International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2022.104.115

Research Article
In silico Screening of Phytochemicals as Potential Inhibitors of SARS-CoV-2 Mpro and Human ACE-2

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Abstract

Background and Objective: An enzyme that inhibits the receptor could make it more difficult for coronavirus to reach cells. The key protease necessary for coronavirus proteolytic maturation is the recognized coronavirus 3-chymotrypsin-like protease 3CLpro, also known as Mpro. This Mpro is needed for immune control and the cleavage of the polyproteins pp1a and pp1ab, making it a promising target for anti-COVID-19 drugs. As a result, inhibiting the Mpro enzyme inhibits viral maturation. Bioactive constituents obtained from some selected indigenous plants of India, which have been reported to have antiviral potential, were subjected to virtual screening against ACE-2 and Mpro in the current study. **Materials and Methods:** Cresset's Flare 4.0 was used to establish the 3-D structure of all the compounds. Complete optimizations of these constructed structures were carried out. While performing the minimization, the spin state of the wave function was set to the singlet and standard SCF convergence was used for optimization, all other parameters were left at their default values. The Protein Data Bank (https://www.rcsb.org) was used to download the 3-D structures of Mpro from COVID-19 (PDB ID 6LU7) and ACE-2 receptor from Human (PDB ID 1R4L). **Results:** The findings show that these phytochemicals can bind to ACE-2 and Mpro more effectively as compared to reference compounds and act as inhibitors. **Conclusion:** The findings of virtual screening of these bioactive constituents revealed that most of them are more active than the reference compounds. Therefore, they could be used to produce antiviral drugs against Coronavirus in the future.

Key words: Molecular docking, COVID-19, phytoconstituents, angiotensin-converting enzyme-2, 3-chymotrypsin-like protease, *Tinospora cordifolia*, *Withania somnifera*

Citation: Chaudhary, A., R. Tomar, S.M.B. Asdaq, M. Imran and S.I. Alaqel *et al*., 2022. *In silico* screening of phytochemicals as potential inhibitors of SARS-CoV-2 Mpro and human ACE-2. Int. J. Pharmacol., 18: 104-115.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tinospora cordifolia is a potent immunomodulator and influences humoral, cellular and nonspecific immunity. *Tinospora cordifolia* contains macromolecular polysaccharides that regulate host immunity by acting specifically on the receptor, initiating the signal pathway and secreting macrophages, T cells and B-cells, natural killer cells and cytokines[1]. *Tinospora cordifolia* increases melatonin levels in the pineal gland as well as levels of important immunomodulatory cytokines such as interleukin-2, interleukin-10 and TNF-α[2]. The aqueous extracts also influence cytokine synthesis and stimulate the immune system[3]. It also contains vitamin C, which boosts immunity[4]. *Tinospora cordifolia* also inhibits photosensitization-induced protein degradation, plant-derived (1, 4)-alpha-D-glucan and arabinogalactan 19[5] activates macrophages via Toll-Like Receptor-6 (TLR6) signalling and cytokine production and improves immunity[6]. The methanolic extract improves immunity by influencing the -amylase function and cellularity of bone marrow in rats, 11-hydroxymustakone, N-methyl-2 pyrrolidone, N-formylannonain and cordifolioside are primarily responsible for immunomodulatory activity[7].

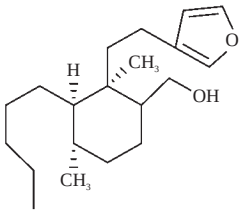
The pathogenesis of pneumonia is a complex reaction, the viral infection produces an immunogenic response or cytokine storm that causes extensive tissue injury with dysfunctional coagulation, pulmonary inflammation and microvascular thrombus formation[8]. Interleukin 6 that is the main promoter of this incidence that interacted with the cells and tissues and stimulate the growth or inhibition of cell. In any kind of inflammatory condition its level rises. Not only the Interleukin 6 but the SARS-CoV-2 also binds Toll-Like Receptor (TLR) and induces the release of pro-Interleukin-1β, which is converted into active Interleukin-1β causes the inflammatory and finally fibrosis[9].

Various mechanisms are considered in the treatment of COVID-19 patients that include the suppression of the inflammatory response, antioxidant effects and immunomodulatory effects[9]. Considering these properties various herbal plants are screened out to find out the suitable treatment of COVID like that belongs to the family Menispermaceae[3], which is a large family of about seventy species, having a long and smooth, flashy climber stem and well distributed in India[10,11]. In the Indian tribal medicine or folklore system, *Tinospora cordifolia* has a special place, all over India hundreds of tribal communities are using *Tinospora cordifolia* for treating various ailments e.g., cough, fever, ear pain, fractured bone, cancer asthma, leucorrhoea, anti-snake venom, acidity and skin disease[6,12]. *Tinospora cordifolia* contains alkaloids, diterpenoid lactones, glycosides, sesquiterpenoid, polysaccharides, steroids, phenolics and aliphatic compounds, which have immunomodulatory, antioxidant, anti-inflammatory, analgesic, antipyretic, hypoglycemic, antibacterial and anticancer potential[13,14]. Some of the principle phytoconstituents are given in the following Table 1. Chemical structures of important phytoconstituents are given in Fig. 1.

