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A PRELIMINARY *IN-SILICO* ANALYSIS OF *OCIMUM SANCTUM* FLAVONOIDS, ORIENTIN AND VICENIN-1, AS POTENTIAL DRUGS AGAINST SARS-COV-2 INFECTION

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ABSTRACT: The SARS-CoV-2 virus is the causative agent of the Covid-19 pandemic. The major proteins of this virus, like covid main protease, RNA dependent, RNA polymerase, are being extensively studied with the hopes of finding an effective molecule that would bind to these proteins and lead to their inhibition. The inhibition of these critical viral proteins would hamper SARS-CoV-2 infection. With most of the currently available drugs falling short of providing an absolute cure and the reported side-effects of the current medications, a lot of phytochemicals are being investigated for their efficacy against the viral proteins. In our preliminary *in-silico* analysis, we found that the *Ocimum Sanctum* (Holy Basil/Tulsi) flavonoids, orientin, and vicenin-1, showed energetically favoured docking with the functionally critical amino acid residues of covid main protease, RNA dependent RNA polymerase, Spike protein, nucleocapsid, and 3a proteins of SARS-CoV-2 virus. These results hold promise for orientin and vicenin-1 to be further investigated as potential therapeutic drugs against SARS-CoV-2 infections.

INTRODUCTION: Covid-19 is now a worldwide pandemic bringing the world to a complete pause. The causative agent for this pandemic is a single-stranded RNA virus belonging to the beta coronavirus genera. Other viruses from this genera include Severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle Eastern respiratory syndrome coronavirus (MERS-CoV). It has officially been named as the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The approximately 32 kb RNA genome of the SARS-CoV-2 virus codes for the core viral proteins like RNA dependent RNA polymerase (RdRP), covid main protease (M^{pro}), the structural proteins like S (spike) protein, and the N (nucleocapsid) protein, and accessory proteins like 3a ion channel [1, 2].

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M^{pro} is a non-structural protein that is necessary for viral replication. M^{pro} cleaves the primary SARS-CoV-2 polyprotein into functional units that are involved in viral replication [3]. M^{pro} is highly conserved across the coronavirus family and has a similar site of cleavage [4]. M^{pro} has a cleavage site that is not shared by human proteases (Leu-Gln Ser-Ala-Gly), allowing the easy targeting of M^{pro} via inhibitors as human proteases would be immune to such inhibitors [5]. A lot of effort has gone into the search for a clinically feasible inhibitor of M^{pro}. Drugs like lymecycline and mizolastine, with clinical approval for use against other diseases like malaria, are also being tested as potential inhibitors of M^{pro} [6].

The RNA-dependent RNA polymerase (RdRP) of SARS-CoV-2 is involved in the replication and transcription of the viral RNA. The RdRP is a common protein present across several viruses like Ebola, SARS-CoV, etc. [7]. The SARS-CoV-2 RdRP shares several structural motifs with the other viral RdRPs and has a conserved reaction mechanism.

RdRP forms a complex with other viral proteins, and it is critical in the initiation and elongation of the viral RNA [8]. Viral RdRP forms a major target for potential anti-viral drugs. Drugs like remdesivir, sofosbuvir, favipiravir target the RdRP enzyme, and these have been approved for clinical use against infections caused by ebola, hepatitis C, and influenza viruses [7, 9]. The viral S protein is a glycoprotein that is highly conserved across all human coronavirus families. The S protein is the first point of contact for the SARS-CoV-2 virus with the host angiotensin-convertin enzyme-2 (ACE-2) protein. Thus S protein becomes indispensable for viral attachment and entry into the host cell. S protein also becomes important for mounting an immune reaction by the host, and as such, the S protein stays well camouflaged with polysaccharide molecules [10]. The structural nucleocapsid protein is conserved across the coronavirus family. The N protein binds to the viral RNA forming the ribonucleoprotein core, and it facilitates the entry of the viral RNA into the host cell. N protein is immunogenic as antibodies against N protein have been recovered from the sera of SARS-CoV-2 patients [11]. Both the N and S proteins are being investigated as targets for drug intervention, and several molecules have had limited success rates. Most drugs under study are small molecule inhibitors like EK1C4 and other peptide inhibitors [10, 12]. The SARS-CoV-2 3a protein is an accessory protein that oligomerizes to form a cation channel. A high level of sequence similarity has been observed between the 3a proteins from other coronavirus families. The 3a protein has pro-apoptotic activities in the host cells. The functioning of the SARS-CoV-2 3a protein is under intensive study, and the search for potential inhibitors is under pursual [2].

