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In silico evaluation of the anti-SARS-CoV-2 activity in bael tree (*Aegle marmelos* (L.) Corrêa)

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Abstract: SARS-CoV-2 (Severe Acute Respiratory Syndrome-Coronavirus-2) is the cause of COVID-19, a highly contagious respiratory infection. The COVID-19 pandemic has induced serious adverse impact on the human population. It is a rapidly mutating virus which induced multiple epidemic waves globally and therefore the currently developed vaccines' efficacy is dubious. Since time immemorial plant-derived medications have been utilized to treat viral infections and many plant-derived drugs used in modern medicine found to have multiple therapeutic activities and prescribed for more than one disease. Identification of such type of phytochemicals with simultaneous inhibitory activity on different stages of pathogenicity is the best way to eradicate the mutating pathogen.

Method: A total of 150 phytochemicals from *Aegle marmelos* (L.) Correa, 135 were procured from databases and 15 phytochemicals were identified through LC-MS analysis of the methanolic extract. These were screened against the five therapeutic targets of SARS-CoV-2 namely spike protein, ACE2, M^{pro}, RdRp, and COX-2 through molecular docking using AutoDock Vina in PyRx 0.8 software. Further H- bond interactions, drug-likeness and ADMET were analyzed to identify the lead compound with multi-targeting properties.

Results: Overall results revealed that out of 150 phytochemicals screened, 38 of them have free energy of binding ≤ -6 kcal/mol against all five forenamed targets, making up 25% of the phytochemicals with multi-targeting properties, and the compound rutin was selected as the best lead molecule against SARS-CoV-2 based on binding energies, hydrogen bond interactions, pharmacokinetic analysis, and drug-likeness studies.

Keywords: SARS-CoV-2, COVID-19, *Aegle marmelos*, *in silico* screening

INTRODUCTION

SARS-CoV-2, a new β-coronavirus, is the cause of COVID-19, a highly contagious respiratory infection. As of April 26, 2023, the COVID-19 pandemic had affected people worldwide, resulting in 76,444,74387 confirmed cases and 69,15,286 deaths (https://covid19.who.int/). Since the beginning of the pandemic, multiple waves have been reported worldwide, despite the fact that various efforts were tried to curb the viral infection. The propensity of viral mutation resulted in the formation of hyper-evolved variants with great transmissibility, raising doubts about the effectiveness of viral medications and immunizations. The anti-viral ability of various phytochemicals has previously been proven, and plant-derived medications have been utilized to treat viral infections since time immemorial [1]. Due to their simultaneous action on several targets, herbal medications have the advantages of being more affordable, having fewer side effects, and possessing a variety of pharmacological qualities. Recently, drug discovery for complicated diseases like

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cancer, COVID-19, and drug-resistant disorders has heavily embraced "polypharmacology," or a multi-target drug strategy. A single drug targeting simultaneously different molecular targets at various phases of pathogenicity can be a more efficient therapeutic strategy in viral infections like COVID-19 since these viruses undergo continuous mutations. Many plant-derived compounds show multi-targeting properties against SARS-CoV-2 target proteins [2].

Numerous authors claim that Rutaceae plants, also known as citrus plants, have potential antiviral properties. *Aegle marmelos* (L.) Correa, also called bael, belongs to Rutacea family and is the only plant belonging to the genus *Aegle*. It has been used as antiviral, antibacterial, antioxidant, anti-diarrheal, anti-dyslipidemic, gastroprotective effects, anti-diabetic, anticancer and chemopreventive and anti-cobra venom agents [3,4]. The present investigation was aimed to demonstrate the anti-SARS-CoV-2 activity of phytochemicals derived from *A.marmelos* against molecular targets; spike protein (SP), angiotensin converting enzyme-2 receptor (ACE2), main protease (M^{pro}), RNA dependent RNA polymerase (RdRp) and, cyclooxygenase-2 (COX-2) through *in silico* method.

MATERIALS AND METHODS

Preparation of plant extract and LC-MS/MS analysis

The 200g fresh leaves of *A. marmelos* were collected from ~ 10 year old plants growing in the conservatory of KSCSTE-Jawaharlal Nehru Tropical Botanic Garden and Research Institute during the months of August, 2022 and dried at room temperature for 3 days and macerated using a blender and extracted in a Soxhlet using methanol. The extract was concentrated using a rotary evaporator and determined the total yield.