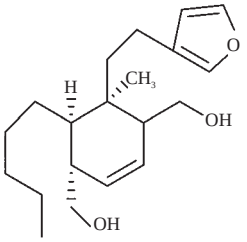
Withania somnifera (family Solanaceae) is a green shrub found in the drier parts of Egypt, Morocco, Jordan, South Africa, Baluchistan, Afghanistan and India[15]. It is also known as Ashwagandha or Winter Cherry. This plant has a variety of pharmacological effects, including antioxidant, immunomodulatory, anti-inflammatory, anticancer, antistress, antiaging, cardiovascular, neuroprotective and adaptogenicproperties[16-19]. The chemical constituents identified from this plant (Fig. 2), such as withanolide A, withaferin A and withanolide D, are effective and potent compounds[20]. This plant also contains somniferous, somnine, somniferine, withanmine, pseudowithamine and withanaminine as chemical constituents. Various phytochemical groups, such as steroidal lactones, saponins, tannins, alkaloids and so on, have been identified, isolated and extracted. 12 alkaloids, 40 withanolides and many sitoindosides have been confirmed and identified from roots, berries and aerial parts. Withaferin A (4-, 27-dihydroxy-1-oxo-5, 6-epoxy with a-2-24-dienolide) was the first component identified from a South-Asian strain whose structure was first elucidated by Lavi *et al.*[21]. Withaferin D is an antitumor and Withanolide E has immunosuppressive, antibacterial and insect antifeedant properties[22]. *Withania somnifera*

Table 1: Chemical constituents obtained from *Tinospora cordifolia*

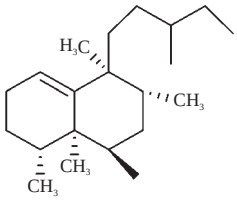
Alkaloids	11-hydroxymustakone, N-methyl-2-pyrrolidone, berberine, palmatine, N-formyl-annonain, tembetarine, isocolumbin, magnoflorine, choline, tetrahydropalmatine
Steroids	β-sitosterol, ecdysterone
Polysaccharides	(1,4)-alpha-D-glucan
Glycoside	Syringin, tinocosdiside and cordifolioside A,18-norclerodane glucoside, cordiosides and palmitosides
Clerodane furanoditerpene	Glucosides (amritosides A, B, C and D)