The current range of therapeutics mainly includes anti-retroviral drugs like remdesivir, plasma therapy, and inhibitors of host cell proteins like ACE-2 and protein synthesis machinery. All of these methods have had limited success rates. They have caused both short-term and long-term side effects in treated individuals. Looking at alternative and safer sources for a prospective cure, we turn towards flavonoids which have shown anti-viral properties against major human viruses like HIV-1, HCV, and coronaviruses [13]. *Ocimum sanctum* (Holy Basi/Tulsi) has been regarded as a medicinal plant for hundreds of years across South East Asia and has been dubbed as an 'Elixir of Life. The list of medicinal properties of tulsi includes anti-microbial, anti-viral, anti-diabetic, anti-cancer, to name a few. Tulsi has been a regular part of the diet of the people from India, Myanmar, Thailand *etc*. [14]. Amongst its many medicinal properties, the anti-viral aspect of tulsi can be further deliberated upon in the current scenario of the Covid-19 pandemic. In this discussion, we emphasize upon the two flavonoids which are components of Tulsi; namely; orientin and vicenin-1 [15]. These two flavonoids have anti-cancer and radioprotective effects. Our preliminary *in silico* analysis reveals that both of these flavonoids have the potential to bind to the critical SARS-CoV-2 proteins, RdRP, M^{pro}, S, N, and the 3a accessory protein. The binding occurs at amino acid residues which are crucial for the normal functioning of these viral proteins. Thus the two flavonoids, orientin, and vicenin-1, obtained from Tulsi, can be further checked for their ability to act against SARS-CoV-2 infections.

MATERIALS AND METHODS:

Molecular Docking Analysis:

Ligand Preparation: The 3D structures of the flavonoids, orientin, and vicenin-1, were retrieved from the PubChem database [16] in .sdf format. The 3D structures of flavonoids in their .sdf format were then converted to .pdb files using the Open Babel software [17]. The .pdb file format of the flavonoids was converted to a .pdbqt format using the MGL tools software [18]. The MGL tool software was used to remove water molecules, add polar H atoms and compute the Geisteger charges in the .pdbqt files of the flavonoids. The inhibitors that we checked in this study were converted to .pdbqt files using the same method. The structures of orientin and vicenin-1 are shown in **Fig. 1**.

Preparation of Protein Molecules: The crystal structures of the various proteins that we studied were obtained from the Protein Data Bank (RCSB) in .pdb format [19]. The Autodock 4.2 function of MGL tools were used to remove water molecules and heteroatoms the .pdb files. The polar H atoms were added, and the Kollmann charges were calculated. For setting the flavonoid/ligand binding site, the amino acid residues important for the functioning of the individual proteins were selected. The grid map was set using the grid box

set at 60 x 60 x 60 Å (x, y, and z), and the grid spacing was kept at 0.5 Å in such a way that the amino acids selected for the docking analysis are well covered. The amino acids against which the docking was performed and their functions have been listed in **Table 1**. The autogrid function was used to generate the autogrid files. For autodock analysis, protein and ligand of interest were selected. The search parameter was set at the Genetic algorithm, and the Lamarckian output option was chosen [18]. For analysis of the protein-ligand interactions, Discovery Studio software was used [20].

