinder the conformational changes that occur when RBD binds to ACE2 initiating cleavage. Henceforth, the viral infection can be attenuated at an early stage.

CONCLUSION

The evident use of bee products can be traced back to 15,000 BC (Eroglu, 2020). However, the use of bee pollen as a nutritional and medicinal product became popular much later. Bee products like propolis, honey, and royal jelly are known for antiviral properties nevertheless, bee pollen as an antiviral agent does not have much clinical data. Bee pollen has well-established anti-microbial properties among other health benefits and knowing the preferred flora of *Apis dorsata* it can be hypothesized to have potent antiviral

properties. In recent years, bee products are being extensively studied for their properties against SARS-CoV-2. Since, the Covid pandemic crippled more than two hundred countries (Rath *et al*., 2020), it becomes essential for researchers to develop potential antiviral drugs. Currently, we do not possess such impactful antiviral medicines. This study aims to aid in screening plausible phytochemicals with potential antiviral activity. Based on obtained predictions we can take *Apis dorsata* bee pollen for further antiviral studies. As the selected phytochemicals showed high affinity towards not only the spike protein of the SARS-CoV-2 but also the S-protein and ACE2 complex. Moreover, the easy accessibility of the product makes it an ideal candidate for further *in vitro* & *in vivo* studies to validate apitherapy. In order to further validate the obtained results, microscopy-based assays and viral internalization assays can be performed to ascertain the interaction of SARS-CoV-2 on the cell surface as well as its internalization upon treatment with bee pollen extracts. Furthermore, to validate the toxicity results *in vitro* assays need to be performed for a better understanding of how these phytochemicals present in *Apis dorsata* bee pollen could potentially be toxic or non-toxic.

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