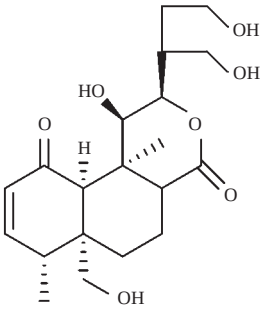
Tinosporafuranol



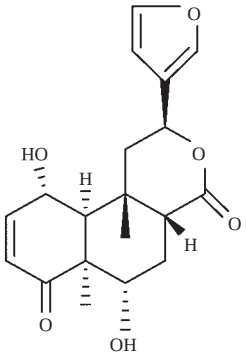
Tinosporaclerodanol



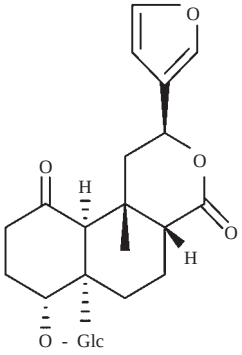
Tinosporafurandiol



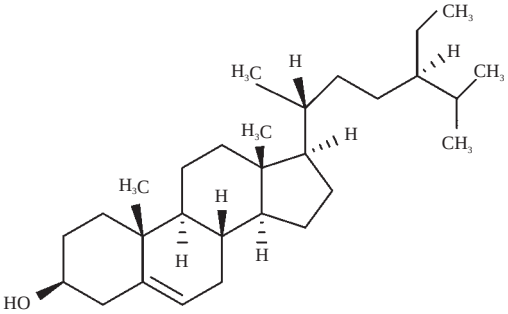
Tinosporaclerodanoid



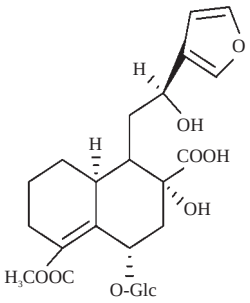
Tinocordin



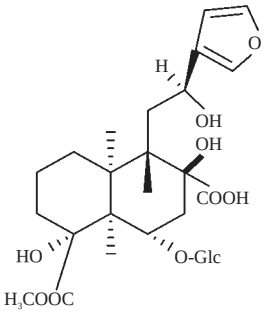
Tinosporaside



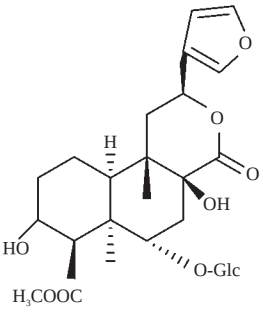
Beta-Sitosterol



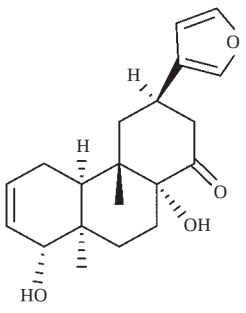
Amritoside A



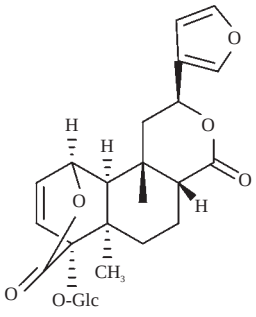
Amritoside B



Amritoside C



Amritoside D



Palmatoside C

Fig. 1: Continue

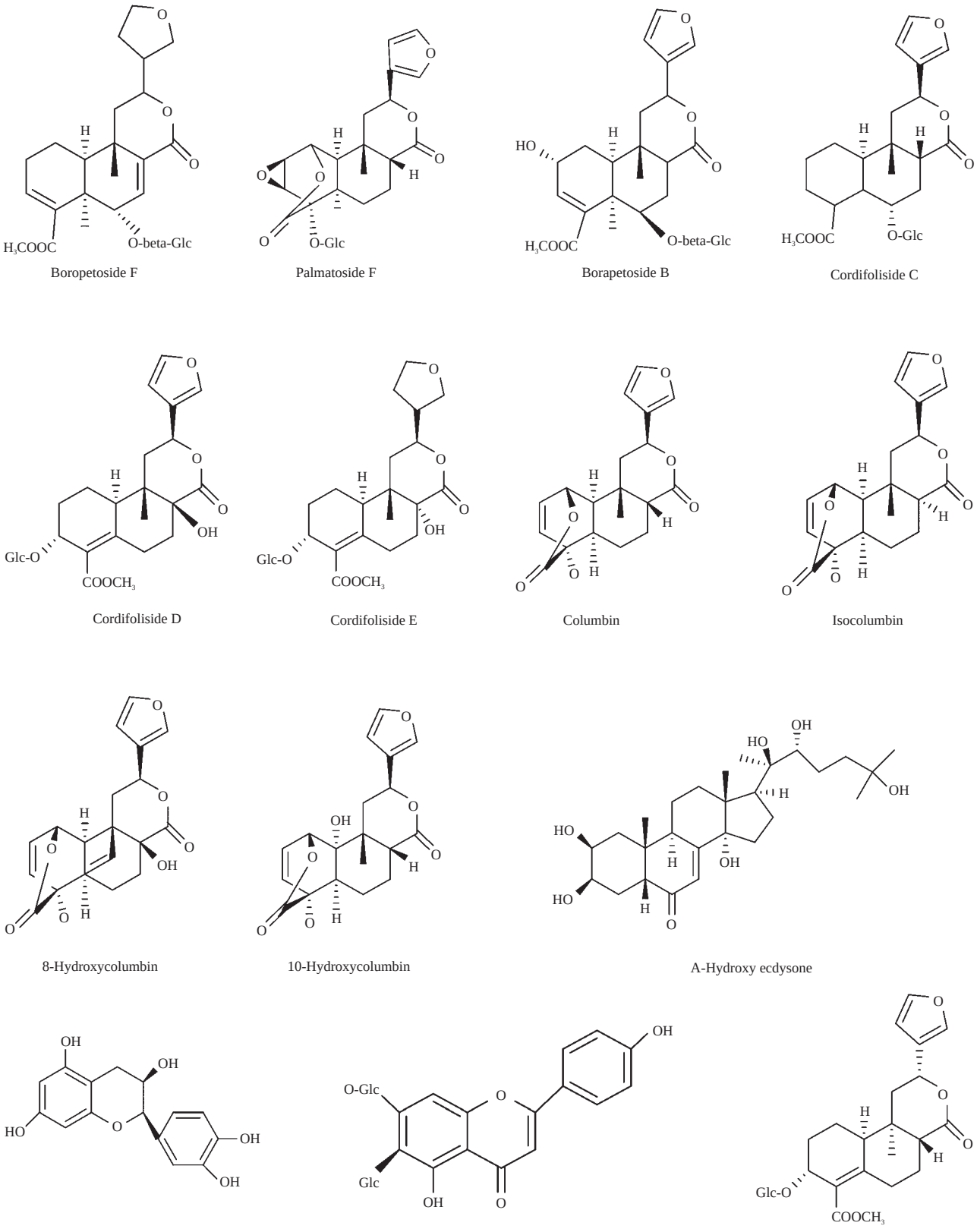


Fig. 1: Chemical constituents of *Tinospora cordifolia*

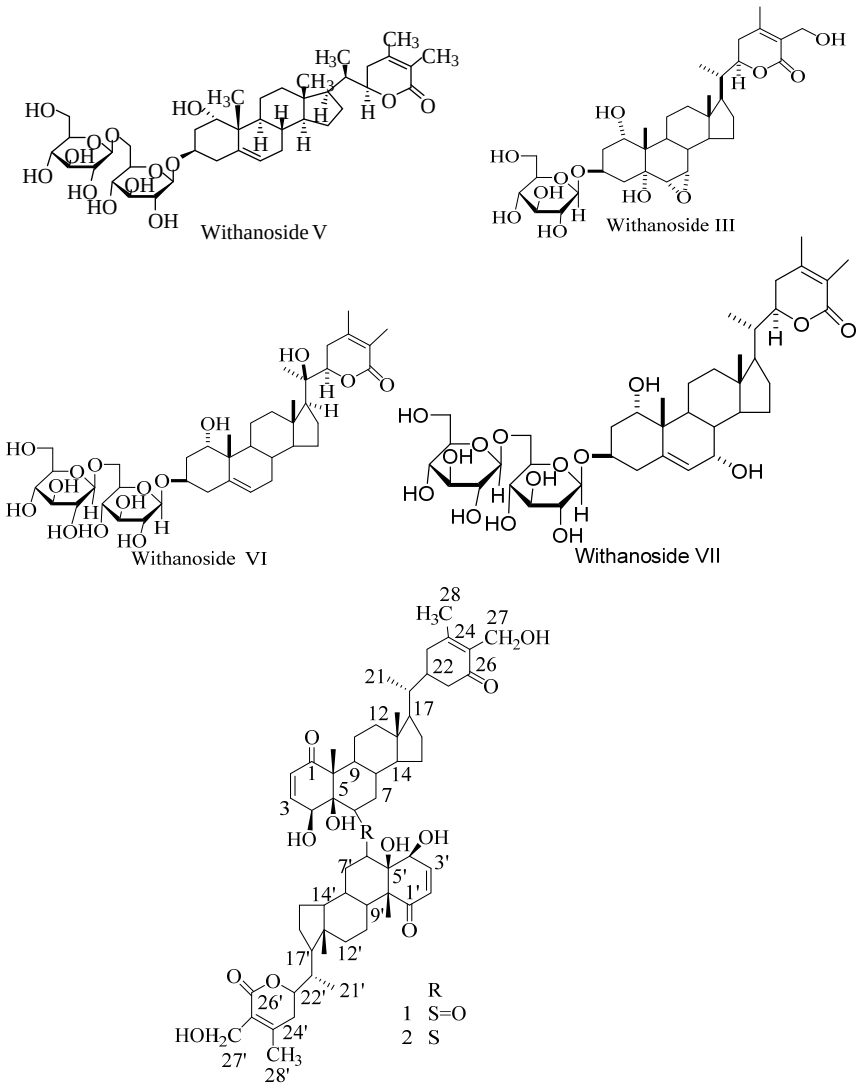


Fig. 2: Chemical constituents of *Withania somnifera*

cholinesterase inhibitory efficiency, combined with calcium antagonistic capability, has made it a viable drug molecule for the treatment of Alzheimer's disease[23].

In our quest to find potent and effective phytochemicals against SARS-CoV-2 we used virtual screening of phytochemicals from *Tinospora cordifolia* and *Withania somnifera* to look for possible and unique Coronavirus inhibitors. We used two enzymes in this study: Mpro from the virus cell and ACE-2 receptor from the host cell. The 3D structure of Mpro from COVID-19 PDB ID 6LU7 and the ACE-2 receptor from Human PDB ID 1R4L were obtained from the Protein Data Bank (https://www.rcsb.org). Using the Cresset FLARE 4.0 program, bioactive compounds derived from plants were docked in the active sites of the enzymes to find their binding affinity with the receptors and to compare that with reference compounds.

MATERIALS AND METHODS

Study area: The study was carried out simultaneously at Meerut Institute of Engineering and Technology, Meerut, India and Taif University, Taif, Saudi Arabia from July, 2020-2021.

Methodology: Cresset's Flare 4.0 was used to establish the 3-D structure of all the compounds. Complete optimizations of these constructed structures were carried out. While performing the minimization, the spin state of the wave function was set to the singlet and standard SCF convergence was used for optimization, all other parameters were left at their default values.