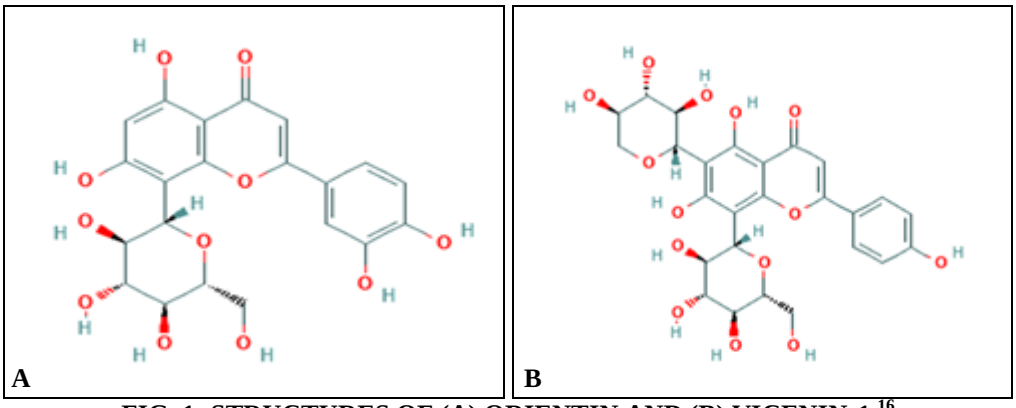

FIG. 1: STRUCTURES OF (A) ORIENTIN AND (B) VICENIN-1 [16]

TABLE 1: SARS-COV-2 PROTEINS AND THE AMINO ACIDS CHOSEN FOR DOCKING

Protein	PDB id	Amino acid selected for docking	Location of selected amino acids in the protein	Reference
Covid Main Protease	7C2Q	THR26, SER46, CYS44, GLY143, CYS145, GLU166, ARG188, THR190, GLN192	Present in the substrate-binding site of the protease	21
RdRP of SARS-CoV-2	6M71	TYR619, SER759, ASP760, ASP761, TRP800, GLU811	Present in the active site of the RNA polymerase	22
Spike protein	6XM4	ASN317, PHE318, ARG319, VAL320, GLN321, PRO322, CYS590	Regulation of positioning of Receptor-Binding domains of S protein	23
N protein	6M3M	ALA51, ARG89, ALA91, THR92, ARG108, TYR110, TYR112	Present in the active site of the N protein	24
3a protein	6XDC	TYR141, TYR154, GLU181, THR190, LYS192	Present in the tetramerization domain of the 3a protein	2

RESULTS AND DISCUSSION: Our studies showed that both the flavonoids showed a potent binding ability against the tested SARS-CoV-2 proteins implying their potential efficacy against infection. The binding energy values, constant inhibition values, and the predicted nature of the bonds have been listed in **Table 2**. The corresponding 2D and 3D images of the interactions have been listed in **Fig. 2** and **3**.

TABLE 2: BINDING ENERGIES, INHIBITION CONSTANT VALUES AND TYPES OF BONDS INVOLVED IN DOCKING OF ORIENTIN AND VICENIN-1 TO SELECTED AMINO ACIDS OF SARS-COV-2 PROTEINS

Protein	Flavonoid used	Binding energy (kcal/mol)	Inhibition constant	Types of Bonds
Covid Main Protease	Orientin	-5.04	201.79 mM	Hydrogen Bonding, Pi Alkyl, Pi Cation
	Vicenin-1	-4.54	467.71 mM	Hydrogen Bonding, Donor donor, Pi Alkyl, Pi Cation
RdRP of SARS-CoV-2 Spike protein	Orientin	-5.21	176.32 µM	Hydrogen bonding, Pi Anion, Carbon Hydrogen
	Vicenin-1	-4.69	363.21 mM	Hydrogen Bonding, Pi Alkyl, Pi Cation
	Orientin	-4.88	265.78 mM	Hydrogen bonding, Pi Alkyl, Pi Lone Pair, Pi Sigma Carbon Hydrogen
	Vicenin-1	-5.20	154.21 µM	Hydrogen bonding, Pi Alkyl, Carbon Hydrogen
N protein	Orientin	-4.44	556.45 mM	Hydrogen bonding, Pi Alkyl, Carbon Hydrogen
	Vicenin-1	-3.31	3.74 mM	Hydrogen bonding, Pi Alkyl, Pi Sigma, Pi-Pi stacked

3a protein	Orientin	-4.46	537.76 µM	Hydrogen bonding, Pi Alkyl, Pi Sigma , Unfavourable Acceptor-Acceptor Bond, Carbon Hydrogen
	Vicenin-1	-4.49	513.28 µM	Hydrogen bonding, Pi Alkyl, Pi Anion, Pi Sulphur, Carbon Hydrogen