Phytochemical profiling of methanolic extracts was determined using LC-MS/MS (Agilent Technologies, USA), Model no. 1290 Infinity UHPLC system, 1260 Infinity Nano HPLC with chip cube 6550 iFunnel Q-TOFs.

Selection and preparation of target protein

The crystallographic structure of the target proteins namely, spike protein (S protein, PDB ID:6M0J), Angiotensin Converting Enzyme-2 receptor (ACE2, PDB ID: 1R4L), main protease (M^{pro}, PDB ID: 7BUY), RNA-dependent RNA polymerase (RdRp, PDB ID:7BV2) and cyclooxygenase-2 (COX-2, PDB ID: 5IKR) retrieved from Protein Data Bank (https://www.rcbs.org). The target proteins were prepared for docking using AutoDock tools and then transformed into the pdbqt format. ProtParam server was used to assess the aforementioned target proteins, and the PDBSum server to find the active sites of the proteins.

Selection and preparation of ligands

The structure of 135 phytomolecules were procured from the Pubchem and FoodB databases and through literature survey and 15 phytochemicals were determined through LC-MS analysis and all the phytochemicals 3D structures were obtained from the database, PubChem.

Molecular docking

Molecular docking was carried out using the tool AutoDock Vina integrated in PyRx 0.8 version, which applying the Lamarckian Genetic Algorithm and Empirical Free Energy Scoring Function [5]. The proteins were loaded as rigid and the ligands as flexible. The top five hits with the least binding energy were further studied using Pymol and Discovery studio visualizer.

Physiochemical and pharmacokinetic analysis

The physiochemical and pharmacokinetic properties were analyzed using SwissADME and Pkcsm online tools.

RESULTS AND DISCUSSION

LC-MS/MS analysis of methanolic extracts

The percentage yield of methanolic extracts was 2.16%. Fifteen compounds were identified through LC-MS/MS analysis of methanolic extracts, which include alkaloids, phenols, and terpenoids (Figure 1).

Compounds	Molecular formula	RT	m/z
1-(1-Pyrrolidinyl)-2-propanone	C7 H13 N O	5.09	150.0882
2-Ethyl-5-imino-1-cyclopenten-1-ol	C7 H11 N O	6.668	148.0726
Agaritinal	C12 H15 N3 O4	7.393	266.1123
S-Furanopetasitin	C24 H32 O5 S	8.796	433.2048
Linalool 3,7-oxide beta-primeveroside	C21 H36 O11	8.708	465.2308
Trilobolide	C27 H38 O10	12.689	545.2343
Tiliacorine	C36 H36 N2 O5	13.157	577.2596
Prosapogenin	C36 H54 O11	16.225	685.3539
Avadharidine	C36 H51 N3 O10	16.601	685.3529
Primidolol	C17 H23 N3 O4	17.08	334.1743
Verimol C	C18 H20 O4	18.718	323.1252
Geranyl 2-ethylbutyrate	C16 H28 O2	19.125	275.1976
Citronellyl hexanoate	C16 H30 O2	21.487	277.216
16-Hydroxy hexadecanoic acid	C16 H32 O3	22.965	295.224
Ganosporelactone A	C30 H40 O7	23.707	535.2661

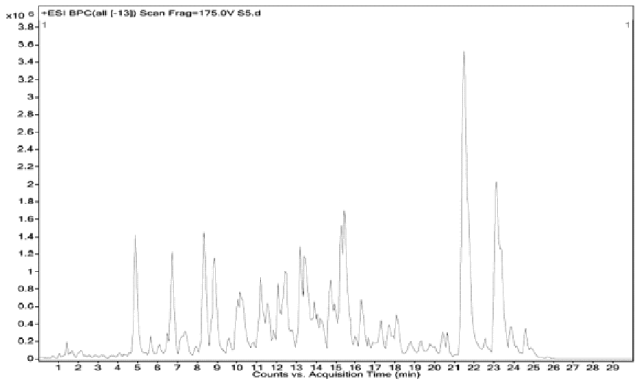


Figure 1: Phytoconstituents from *A.marmelos* methanolic extract with the molecular formula, retention time, and mass/charge ratio and LC-MS profile