Enzyme use: We used two enzymes in this study: Mpro from the virus cell and the ACE-2 receptor from the host cell. The Protein Data Bank (https://www.rcsb.org) was used to download the 3-D structures of Mpro from COVID-19 (PDB ID 6LU7) and ACE-2 receptor from Human (PDB ID 1R4L). Flare 4.0 was used to extract all water molecules, ions and ligands from the protein molecule.

Protein preparation wizard: The Protein Preparation Wizard was used to prepare the protein structure for the docking analysis. The correct bond orders were allocated and the protein was given hydrogen atoms. The imperf usefulness then performed a restrained minimization of the hydrogen atoms' average Root-Mean-Square-Deviation (RMSD), leaving heavy atoms in place.

Docking: During docking, an interaction grid for protein structure was generated. In the active site of chain A, a grid was created for Mpro protein (6LU7). The receptor grid for ACE-2 protein (1R4L) was developed by using bound inhibitor AXX5804 as the reference structure for defining the active site. The grid box was based on the bound ligand in the protein structure by selecting the ligand from the workspace.

The bound ligand was extracted from the prepared protein structure to establish a docking protocol as defined by A. Chaudhary *et al.*[24]. The extracted ligand's atom and bond form were corrected and re-docked using the above-mentioned grid parameters and finally, the RMSD was calculated. The collection of prepared ligands was docked into the active site after the docking protocol was validated.

RESULTS AND DISCUSSION

Molecular docking studies were performed to investigate the binding ability of the chemical constituents from *Withania somnifera* and *Tinospora cordifolia* against COVID-19 by targeting Mpro and ACE-2. The first and most important step in any molecular docking analysis of ligands of interest is to develop a docking protocol and validate it.

