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Evaluation of Phytochemicals of *Cassia occidentalis* L. for their Binding Affinities to SARS-CoV-2 3C-Like Protease: An *in Silico* Approach

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Authors' contributions

This work was carried out in collaboration among all authors. Author MR coordinated the study and wrote the first draft of the manuscript. Authors TAB, MAHR, AH, RJ and KJ performed the molecular docking simulations and managed the literature searches. All authors read, contributed and approved the final manuscript.

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ABSTRACT

Aims: Corona virus SARS-CoV-2, otherwise known as COVID-19 has created a pandemic resulting in social and financial crisis throughout the world. The virus has no known drugs or vaccines for preventive or therapeutic purposes. The objective of the present study was to screen phytochemicals from *Cassia occidentalis* L. in virtual screening (*in silico*) studies to evaluate their potential of binding to the main 3C-like protease of the virus and so stop its replication.
Study Design: Molecular docking approach was used for virtual screening studies.
Place and Duration of Study: University of Development Alternative between April and July 2020.
Methodology: Molecular docking (blind) were done with the help of Autodock Vina. We have used the pdb file (6LU7) of the main protease of SARS-CoV-2 3C-like protease or SARS-CoV-2 $3CL^{pro}$ (monomeric form) to study binding of the phytochemicals.

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Results: Of the nine phytochemicals studied, the C-glycosidic flavonoids, cassiaoccidentalins A-C demonstrated excellent binding affinities to the protease. The compounds bound to the active site of the protease with binding energy values of -8.2 to-8.4 kcal/mol.
Conclusion: The *in silico* studies suggest that the compounds merit actual COVID-19 inhibitory tests and have potential for anti-COVID-19 use.

Keywords: COVID-19; molecular docking; Cassia occidentalis; phytochemicals; 3C-like protease.

1. INTRODUCTION

Corona viruse(s) belong to the Coronaviridae family, the name "corona" being derived from the outer fringe, or "corona" of embedded envelope protein [1]. In the last two decades, the world has witnessed the emergence of three zoonotic corona viral diseases of major significance in terms of becoming a pandemic, namely the Severe Acute Respiratory Syndrome (SARS) in 2002, Middle East Respiratory Syndrome (MERS) in 2012, and the SARS-CoV-2 or COVID-19 in late December of 2019. The latter virus, as of July 25, 2020 has caused a total of 15,969,465 infected cases and 643,393 deaths along with severe social and economic disruptions in practically every country in every region of the world [2]. Although work is frantically going on in a number of countries in the world, no drugs or vaccines have yet been discovered with proven action against SARS-CoV-2 or for that matter SARS or MERS.

The SARS-CoV-2 genome encodes two polyproteins, which are cleaved into mature non-structural proteins (NSPs) by a chymotrypsin-like protease $3CL^{pro}$ (3C-like protease) and PL^{pro} (Papain-like Protease) [3]. The NSPs, in turn play a major role in the transcription/replication of the virus during infection [4]. The key residues involved in substrate binding in SARS 3C-like protease include the Cys145-His41 dyad and His163/His172/Glu166, the latter possibly providing the opening gate for the substrate [5]. However, the two 3C-like proteases in SARS and SARS-CoV-2 differ by only 12 amino acids and there is a high level of alignment of the key residues involved in substrate binding [5]. Most anti-viral drug designs have been therefore directed against the two proteases and mostly the 3C-like protease. The same applies to screening of potential anti-COVID-19 compounds *in silico* (mostly molecular docking studies). Among the compounds evaluated by molecular docking for binding affinities to 3C-like protease have been phytochemicals or compounds derived from plants [6].

