

**In Silico Studies on Phytochemicals of *Pimpinella Anisum* L. (Apiaceae) as Potential Inhibitors of SARS-CoV-2 3C-Like Protease**

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**Abstract:** Objectives As of October 13 2020, the current pandemic (COVID-19) caused by the coronavirus SARS-CoV-2 has resulted in 38,167,109 infections and 1,087,118 deaths and has practically affected nearly all countries of the world. One of the characteristics of this virus-induced disease is severe respiratory distress, hence the name Severe Acute Respiratory Syndrome Coronavirus. So far there has been no discovery of therapeutics in the form of vaccines and drugs, which can control this virus. *Pimpinella anisum* (anise in English) is used in the three major traditional medicinal systems of India (Ayurveda, Siddha and Unani) to control respiratory distress. It was of interest to evaluate some of the phytochemicals reported in anise for their binding and possible inhibition of the main protease or 3C-like protease of SARS-CoV-2, which plays an indispensable part in replication of the virus. Methods Binding studies were carried out in silico using AutoDock Vina program to carry out molecular docking of the phytochemicals to 3C-like protease. Results Of the eleven phytochemicals studied, five compounds, namely cyanidin, malvidin, peonidin, petunidin and pelargonidin demonstrated high binding energy values to the protease (more than -7.5 kcal/mol). These five compounds were followed by isorhamnetin with a binding energy of -7.4 kcal/mol. The compounds bound at or close to the catalytic site of the protease, indicating that they can possibly inhibit the proteolytic activity. Conclusions Several phytochemicals from *Pimpinella anisum* merits further studies both in vitro and in vivo to evaluate their ability to inhibit SARS-CoV-2 and their use as therapeutics.

**Keywords:** COVID-19, Molecular docking, Pandemic, phytochemicals, *Pimpinella anisum*, SARS-CoV-2

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**Introduction**

Corona viruses are so named because of the spike like protrusions on their surface, which resembles a corona. These viruses are zoonotic, meaning that they originally come from an animal host prior to infecting humans. More often there are two animal hosts, a primary and an intermediary animal host. Following infection of humans, these viruses gain the ability for human to human transmission.

Thus far, seven species of coronaviruses are known to cause sickness in humans. Four of them are responsible for common cold-like symptoms, which go away by itself and seldom becomes dangerous; of the four, HCoV-OC43 and HCoV-229E, were identified in the mid 1960’s, HCoV-NL63 and HCoV-HKU1 are more recent discoveries [1-4]. Apart from the four mentioned coronaviruses, three more coronaviruses have emerged in the last two decades. The Severe Acute Respiratory Syndrome corona virus (SARS-CoV) was first reported from China in 2003; the Middle East Respiratory Syndrome corona virus (MERS-CoV) was reported in 2012 from Saudi Arabia [5, 6].

The third severe coronavirus was first detected in Wuhan of China in December 2019 and is known as Severe Acute Respiratory System coronavirus-2 or SARS-CoV-2 [7]. As of October 13 2020, the current pandemic (COVID-19) caused by the coronavirus SARS-CoV-2 has resulted in 38,167,109 infections and 1,087,118 deaths.
and has practically affected nearly all countries of the world and has brought in world-wide economic disaster in its wake. The primary symptoms of COVID-19 are dry cough and fever. This can progress to respiratory distress and pneumonia [8].

Despite strenuous efforts by scientists all over the world, no vaccines or drugs are still available against COVID-19, which can completely cure the disease. As a result, treatments for this disease are mainly symptomatic. Since allopathic medicine has not been successful in finding a cure, attention has to some extent switched to traditional medicines of various countries. In this aspect, Ayurveda can be of use in COVID-19 prophylaxis [9].

There are three major traditional medicinal systems in India, namely Ayurveda, Siddha, and Unani, all of them relying on medicinal plants for both preventive and therapeutic purposes. It was of interest to find out whether the three systems have any common plants for use against any diseases causing respiratory distress. Interestingly, seeds of *Pimpinella anisum* L. (Apiaceae family), known in English as anise is used in Siddha medicine for ‘iraippu noi’, comparable to bronchial asthma [10]. In Ayurveda, the plant is known as ‘svetapuspa’, and among other uses, seeds are used for coughs [11].

In the Unani system of medicine, the seeds of the plant are used for coughs and considered beneficial for the lungs [12]. We then decided to evaluate phytochemicals present in the seeds of the plant *in silico* studies for their binding affinities to a suitable target of SARS-CoV-2. For a suitable target, we selected the 3C-like protease (3 chymotrypsin-like protease of the virus, also known as C\(^\text{pro}\) or M\(^\text{pro}\)). Inhibition of this protease will lead to inhibition of viral replication, because this protease plays a significant role in cleaving viral polyproteins into functional proteins necessary for viral replication [13].