To see if the docking procedure is chosen was sufficient for determining the proper binding mode of ligands binding to the active site, the bound ligand was removed from the initial X-ray crystallographic protein structure and re-docked using Flare 4.0. As compared to the co-crystallized X-ray structure, the set docking protocol successfully docked the extracted bound ligand within the protein with an almost identical binding mode.

Captopril and chloroquine were used as reference compounds in molecular docking experiments on the ACE-2 receptor. A total of 34 chemical constituents from *Withania somnifera* and *Tinospora cordifolia* were tested for their ability to inhibit ACE-2. The results of simulated screening Table 2 showed that 24 out of 34 chemical constituents outperformed both reference compounds in docking ratings. In contrast to reference compounds, the findings show that these phytochemicals can bind more effectively and serve as inhibitors.

For molecular docking studies on the Mpro receptor, PRD_002214 (ChemID 4883311) was used as a reference compound. A total of 34 chemical constituents from *Withania somnifera* and *Tinospora cordifolia* were screened for their inhibitory potential against Mpro. Results of virtual screening Table 3 revealed that all 34 chemical constituents have better docking scores than the reference compound. The results, in comparison with the reference compound, demonstrates that these phytochemicals can bind more efficiently and act as inhibitors.

Against ACE-2, Withanoside VII showed the best docking score (-14.812) among the screened constituents. Study of interaction between Withanoside VII and ACE-2 (Fig. 3) revealed that Withanoside's hydroxyl group on carbon at 5-position forms a hydrogen bond with alanine amino acid present at 348 positions in chain A. Hydroxyl group on carbon

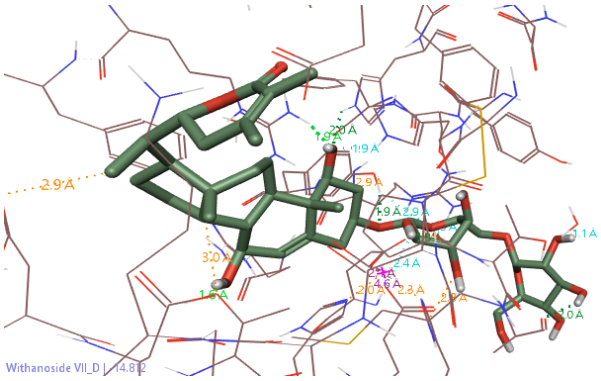


Fig. 3: Binding model of withanoside VII and its interactions with ACE-2 binding pocket

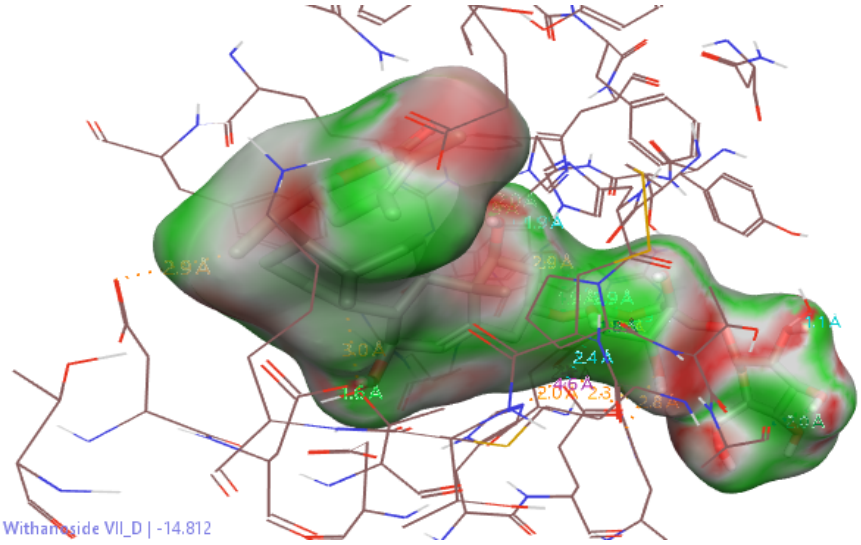


Fig. 4: Electrostatic complementarity of withanoside VII with ACE-2 binding pocket

Red colour regions represent perfect electrostatic clash with the binding site whereas green colour regions represent perfect electrostatic complementarity

Table 2: *In silico* screening results of the phytochemicals from *Withania somnifera* and *Tinospora cordifolia* against ACE-2