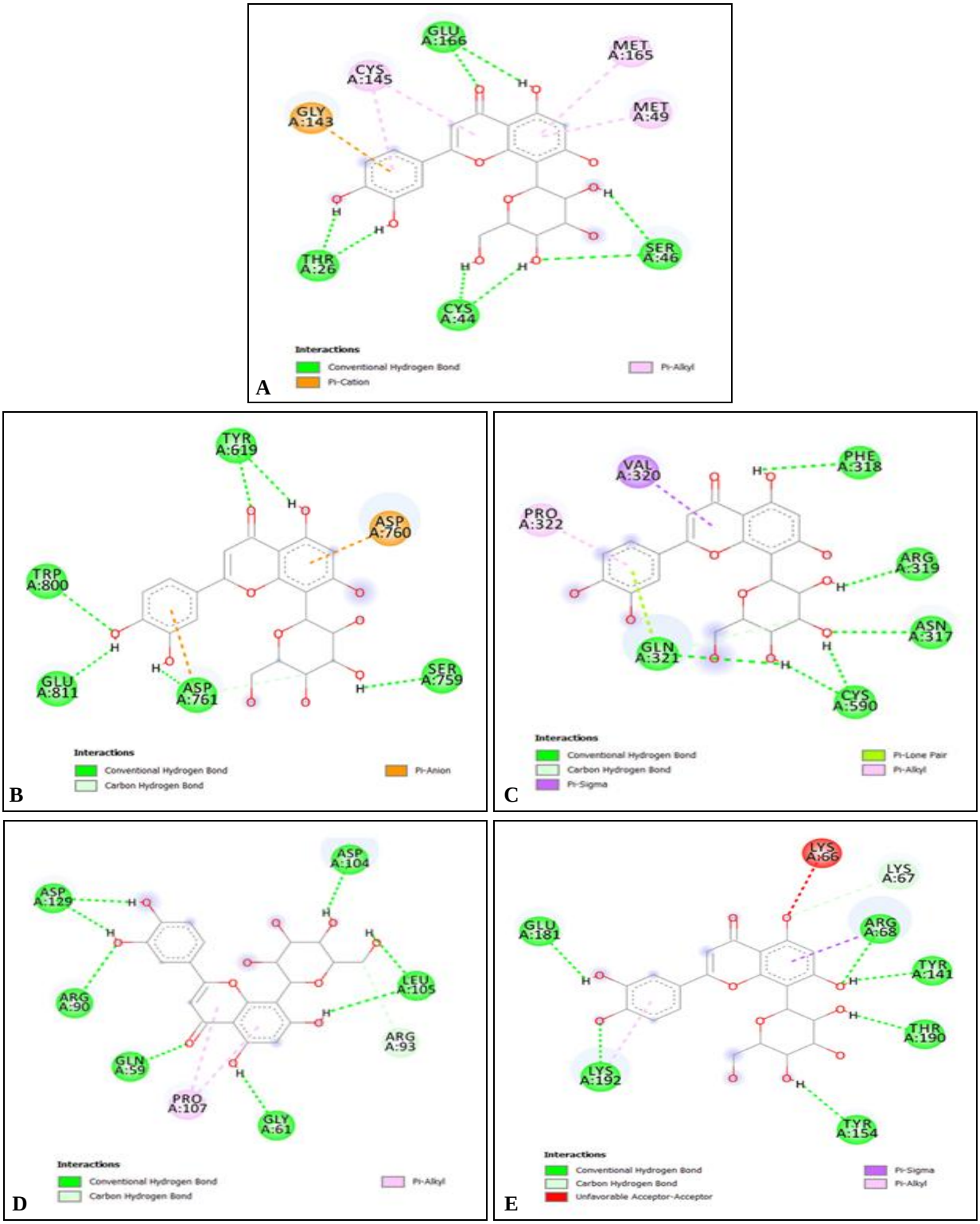

FIG. 2: 2D IMAGES OF THE INTERACTION OF ORIENTIN WITH THE SELECTED AMINO ACID RESIDUES OF (A) COVID MAIN PROTEASE, (B) RDRP, (C) S PROTEIN, (D) N PROTEIN, (E) 3A PROTEIN