Plants have been an excellent source for many modern drugs like aspirin, atropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine to name only a few [7]. It is quite likely that anti-viral drugs will be discovered from plants. In fact, a fairly recent review lists dozens of plants with activity against various viruses [8]. *In silico* studies in the form of determining binding energies through molecular docking of a phytochemical to a given viral target protein saves precious time and expenditures in searching for anti-viral drugs, for this system gives the scientist a starting basis for performing more laborious anti-viral wet laboratory studies. Ethnic reports of traditional plant uses can provide a direction for selection of a plant and its phytochemicals for further molecular docking studies. The recent malarial drug artemisinin (Qinghaosu in Chinese) was discovered in a similar manner from *Artemisia annua* L. by going through activity reports of the plant in "A Handbook of Prescriptions for Emergencies" by Ge Hong in the time of the Jin Dynasty (284-346 AD) [9]. The objective of the present study was to evaluate the binding affinity (through molecular docking) of several phytochemicals reported to be present in a plant *Cassia occidentalis* L. (Fabaceae) found in Bangladesh, India and other countries in Southeast Asia, and parts of Australia, USA and eastern Africa to the 3C-like protease of COVID-19. The reason for selection of this plant was that the plant is considered an Ayurvedic plant and used as analgesic, anti-pyretic, and anti-inflammatory agent [10], which properties may prove useful in COVID-19 treatment.

2. METHODOLOGY

We used the pdb file (6LU7) of the main protease of SARS-CoV-2 3C-like protease as published before [11]. Inhibitor (N3) was removed from the pdb file before using the protein's structure. The reported interacting residues of N3 with the protease amino acids included His41, Met49, Phe140, Leu141, Asn142, Gly143, His163, His164, Glu166, Leu167, Pro168, Gln189, Thr190, and Ala191. The active site residues of

SARS-CoV-2 3C-like protease are His41 and Cys145. Monomeric form of protein was used for molecular docking.

2.1 Compounds Used in Docking Studies

We have studied nine major phytochemicals present in *Cassia occidentalis*. Ligand molecules were downloaded from Pubchem in sdf format. They were optimized with the force field type MMFF94 using Openbabel software and saved as pdbqt format as described before [12].

2.2 Ligand Molecular Docking Studies

Blind molecular docking was done using AutoDock Vina as previously described [12]. The predicted binding affinity values were taken as an average of values from five independent runs of the docking program. The figures show the docked pose of the phytochemicals bound to SARS-CoV-2 main protease as obtained from PyMOL and displayed in Discovery Studio.

2.3 Phytochemicals

Phytochemicals present in *Cassia occidentalis* were obtained from published report [10] and their 3D structures were obtained from PubChem. It was not possible to cover all phytochemicals of *Cassia occidentalis*; we concentrated essentially on the major phytochemicals of the plant. The structures of the phytochemicals are shown in Fig. 1.