Compound name	Slog P	TPSA	Flexibility	Rof5	LF rank score	LF free energy of binding	LF virtual screening score
Withanoside III	3.7	158.4	13.9	1	-12.295	-13.79	-17.17
Withanoside V	2.8	198.9	17.9	3	-13.546	-15.953	-18.191
Withanoside VI	3.8	225.1	20.7	3	-11.224	-11.789	-17.15
Withanoside VII	3.2	245.3	21.7	3	-14.812	-12.789	-20.304
Withanolide sulfoxide	8.4	175.5	15.8	2	-1.34	-6.619	-13.831
Withanolide dimer	9.4	158.4	15.8	2	-5.57	-15.639	-18.261
Sominone	5.4	66.8	6.7	1	-10.72	-12.029	-12.224
Tinosporafuranol	5.3	33.4	10.0	1	-8.877	-8.178	-9.451
Tinosporaclerodanol	4.2	53.6	11.5	0	-9.067	-9.213	-9.866
Tinosporafurandiol	6.8	0.0	5.1	1	-3.385	-8.042	-8.285
Tinosporaclerodanoid	1.4	124.3	11.1	0	-8.937	-9.344	-10.385
Tinocordin	1.8	97	4.1	0	-10.154	-8.986	-9.686
Tinosporaside	3.5	56.5	2.6	1	-6.021	-8.217	-8.919
Beta-sitosterol D	8.3	20.2	9.3	1	-9.418	-12.107	-12.368
20a-hydroxyecdysone D	4.1	138.4	13.3	0	-13.498	-14.181	-16.333
Amritoside A	3.3	53.6	6.1	0	-9.981	-8.776	-9.647
Amritoside B	4.0	73.8	7.8	0	-8.843	-9.126	-9.26
Amritoside C	3.2	79.9	4.6	0	-10.84	-9.737	--9.783
Amritoside D	3.2	70.7	4.1	0	-10.711	-9.373	-10.632
Palmitoside C	2.7	65.7	3.3	0	-5.739	-7.832	-8.615
Boropetoside F	3.4	35.5	1.9	0	-5.559	-8.514	-9.721
Palmitoside F	2.1	78.3	4.4	0	-5.86	-8.239	-8.918
Boropetoside B	3.3	59.7	3.1	0	-10.745	-9.328	-10.163
Cordifoliside A	3.8	39.4	1.9	0	-6.554	-8.182	-9.725
Cordifoliside B	3.8	39.4	1.9	0	-6.901	-8.253	-9.9
Cordifoliside C	3.9	59.7	2.6	0	-7.623	-8.732	-10.482
Cordifoliside D	3.3	55.8	2.9	0	-9.969	-8.751	-9.28
Cordifoliside E	2.9	86.0	3.4	0	-10.255	-9.288	-10.592
Columbin D	2.2	86.0	4.3	2	-9.832	-8.676	-9.485
Iso Columbin	2.2	106.2	4.3	2	-10.519	-8.096	-9.395
8-hydroxycolumbin	1.6	106.2	5.6	1	-10.037	-8.687	-10.617
10-hydroxycolumbin	1.7	106.2	5.3	0	-10.711	-9.325	-10.097
-Epicatechin	1.9	110.4	3.5	1	-12.852	-10.247	-10.635
Apigenin	2.6	66.8	1	1	-11.939	-7.62	-8.653

Captopril and Chloroquine were used as reference compounds. The rank score of Captopril and Chloroquine was -8.13 and -5.48, respectively

Table 3: *In silico* screening results of the phytochemicals from *Withania somnifera* and *Tinospora cordifolia* against Mpro

Compound name	Slog P	TPSA	Flexibility	Rof5	LF rank score	LF free energy of binding	LF virtual screening score
Withanoside III	3.7	158.4	13.9	1	-8.547	-8.904	-11.169
Withanoside V	2.8	198.9	17.9	3	-8.64	-8.171	-11.883
Withanoside VI	3.8	225.1	20.7	3	-8.525	-41.313	-13.547
Withanoside VII	3.2	245.3	21.7	3	-7.719	-9.841	-12.687
Withanolide sulfoxide	8.4	175.5	15.8	2	-9.098	-10.526	-11.825
Withanolide dimer	9.4	158.4	15.8	2	-9.025	-11.036	-12.387
Sominone D	5.4	66.8	6.7	1	-7.508	-8.222	-9.358
Tinosporafuranol	5.3	33.4	10	1	-5.919	-6.829	-7.532
Tinosporaclerodanol	4.2	53.6	11.5	0	-6.001	-7.502	-7.708
Tinosporafurandiol	6.8	0.0	5.1	1	-2.382	-6.27	-6.553
Tinosporaclerodanoid	1.4	124.3	11.1	0	-8.342	-8.339	-9.363
Tinocordin D	1.8	97	4.1	0	-7.467	-7.016	-7.613
Tinosporaside D	3.5	56.5	2.6	1	-4.751	-6.681	-7.661
Beta-sitosterol D	8.3	20.2	9.3	1	-6.511	-8.014	-8.902
20a-hydroxyecdysone D	4.1	138.4	13.3	0	-8.88	-10.074	-10.74
Amritoside A	3.3	53.6	6.1	0	-7.088	-6.99	-7.165
Amritoside B	4	73.8	7.8	0	-8.04	-7.194	-8.61
Amritoside C	3.2	79.9	4.6	0	-7.778	-7.837	-7.392
Amritoside D	3.2	70.7	4.1	0	-7.861	-7.837	-8.356
Palmitoside C	2.7	65.7	3.3	0	-5.18	-7.393	-8.225
Boropetoside F	3.4	35.5	1.9	0	-4.258	-6.861	-7.448
Palmitoside F	2.1	78.3	4.4	0	-5.208	-7.204	-8.044
Boropetoside B	3.3	59.7	3.1	0	-7.435	-6.545	-7.109
Cordifoliside A	3.8	39.4	1.9	0	-4.541	-6.868	-7.469
Cordifoliside B	3.8	39.4	1.9	0	-4.596	-6.125	-6.95
Cordifoliside C	3.9	59.7	2.6	0	-4.415	-6.677	-7.238
Cordifoliside D	3.3	55.8	2.9	0	-8.599	-7.631	-8.345
Cordifoliside E	2.9	86	3.4	0	-7.194	-7.147	-7.919
Columbin D	2.2	86	4.3	2	-8.028	-6.671	-8.006
Iso Columbin	2.2	106.2	4.3	2	-8.327	-7.132	-8.302
8-hydroxycolumbin	1.6	106.2	5.6	1	-8.339	-7.408	-8.737
10-hydroxycolumbin	1.7	106.2	5.3	0	-8.181	-6.828	-8.072
-Epicatechin	1.9	110.4	3.5	1	-10.165	-6.5	-8.342
Apigenin	2.6	66.8	1	1	-10.166	-5.72	-6.654