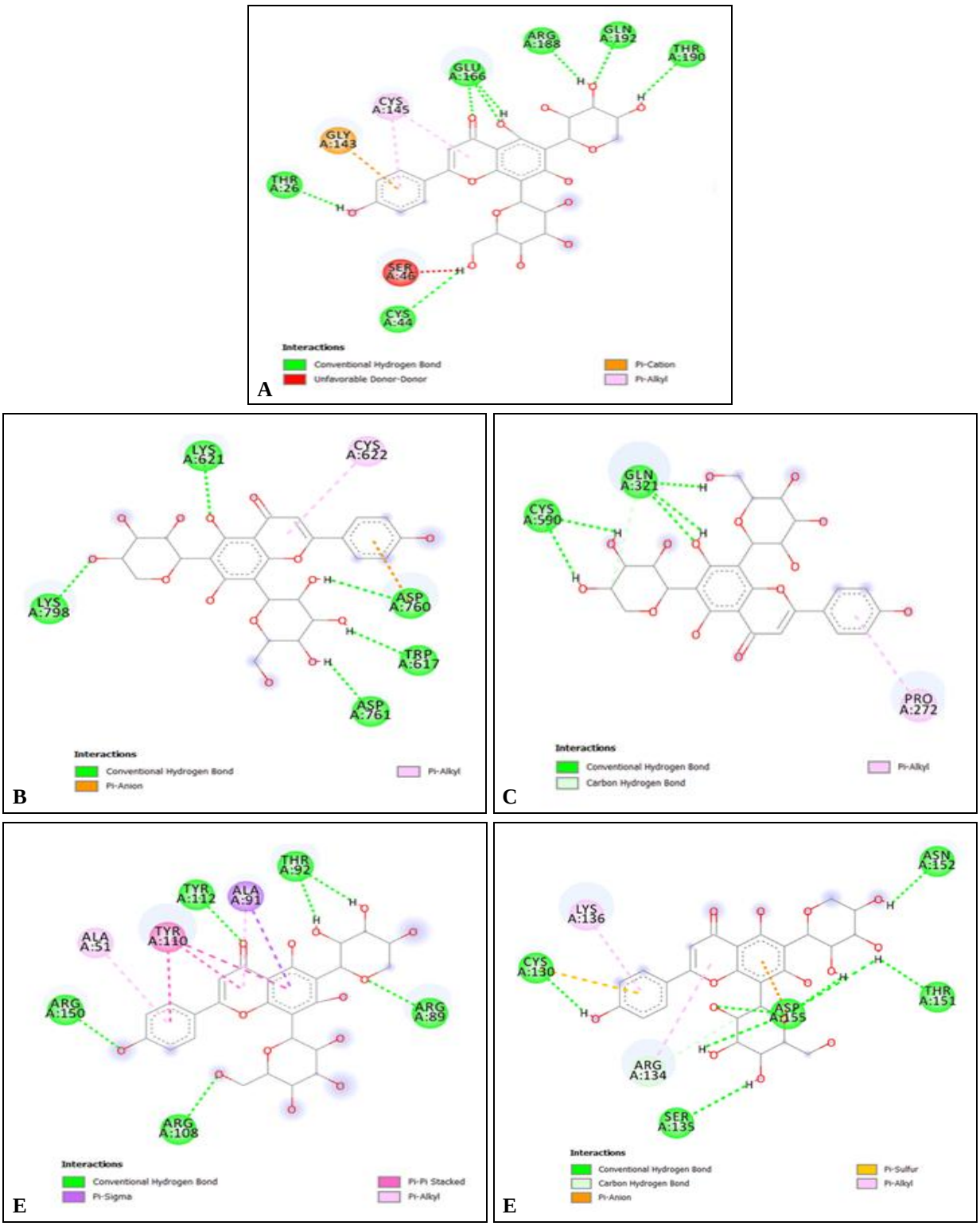

FIG. 3: 2D IMAGES OF THE INTERACTION OF VICENIN-1 WITH THE SELECTED AMINO ACID RESIDUES OF (A) COVID THE MAIN PROTEASE, (B) RDRP, (C) S PROTEIN, (D) N PROTEIN, (E) 3A PROTEIN