Selection of target proteins

In the current investigation, molecular docking experiments were used to screen a total of 150 phytochemicals from *A. marmelos* against the five therapeutic target proteins of SARS-CoV-2, namely Spike protein, ACE2, M^{pro}, RdRp, and COX-2. Spike protein is a prime candidate for therapeutic development since it aids in viral entrance and adhesion. Proteins are made up of 9% helices, 43% sheets, 28% twists, and 47% coils in their three-dimensional structure. While the ACE2 receptor is disrupted, the receptor's structural changes prevent viral entry, making it an ideal drug target. Proteins are made up of 54% -helices, 6% -sheets, 39% twists, and 17% coils in their three-dimensional structure. M^{pro} is a unique therapeutic target for SARS-CoV-2 since inhibition of it prevents viral replication without endangering the host. M^{pro} is a homodimer of protomers A and B, a cysteine protease with a molecular weight of 33.8 kDa. M^{pro} has a 3-dimensional structure made up of 26% helices, 30% sheets, 24% twists, and 42% coils. RNA-dependent RNA polymerase (RdRp), RdRp is considered an important target against SARS-CoV-2 due to its function. Proteins are made up of 43% -helices, 19% -sheets, 23% twists, and 36% coils in their three-dimensional structure. Since SARS-CoV-2 infections are co-related with proinflammatory metabolites like COX-2, suppressing these can be relevant in the therapeutic strategies against COVID-19. The 3-dimensional structure of protein consists of 49% α-helices, 11% β-sheets, 23 % turns and 46% coils.

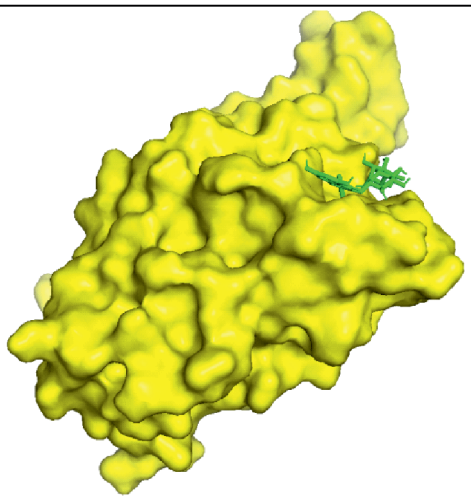
Molecular docking

Out of the 150 phytochemicals of *A. marmelos* docked with five therapeutic target, spike protein (SP), angiotensin converting enzyme-2 receptor (ACE2), main protease (M^{pro}), RNA dependent RNA polymerase (RdRp) and, cyclooxygenase-2 (COX-2), 38, 97, 62, 92 and 86 molecules showed $\Delta G_{bind} \leq -6$ kcal/mol against forenamed target respectively. The best five phytochemicals for each target proteins that showed least docking score were selected as hit molecules for further *in silico* ADMET, drug-likeness and interaction studies. The compound decursinol showed least binding energy (-8.1 kcal/mol) against spike protein forming a single H-bond with Asn343, and rutin formed 4 H-bonds involving three active site residues Tyr449, Gln493 and Asn501. Whereas other hits luvangetin, gamma-crane and beta-amyrin interacted only via hydrophobic interactions. Similarly, the compound rutin showed the strongest interaction (-10.8 kcal/mol) against ACE2 receptor establishing four H-bonds with active site residues Arg273 and Pro346. All other hits marmesinin, betulinic acid, beta-amyrin, and allocryptopine showed both H-bonds and hydrophobic interactions. It is noteworthy that the compound rutin shows inhibiting activity against all five targets, and interacts with active site residues of the respective targets (Figure 2).

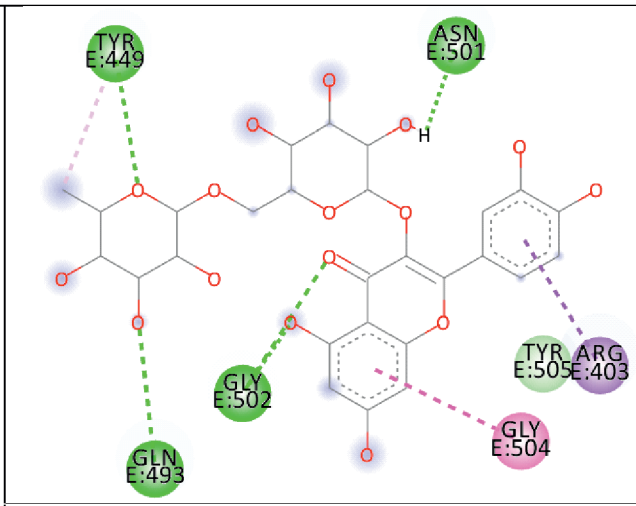
Post-docking analysis

All the compounds possess more than 90% intestinal absorption except rutin, marmesisnin, and aegelinoside A. Similary,