PRD_002214 (Chem ID 4883311) was used as a reference compound. The rank score of PRD_002214 was 0.609

at position 6 and 17 also forms a hydrogen bond with the amino acid residue at position 815, 346 and 375 in chain A. Oxygen at position 18, 21 and 23 interacts with glutamine (402), histidine (378) and tyrosine (515) in chain A, respectively. Hydroxyl group at carbon 31 and carbon atom at position 34 interact with threonine 371. The hydroxyl group at carbon 28 interacts with histidine (345 and 505) and arginine 273. Carbon at position 55 interacts with aspartame 367.

Electrostatic complementarity of Withanoside VII with ACE-2 binding pocket was also evaluated Fig. 4. Electrostatic complementarity evaluation revealed that there is more green region (electrostatic complementarity) on the molecular surface in comparison to the red region (electrostatic clash)which further validated the favourable interaction of Withanoside VII with ACE-2 binding pocket. Electrostatic fields cloud of Withanoside VII in ACE-2 binding pocket represented the positive electrostatic fields regions (Magenta colour) and negative electrostatic field regions (blue colour) which are going to be important for designing similar molecules in future. Withanoside V, III and VI also showed a good docking score (-13.546, -12.295 and -11.224 respectively) which revealed that Withanosides have a strong affinity for ACE-2 receptors. 20a-Hydroxyecdysone D (docking score: -13.498) and Epicatechin (Docking score: -12.852) have also shown favourable docking results. A good docking score of Epicatechin imparts that the binding pocket of ACE-2 receptor can accommodate not only large molecules like Withanosides but also Epicatechin like small molecules.

Against Mpro, Apigenin showed the best docking score (-10.166) among the screened constituents. A study of the interaction between Apigenin and Mpro revealed that Apigenin's hydroxyl group on carbon at position 14 forms a hydrogen bond with the oxygen atom of glutamine (at position 14 in chain A) (Fig. 5). Oxygen at position 4 forms a hydrogen bond with the amino group of glycine (at position 71 in chain A). The hydroxyl group on carbon at position 3

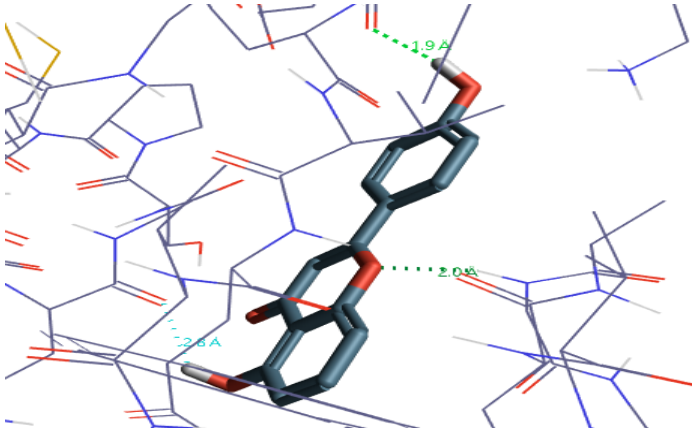


Fig. 5: Binding model of apigenin and its interactions with Mpro binding pocket

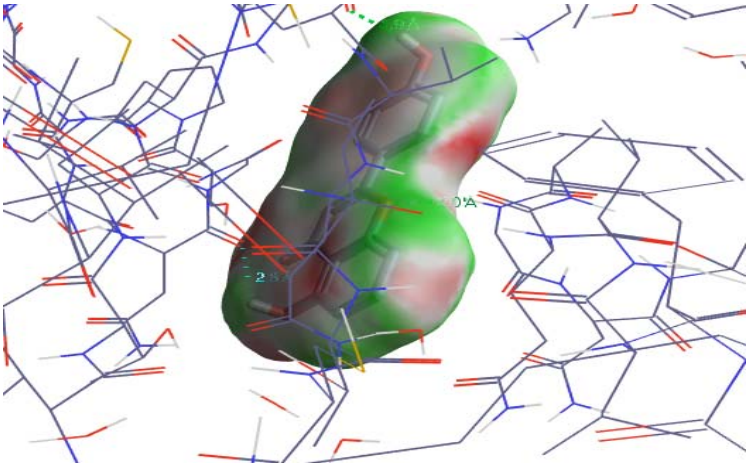


Fig. 6: Electrostatic complementarity of apigenin with Mpro binding pocket
Red colour regions represent perfect electrostatic clash with the binding site whereas green colour regions represent perfect electrostatic complementarity

forms a hydrogen bond with the carbonyl group of asparagine (at position 119 in chain A). Electrostatic complementarity of Apigenin with a Mpro binding pocket was also evaluated (Fig. 6). Electrostatic complementarity evaluation revealed that there is more green region on the molecular surface in comparison to the red region which further validated the favourable interaction of Apigenin with the Mpro binding pocket. Electrostatic fields cloud of Apigenin in Mpro binding pocket revealed that positive electrostatic fields regions are favourable for interaction with binding pocket. Epicatechin (docking score: -10.165) also showed receptor affinity similar to Apigenin. It revealed that Mpro has a potent binding affinity for flavonoids like Apigenin and Epicatechin.