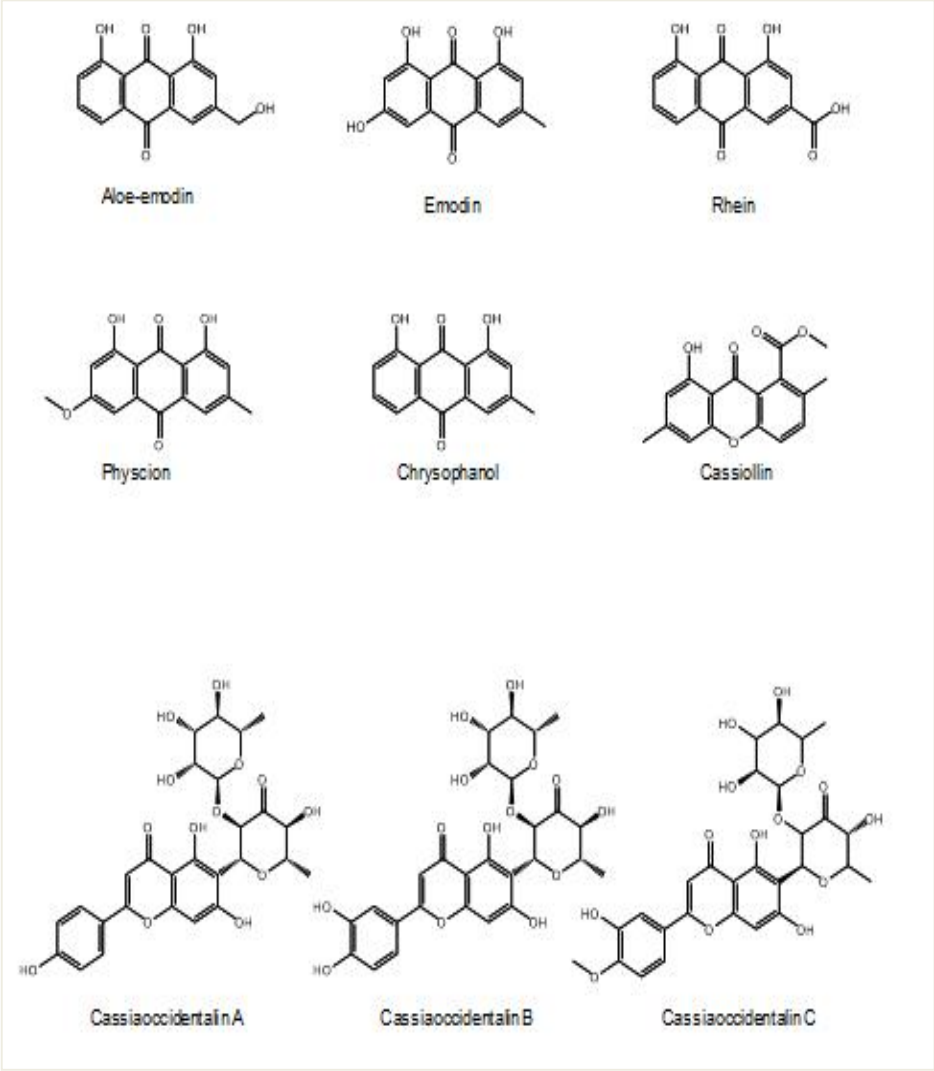

Fig. 1. Structure of *Cassia occidentalis* phytochemicals screened in the present study

3. RESULTS AND DISCUSSION

The nine phytochemicals of *Cassia occidentalis* evaluated in the present study for their binding affinities to the SARS-CoV-2 3C-like protease all demonstrated good binding affinities to the protease as can be seen from their binding energies in Table 1.

Table 1. Binding affinity of COVID-19 3C-like protease and phytochemicals of *Cassia occidentalis*

Phytochemical	Binding energy (ΔG = kcal/mol)
Aloe-emodin	-7.0
Cassiaoccidentalin A	-8.2
Cassiaoccidentalin B	-8.2
Cassiaoccidentalin C	-8.4
Cassiollin	-7.3
Chrysophanol	-7.1
Emodin	-7.2
Physcion	-7.2
Rhein	-7.3

However, of the nine phytochemicals studied, the C-glycosidic flavonoids, cassiaoccidentalins A-C demonstrated better binding affinities to the protease. The compounds bound to the active site of the protease with binding energy values of -8.2 to -8.4 kcal/mol.

The interaction of aloe-emodin (binding energy = -7.0 kcal/mol) is shown in Fig. 2. The compound interacts with His41, Gly143, Ser144, Cys145 and Glu166, His41 and Cys145 being the active site residues of SARS-CoV-2 3C-like protease. The 3C-like protease monomer contains 3 domains of which the first two domains comprises of residues 8-101 and residues 102-184, respectively. These two domains are responsible for the chymotrypsin-like catalysis. The main interaction of aloe-emodin is with the second domain amino acid residues of the protease including the cluster of Gly143, Ser144 and Cys145, and Glu166. The hydrogen bonds stabilize the bonding but are weak intermolecular interactions, which possibly accounts for the comparatively low binding energy of-7.0 kcal/mol.

The interaction of cassiaoccidentalin C (binding energy = -8.4 kcal/mol) is shown in Fig. 3. The compound interacts with Thr24, His41, Met49 and Cys145 of the protease. The interactions include the His41-Cys145 dyad. Furthermore, the two hydrogen bonding interactions of Thr24 and His41 are stabilized and made stronger with the two hydrophobic interactions with Met49 and Cys145. Notably, the compound binds to both protease domains 1 and 2.