Protein-lead molecule interaction



1a



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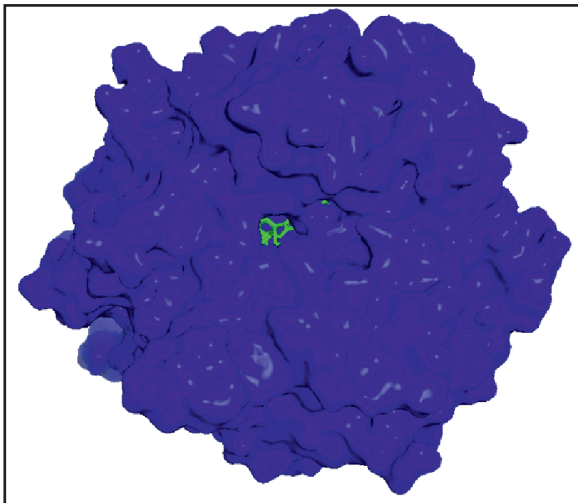

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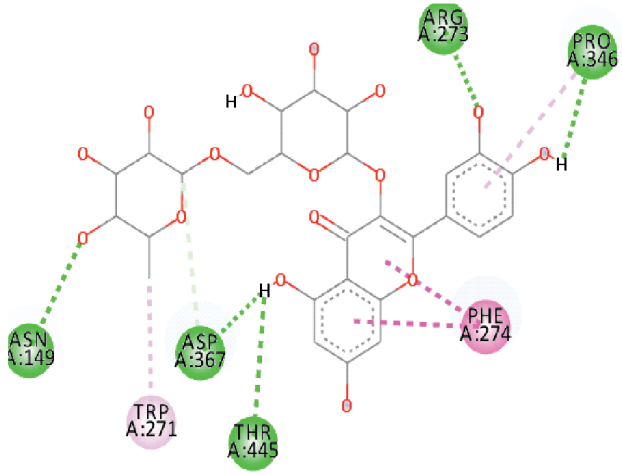

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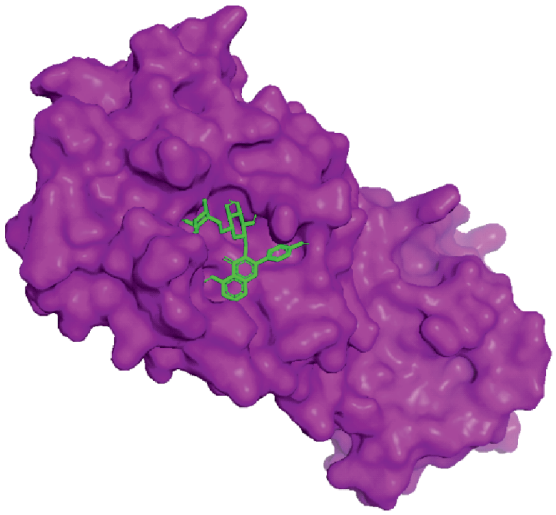

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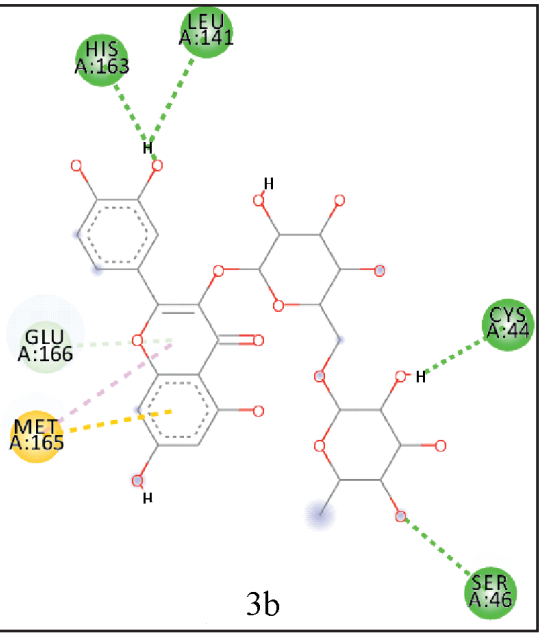