The other advantage of selecting this protein is that high resolution structures are available for this protease enabling better molecular docking studies. Some salient features of the 3C-like protease are (I) the protease contains three domains composed of amino acid residues 8-101 in domain I, residues 102-184 in domain II, and residues 201-303 in domain III. Domain III is not involved in interacting with the substrate, but plays a part in enzymatic activity. A catalytic dyad is formed in the protease by His41 and Cys145. Any inhibitor or substrate binds to the cleft, which is between domains I and II. The N-terminal seven amino acids are necessary for dimerization [14-16].

**Methods**

### Three-Dimensional Structure of SARS-CoV-2 Major Protease (3C-like Protease)

We used the pdb file (6LU7) of the main protease of SARS-CoV-2 3C-like protease (SARS-CoV-2 3CL\(^\text{pro}\)) as published by Professor Rao and others [15]. Prior to using in our molecular docking studies, an inhibitor N3 bound to the protease was removed. The reported interacting residues of N3 with the protease amino acids include His41, Met49, Phe140, Leu141, Asn142, Gly143, His163, His164, Glu166, Leu167, Pro168, Gln189, Thr190, and Ala191. Monomeric form of protein was used for molecular docking.

**Ligand Molecular Docking Studies**

Molecular docking (blind) was conducted using AutoDock Vina [17]. We report the average ΔG values from five independent runs of the docking program. The pose of phytochemicals bound to SARS-CoV-2 main protease as shown in the Figures was obtained from PyMOL and displayed in Discovery Studio [18]. Phytochemicals of *Pimpinella anisum* were obtained from a previously published review paper [19]. Ligand molecules were downloaded from Pubchem in sdf format. They were optimized with the force field type MMFF94 using Openbabel softwares and saved as pdbqt format.

**Virtual Screening of the Phytochemicals**

We chose Lipinski’s rule of five (Ro5) with the following filters, namely molecular weight (less than or equal to 500), H-bond donors (less than or equal to 5), H-bond acceptors (less than or equal to 10), MLOGP (less than or equal to 4.15), and molar refractivity (between 30 and 140) [20].

**Results and Discussion**

Altogether 11 phytochemicals from *Pimpinella anisum* were evaluated for their binding affinities to 3C-like protease of SARS-CoV-2.
Of the eleven phytochemicals studied, five compounds, namely cyanidin, malvidin, peonidin, petunidin and pelargonidin demonstrated high binding energy values to the protease (more than -7.5 kcal/mol). These five compounds were followed by isorhamnetin with a binding energy of -7.4 kcal/mol. The results are shown in Table 1. The binding affinities suggest that flavonoids have higher binding affinity than terpenoids.

Table 1: Binding energy of *Pimpinella anisum* phytochemicals to 3C-like protease of SARS-CoV-2. Phytochemicals with high binding affinities to 3C-like protease of SARS-CoV-2 are marked with an asterisk

<table>
<thead>
<tr>
<th>Phytochemicals (Groups)</th>
<th>Binding energy (ΔG = kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-cedrene (Sesquiterpenoid)</td>
<td>-6.0</td>
</tr>
<tr>
<td>Camphor (Terpenoid)</td>
<td>-5.6</td>
</tr>
<tr>
<td>cis-Dihydrocarvone (Monoterpenoid)</td>
<td>-4.9</td>
</tr>
<tr>
<td>Cyanidin (Anthocyanidin or 7-hydroxy flavonoid)</td>
<td>-8.1*</td>
</tr>
<tr>
<td>isorhamnetin (Flavonol group of flavonoids)</td>
<td>-7.4*</td>
</tr>
<tr>
<td>Limonene (Cyclic monoterpenes)</td>
<td>-5.6</td>
</tr>
<tr>
<td>Malvidin (O-methylated anthocyanidin)</td>
<td>-8.0*</td>
</tr>
<tr>
<td>Peonidin (O-methylated anthocyanidin)</td>
<td>-7.7*</td>
</tr>
<tr>
<td>Petunidin (O-methylated anthocyanidin)</td>
<td>-7.5*</td>
</tr>
<tr>
<td>Pelargonidin (Anthocyanidin)</td>
<td>-8.0*</td>
</tr>
<tr>
<td>Anethole (Anisole)</td>
<td>-5.9</td>
</tr>
</tbody>
</table>

The structures of the various compounds given in Table 1 are shown in Figure 1.

![Figure 1: Structures of phytochemicals of *Pimpinella anisum*](image-url)
Earlier studies have also shown similar results that flavonoid group of compounds have higher binding energies and higher inhibitory capacities of 3C-like protease of not only SARS-CoV-2 but also SARS. Polyacylated anthocyanins including phacelanin, gentiodelphin, cyanodelphin, and tecophilin were found to bind to the catalytic dyad of His41 and Cys145 of SARS-CoV-2 3C-like protease in in silico studies [21].

Other flavonoid molecules like caflanone, hesperetin and myricetin showed high affinity to the spike protein, helicase and protease sites on the ACE2 receptor used by the SARS-CoV-2 to infect cells [22]. Vicenin (flavonoid glycoside) and isoorientin (flavone C-glycoside) from Ocimum sanctum L. reportedly showed high binding affinities to 3C-like main protease in molecular docking studies [23]. The 3C-like protease sequence was screened against a medicinal plant library containing 32,297 phytochemicals; a flavonoid group compound, 5,7,3′,4′-tetrahydroxy-2′-(3,3-dimethyl allyl) isoflavone showed the highest binding affinity in silico to the 3C-like protease.