Spike's protein of SARS-CoV-2 interacts with host cells' Angiotensin-Converting Enzyme 2 (ACE-2) receptor. After interacting with ACE-2, the Spikes protein's conformation changes, allowing the viral envelope to fuse with the cell membrane through the endosomal pathway, allowing SARS-CoV-2 to release RNA into the host cell. The genome RNA is encoded into the viral replicase polyproteins pp1a and 1ab, which are then cleaved into small products by viral proteinases. As a result, small compounds such as Epicatechin may reduce the binding effectiveness of spike protein to its receptor, eventually acting as an inhibitor for the attachment of SARS-CoV-2 spike protein to ACE-2 receptors[25].

By discontinuous transcription, the polymerase generates a sequence of subgenomic mRNAs, which are then converted into related viral proteins. In the endoplasmic reticulum and Golgi, viral proteins and genome RNA are assembled into virions, which are then transferred by vesicles and released from the cell. ACE-2, which acts as an entry point into the host

cell, has been identified as a possible candidate for vaccines or therapies. An antibody that blocks the receptor can make it more difficult for coronavirus to invade cells, potentially slowing the outbreak until the virus is eradicated. The key protease necessary for coronavirus proteolytic maturation is the recognized coronavirus 3-Chymotrypsin-Like protease (3CLpro), also known as Mpro[26]. This Mpro is critical for immune modulation and cleaving the polyproteins pp1a and pp1ab, making them appealing and important targets for anti-COVID-19 medicines. Mpro cleavage of pp1a and pp1ab polyproteins produce functional proteins such as RNA polymerase, endoribonuclease and exoribonuclease. As a result, blocking the Mpro enzyme can slow viral development while also boosting the host's natural defences against COVID-19.

COVID-19 therapeutic medication candidates are being investigated using novel approaches to drug design and discovery. The study of the interaction of ligand (drug) molecules inside the binding pocket of a target protein using molecular docking is a potential approach for drug discovery and development[27]. It allows researchers to look at things like hit molecule discovery, lead compound optimization and virtual screening[28-31]. Several existing medications, including oseltamivir[32], lopinavir[33], ritonavir[33], remdesivir[34], favipiravir[35], ribavirin[36], chloroquine and hydroxychloroquine[37] have shown potential efficacy against COVID-19. Protease inhibition accounts for the majority of these drugs[37]. This research looked into docking with the COVID-19 Mpro and ACE-2. Chloroquine has also been shown to have anti-SARS-CoV activity, which may be due to ACE-2 glycosylation depletion[38]. It is reported that these medications may also interfere with post-translational modification in viral protease and glycosyltransferases in the endoplasmic reticulum or trans-Golgi complex vesicles at low pH[38]. Therefore, docking of chemical constituents from *Tinospora cordifolia* and *Withania somnifera* was performed against Mpro and ACE-2 by taking chloroquine as one of the reference compounds.

ACE inhibitors are reported to reduce the risk of COVID-19 disease without any serious side effects which increase the risk of ICU care[39]. Researchers have reported that formulating antiviral drugs which inhibit SARS-CoV-2 Mpro could have potential clinical use[40]. Potential known inhibitors of Mpro and ACE-2 were used as a reference for the study. In the study, most of the phytochemicals screened were found to be better inhibitors than reference compounds. The present study revealed that chemical constituents from *Tinospora cordifolia* and *Withania somnifera* have the potential to inhibit viral Mpro and human ACE-2 receptors. With anolides, 20a-Hydroxyecdysone D, Epicatechin, Apigenin and similar compounds can be further studied to develop novel and potent agents against COVID-19.

CONCLUSION

The findings of virtual screening of these bioactive constituents revealed that most of them are more active than the reference compounds. These phytochemicals can bind to ACE-2 and Mpro more effectively and function as inhibitors. Therefore, these bioactive ingredients could be used to produce antiviral drugs against Coronavirus in the future.

SIGNIFICANCE STATEMENT

This study discovers that plant-derived chemical constituents can also inhibit ACE-2 and Mpro more effectively than some existing drugs and can be beneficial for the prevention and treatment of COVID-19. This study will help the researcher to uncover and develop novel inhibitors of Mpro and ACE-2. Thus, a new medication that can give relief from COVID-19 complications, may be arrived at.

ACKNOWLEDGMENTS

The authors are thankful to AlMaarefa University, Riyadh, Saudi Arabia, for providing support to do this research. Abdulhakeem S. Alamri would like to acknowledge Taif University for support No. TURSP (2020/288).

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