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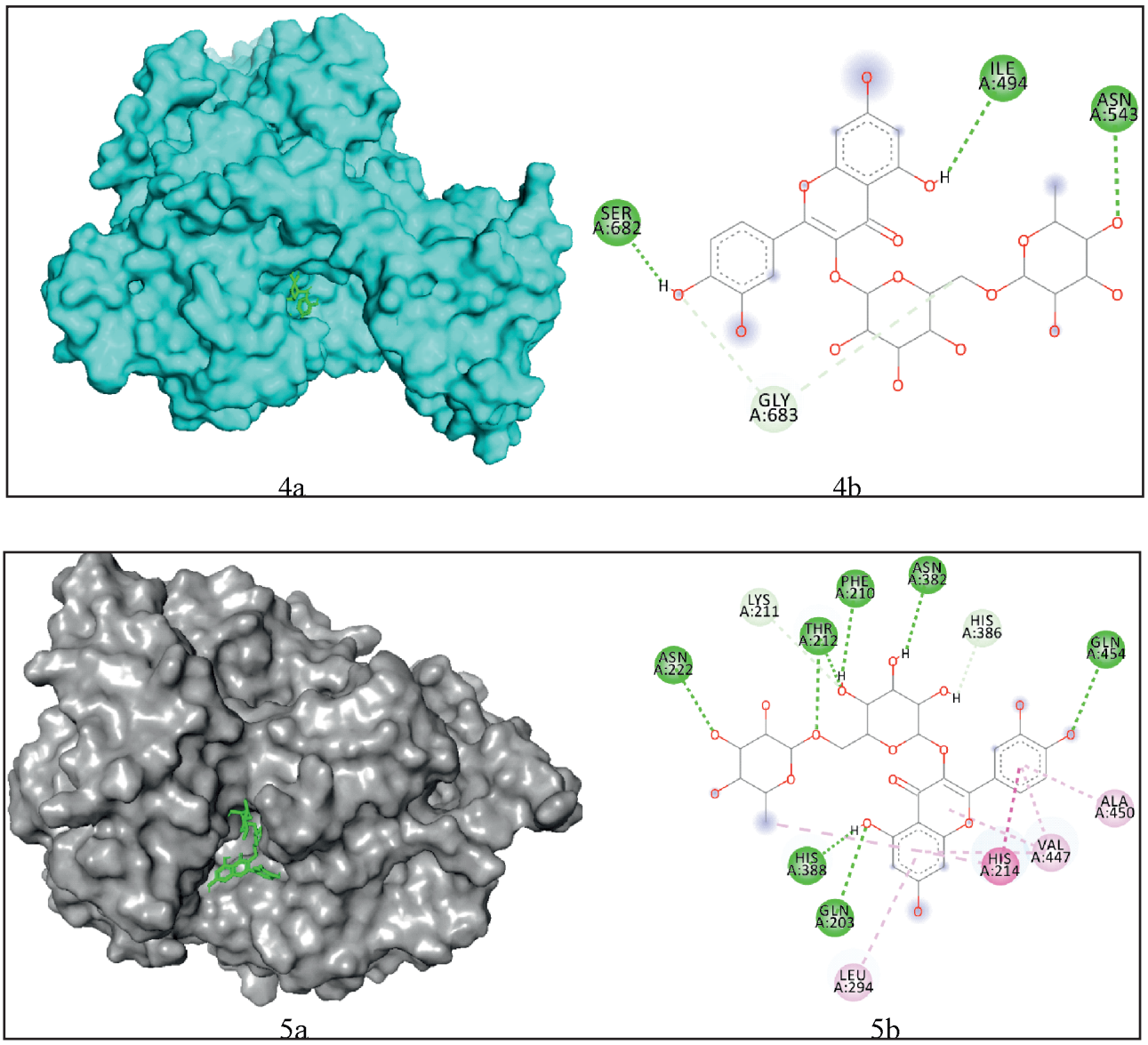

Figure 2: Docking between target proteins and the best lead molecule rutin (a) 3D view and (b) 2D view. 1) Spike protein, 2) ACE2 3) M^{pro} 4) RdRp 5) COX-2

the hits are either P-glycoprotein substrates or inhibitors. The compounds rutin, marmesisnin, decursinol, aegelinoside A, and seselin act as neither cytochrome P450 isozyme substrates nor inhibitors. For toxicity analysis, compounds piperitol, 2-phenylchromone and 2-hydroxyflavone showed AMES toxicity and compounds marmesinin, betulinic acid, and aegelinoside A showed hepatotoxicity.

CONCLUSION

The overall results indicated that the compound rutin has an inhibitory effect on multiple therapeutic targets of SARS-CoV-2. Further *in vitro* and *in vivo* studies are required to propose these compounds as efficient drugs.

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