Investigating the Antiviral Activity of Mediterranean Herbs and Spices against SARS-CoV-2 using *in silico* Methods

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Background and Aim

The virus SARS-CoV-2 is responsible for the COVID-19 pandemic, which killed millions of people world-wide. Though effective vaccines have been developed, their method of administration remains invasive. Alternative and simpler solutions are still required in order to combat the spread of the virus. So far, the search for antiviral small-molecule inhibitors has mostly focused on repurposing existing drugs. [1]

Plants, particularly herbs, contain a vast array of chemicals which evolved to defend the plants from pests and pathogens, including viruses. In fact, plants have historically served as the original source of a very large number of important drugs, most of which are still in use today.

Many of the compounds which are found inside plants remain unknown.

The Aim of this Project is to use *in silico* methods to select and to subsequently test a set of small molecules, known to be found in Mediterranean plant species, for their ability to bind to the target proteins and prevent SARS-CoV-2.

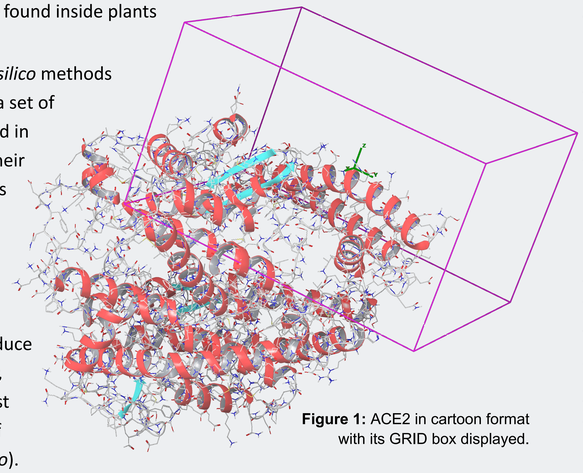

Figure 1: ACE2 in cartoon format with its GRID box displayed.

Research Approach and Project Overview

In silico methods attempt to reproduce biological systems computationally, in order to reduce the time and cost of running thousands or millions of experiments in a laboratory (*in vitro*).

A **literature review** was first conducted to identify which biological systems should be targeted in order to prevent SARS-CoV-2 in the most effective manner/s. Known small-molecule inhibitors of these systems were also identified at this stage.

Once the targets were selected, a computational **model** was built using protein X-ray structures which are available in literature.

The model was subsequently **validated** using small molecules which are known to be inhibitors.

Next, a database of chemical compounds known to be found in plants was prepared. The antiviral potential of these chemical compounds was **predicted** using the previously constructed model.

In the next phase of the project, the compounds which were predicted to be the best inhibitors will be physically acquired and tested in the lab on SARS-CoV-2 sensitive cell-lines.

Using the results obtained, the model and be improved, and better predictions can then be made.

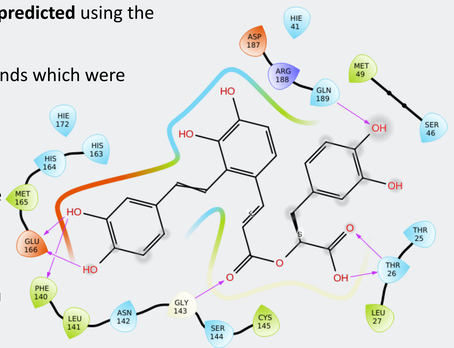

Figure 2: A 2D Ligand-Interaction diagram showing Salvianolic acid, a known inhibitor of 3CLpro, docked in the active site of the enzyme.

Methodology

Two protein targets which are crucial to the reproductive cycle of SARS-CoV-2 [2] were selected: 3-Chymotrypsin-like protein (3CLpro) is a viral enzyme which is essential in processing several structural and non-structural viral proteins [3]. The active site of this enzyme is well-defined, and several small-molecule inhibitors which bind to this site antagonistically (often irreversibly) are known.

ACE2 (Angiotensin Converting Enzyme 2) is a human membrane protein which functions usually as part of the system regulating blood pressure. Though SARS-CoV-2 enters human cells through a number of mechanisms, the interaction of its S-protein (Spike-protein) with ACE2 triggers a process which results in the endocytosis of the complete virus, followed by infection of the cell. Hence, inhibiting the binding of this protein to ACE2 will stop the reproductive cycle of the virus.

Figure 3: Flowers of *Borago officinalis* (Fidloqqom), a common herb found in the Mediterranean, including in Malta [9].

Following a detailed search of the RSCB PDB database, two protein entries were short-listed for ACE2: 6M0J [4] and 6LZG [5] and one entry was selected for 3CLpro: 6LU7 [6]. These proteins were downloaded, pre-processed and minimized energetically. In the case of the ACE2 structures, the S-protein was removed from the structure file. A GRID was then prepared using the default options. This represents the **model** of the biological systems mentioned earlier.