The compound reportedly not only interacted with the His41-Cys145 dyad but also with the receptor binding amino acid residues Thr24, Thr25, Thr26, Cys44, Thr45, Ser46, Met 49, Asn142, Gly143, His164, Glu166, and Gln189 [24]. The three compounds showing the highest binding affinities to the 3C-like protease in the present study were cyanidin, malvidin and pelargonidin with binding energies, respectively, of -8.1, -8.0 and -8.0 kcal/mol. The molecular docking of cyanidin and malvidin with 3C-like protease are shown in Figure 2.

Interacting amino acid residues of 3C-like protease with malvidin include Thr111, Asn151, Ile152, Asp153, Phe294, and Asp295. Thus these compound interacts mostly with domain 2 amino acid residues and there are 4 H bonding forces, which will create strong bonding.

The virtual inhibitor N3 interacted with 9 amino acid residues in domain 2. The results suggest that malvidin can be a strong inhibitor of the protease. Interacting amino acids of the protease with cyaniding include Met49, Ser144, Cys145, Met165, and Glu166. What is of importance is that cyanidin could bind to one of the dyad amino acid residues of the protease, namely Cys145, which suggests that the compound can also be a potential inhibitor of the protease. Lipinski’s rule of 5 was followed to predict whether the six phytochemicals of Pimpinella anisum showing binding energy values of -7.4 kcal/mol or above can be regarded as possible drug candidates.

These rules, namely molecular weight (less than or equal to 500), H-bond donors (less than or equal to 5), H-bond acceptors (less than or equal to 10), MLOGP (less than or equal to 4.15), and molar refractivity (between 30 and 140) were applied to the six phytochemicals and the results are shown in...
Table 2. Briefly, all six compounds (cyanidin, isorhamnetin, malvidin, peonidin, petunidin and pelargonidin) showed that the compounds were within the ranges of various indices in Lipinski's rule of five and so can be compared as potential drug candidates for inhibiting 3C-like protease of SARS-CoV-2. The results are shown in Table 2.

Table 2: Applications of Lipinski’s rule of five to selected compounds of Pimpinella anisum

<table>
<thead>
<tr>
<th>Compound’s Name</th>
<th>Molecular weight</th>
<th>Num. H-Bond Acceptors</th>
<th>Num. H-Bond Donors</th>
<th>Log P</th>
<th>Molar Refractivity</th>
<th>No. of Violation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanidin</td>
<td>287.24</td>
<td>6</td>
<td>5</td>
<td>-2.59</td>
<td>76.17</td>
<td>0</td>
</tr>
<tr>
<td>Isorhamnetin</td>
<td>316.26</td>
<td>7</td>
<td>4</td>
<td>2.35</td>
<td>82.50</td>
<td>0</td>
</tr>
<tr>
<td>Malvidin</td>
<td>331.30</td>
<td>7</td>
<td>4</td>
<td>-1.80</td>
<td>87.13</td>
<td>0</td>
</tr>
<tr>
<td>Peonidin</td>
<td>301.27</td>
<td>6</td>
<td>4</td>
<td>-1.84</td>
<td>80.64</td>
<td>0</td>
</tr>
<tr>
<td>Petunidin</td>
<td>317.27</td>
<td>7</td>
<td>5</td>
<td>-1.72</td>
<td>82.66</td>
<td>0</td>
</tr>
<tr>
<td>Pelargonidin</td>
<td>271.24</td>
<td>5</td>
<td>4</td>
<td>-2.29</td>
<td>74.15</td>
<td>0</td>
</tr>
</tbody>
</table>

Various anthocyanins and other flavonoid group of compounds have been reported for their anti-viral activity against various viruses; this list include flavones, flavonols, flavans, isoflavones, and anthocyanidins [25, 26]; among them are pelargonidin and cyanidin, which have shown inhibitory activities against influenza viruses A and B [25,26].

The present study adds more of these class of compounds from Pimpinella anisum, a plant whose seeds are used for a number of medicinal purposes and for removing mouth odor. It is recognized that molecular docking and other in silico studies can at best be pointers, to turn a phytochemical to a drug needs laboratory-based virucidal tests followed by clinical trials.

Conclusion

Six phytochemicals from Pimpinella anisum (cyanidin, isorhamnetin, malvidin, peonidin, petunidin and pelargonidin) showed promising binding affinities to 3C-like protease of SARS-CoV-2 and can be considered as potential drug candidates based on Lipinski’s rule of five. The COVID-19 pandemic shows no signs of going away and is resulting in sharp declines in economy and loss of human life in practically every country. Since allopathic drugs are costly, have adverse effects, and most importantly, have not been shown to cure COVID-19 but treats essentially one or other symptom(s), the time has come to derive drugs from plants used in traditional medicinal systems like Ayurveda, Siddha and others.

References

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