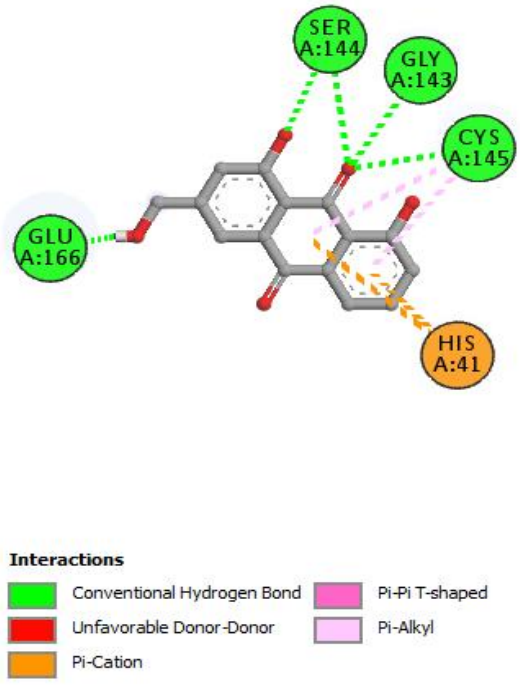

Fig. 2. Interaction of aloe-emodin with SARS-CoV-2 3C-like protease

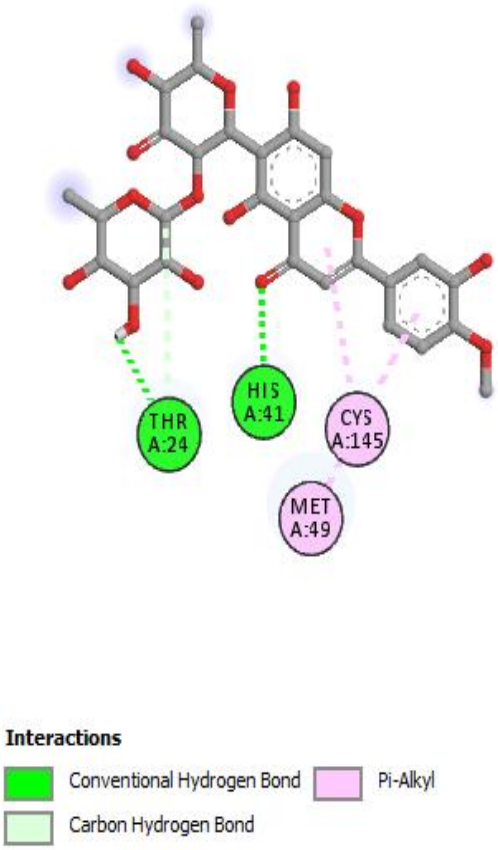

Fig. 3. Interaction of cassiaoccidentalin C with SARS-CoV-2 3C-like protease

Notably, a number of the phytochemicals in the present study have reported anti-viral activities. For instance, aloe-emodin reportedly inhibits influenza virus A via galectin-3 up-regulation [13]. The C-glycosidic flavonoids, cassiaoccidentalins, do not have any published report on their anti-viral activities. This makes them more suitable for study as therapeutic candidates or lead compounds for COVID-19 drugs. Glycosidic derivatives may form an important part for tighter binding to the active site of the protease; for instance arjunglucoside I has been reported to show a strong binding affinity for the active site of the protease in molecular docking studies [14]. The xanthone, cassiollin, also does not have any report on any anti-viral activity of the compound.

The anthraquinone chrysophanol unlike other anthraquinones aloe-emodin and rhein, reportedly inhibited the replication of poliovirus type 2 and 3. The compound has also been found to be effective against Japanese encephalitis virus [15]. Emodin has been shown to have potent inhibitory activities against Coxsackie virus (CVB_5) and respiratory syncytial virus (RSV) [16]. Another anthraquinone, physcion, isolated from *Senna siamea*, has been shown to possess anti-enteroviral activities [17]. Rhein (an anthraquinone) has been reported to demonstrate anti-influenza A virus activity through regulating oxidative stress and various signaling pathways like MAP-kinase (mitogen activated protein kinase) and nuclear factor kappa B (NF-κB) pathways [18]. Overall it can be said that most of the phytochemicals studied had reported anti-viral activities against other types of viruses with the notable exception of the cassiaoccidentalins.

4. CONCLUSION

Phytochemicals from *Cassia occidentalis* demonstrated good binding affinities with the SARS-CoV-2 3C-like protease. The binding energies of the phytochemicals in the present study were -7.0 or more. In molecular docking studies, the phytochemicals appeared to bind to the active site of the protease. The

phytochemicals therefore can be potential drug candidates as inhibitors or lead compounds, especially the cassiaoccidentalins with binding energies of more than -8.0, making them ideal compounds to be tested for SARS-CoV-2 inhibitory studies in actual experiments with the corona virus. Further studies to elaborate the structure activity relationships and molecular docking of the phytochemicals are planned for the future.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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