From **Table 2**, we can observe that the flavonoids orientin and vicenin-1 have a binding energy of -5.04 and -4.54 kcal/mol when docked against the selected active site amino acids of Covid main protease (**Fig. 2a** and **3a**). These values are lower than the binding energy obtained when we docked viomycin against the same amino acids, which stood at -3 kcal/mol. Viomycin is being actively pursued as a promising inhibitor of the SARS-CoV-2 main protease [25], and the ability of the two Ocimum flavonoids to bind better to the critical active site amino acid residues of the main protease

[21] holds promise for the two flavonoids to be checked more intensively. We docked the two flavonoids against the RNA-dependent RNA polymerase of the SARS-CoV-2 virus. The flavonoids, orientin, and vicenin-1, showed favourable binding energies of -5.21 and -4.69 kcal/mol, respectively, after being docked against critical active site amino acid residues [22] **Fig. 2b** and **3b**. Although remdesivir, the major drug inhibitor of RdRP, showed a comparatively lower binding energy of -8.28 kcal/mol but the binding energy of the two flavonoids was still competitively lower than that of the natural substrate, ATP (-4.14 kcal/mol) [26]. Keeping in mind the adverse side effects of remdesivir [27], the higher binding energy of ATP, and the overall safety profile of the two **Ocimum** flavonoids [15], the *in-vivo* efficacy of the two flavonoids against the RdRP can be analyzed soon.

Orientin and vicenin-1 showed a good *in silico* binding with the S protein (-4.88 and -5.20 kcal/mol, respectively), which is a major drug target to prevent SARS-CoV-2 entry into the cell (Figure 2c and 3c). The two flavonoids attached to amino acid residues that were involved in the regulation of the ACE-2 binding domain; and maintaining the immune silence of the S protein [23]. Thus the two flavonoids could be further investigated as peptide fusion inhibitors which are involved in preventing the binding of the S protein with the ACE-2 receptor. Nelfinavir mesylate is the first reported peptide fusion inhibitor against the SARS-CoV-2 virus [28]. We docked nelfinavir mesylate against the amino acids of interest in S protein and observed an almost similar binding energy (-6.06 kcal/mol) for nelfinavir mesylate as compared to the flavonoids in our study. These results further show the potency of the two flavonoids to act as possible peptide fusion inhibitors. Orientin and vicenin-1 showed favourable binding with the selected active site residues of N protein (binding energies of -4.44 and -3.31 kcal/mol) **Fig. 2d** and **3d**. We analyzed the ability of orientin and vicenin-1 to bind to the SARS-CoV-2 3a protein **Fig. 2e** and **3e**. Both the flavonoids showed favourable binding with the amino acids involved in oligomerisation of the 3a protein, which also happens to be a K^+ ion channel (binding energies of -4.46 and -4.49 kcal/mol, respectively, for orientin and vicenin-1). The inhibition of 3a protein oligomerization will affect its activity which will ultimately hinder the release of the virus particles, thereby blocking virulence [29]. The 3a protein inhibitor emodin also showed similar binding energy to the amino acids of interest in our study (-5.1 kcal/mol). Thus the flavonoids, orientin, and vicenin-1 could be further looked at as inhibitors of the 3a ion channel.

CONCLUSION: The study conducted by us showed that the two flavonoids present in Ocimum Sanctum, orientin, and vicenin-1; were able to bind to amino acid residues critical to the functioning of both structural and non-structural proteins of SARS-CoV-2. The flavonoids were able to dock with proteins that are needed at different stages of the virus life cycle, like S protein which is needed by the virus needed for entry into the cell, covid main protease, and RdRP, which are involved in the replication stage, and the N and 3a protein which are required at the later stages of the viral life cycle. Combined with their safety profile, our preliminary study shows that the flavonoids, namely, orientin and vicenin-1, need to be further analyzed as curative drugs against SARS-CoV-2 infection.

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CONFLICTS OF INTEREST: None to declare.

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