A total of 5121 ligands (237 manually-selected compounds and 4848 database compounds) [REF] were docked. Possible tautomers and stereoisomers were generated, for a total of 13544 structures. These were then minimized energetically using the OPLS force-field [7].

Docking was performed using Glide [8] for all the ligands with the default options under standard precision. Post-docking minimization was performed.

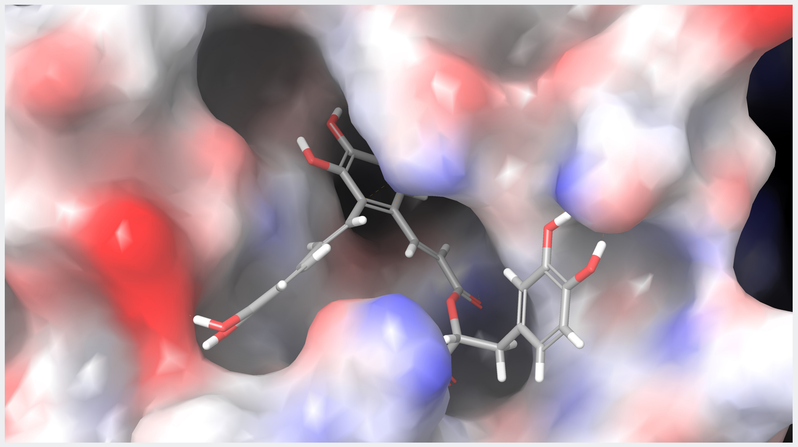

Figure 4: A 3D representation of Salvianolic acid, a known inhibitor of 3CLpro, docked in the active site of the enzyme. Red regions represent positive electrostatic potentials while blue regions represent negative electrostatic potentials.

Results so far

Nine molecules were suggested as good **ACE2** binding inhibitors. These are:

- Luteolin-3'-D-glucuronide
- a yet un-named compound: FT-0700584
- Cinnamic acid
- a yet unnamed compound: 135499080
- Apigenin 7-glucoronide
- Vulgaxanthin I
- Luteolin 7-Glucuronide
- a yet unnamed compound: 162902636
- Cryptochlorogenic Acid

The compounds were selected on the basis of their docking score. Most known inhibitors of the respective proteins scored highly, increasing the confidence (**validity**) of the model.

Five molecules were suggested as good **3CLpro** inhibitors. These are:

- Cyanidin-3-O-arabinose chloride
- Cyanidin 3-O-rhamnoside
- Kaempferol-3-O-pentoside
- Quercetin 3-O-rhamnoside

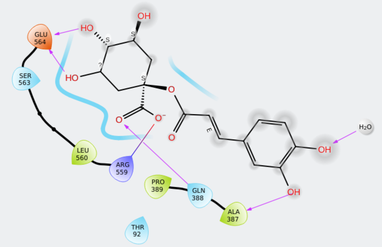

Figure 5: A 2D Ligand-interaction diagram showing cinnamic acid, one of the suggested ACE2 binding inhibitors, docked in place of the SARS-CoV2 spike-protein.

Future Outlook

Limitations exist in the modelling process which reduce the confidence in the results obtained.

For ACE2, the most important limitation is that the active site is large, and prominently exposed to the solvent, without an enclosed pocket to dock into. This favours larger, generic molecules which are not necessarily good antagonistic inhibitors.

For 3CLpro, an important limitation is that a number of inhibitors are known to be irreversible (time-dependent) binders. The energy involved in the reaction is not taken into consideration during modelling.

Other limitations include conformational changes triggered by docking, which are difficult to take into account.

Further improvements and refinements to the methods are planned. These include further testing on other protein targets (such as viral proteases), and the refinement of the model once the laboratory results are available.

The use of a pharmacophore approach might also be attempted to circumvent some of the limitations.

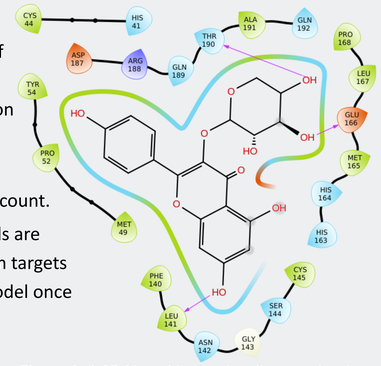

Figure 6: A 2D Ligand-interaction diagram showing kaempferol-3-o-pentoside, one of the suggested 3CLpro inhibitors, docked in the protein.

References:

[1] https://doi.org/10.1002/anie.202008835
[2] https://doi.org/10.1021/acs.jmedchem.0c00606
[3] https://doi.org/10.2147%2FDDDT.S359009
[4] http://doi.org/10.2210/pdb6M0J/pdb
[5] http://doi.org/10.2210/pdb6LZG/pdb
[6] http://doi.org/10.2210/pdb6LU7/pdb
[7] https://doi.org/10.1021/acs.jctc.5b00864
[8] https://doi.org/10.1021/jm051256o
[9] tinyurl.com/3uchdrny

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