Section A-Research paper
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A review on in silico evaluation of natural derived compounds for inhibition of TMPRSS2

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ABSTRACT

The untapped potential of phytocompounds is always been a promising source whenever there is a demand from mankind. A worldwide pandemic, COVID-19, was originated in Wuhan, China and this was first reported to WHO on 31st December 2019 as "Pneumonia of unknown cause". As time went on, more and more people developed the disease as there was no effective therapy or no access to certain immunisations. Exploration of novel scaffolds can lead to the development of new molecules active against the specific targets in Covid-19 disease mechanism. Present manuscript reviews the current studies on in-silico search of a suitable lead molecule active against host cellular protease, TMPRSS2, which is essential for virus invasion into the host cell.

Key Words- Covid-19, in silico, TMPRSS2, viral protein, phytoconstituents.

INTRODUCTION

The pandemic has shaken the entire globe since it was identified in December 2019 in the most infamous fish market of Wuhan, China. The morbidity and mortality caused by Covid-19 are unparalleled and it was soon declared a pandemic. It belongs to a Beta class of Coronavirus, an RNA virus. This single stranded membrane enveloped RNA-virus is pathogenic to human race [4]. The virus is named corona because of the projecting out transmembrane spike glycoproteins (S protein) from its periphery which makes them look like a crown [5]. Because to its phylogenetic relationships to the SARS-CoV virus, it was named as 2019-nCoV or SARS-CoV-2. In just three months, it spread widely and developed into a pandemic. This particular viral infection is continuing to return as a new wave. India reported its first case on January 30, 2020, in the Kerela district of Thrissur [1-3].

SIGNIFICANCE:

The S1 unit of the viral glycoprotein attaches to the host cellular receptor angiotensin-converting enzyme 2 as the initiation phase of viral infection (ACE2). Because of its vulnerability to the viral S protein, the host cellular protease known as transmembrane protease serine 2 (TMPRSS2) is key for the entry of the virus into the host cell. The breaking of the viral S protein at the S1/S2 and S2' sites, which fosters the fusion of the viral part with the host cellular membrane, makes TMPRSS2 the most key aspect in the infection rate and spread of the SARS-CoV-2 virus. [6]. In cells lacking TMPRSS2, SARS-CoV-2 may additionally use the endosomal cysteine proteases CatB/L to trigger its glycoprotein. As a result, it was established that CatB/L, but not TMPRSS2, is key for the entry and development of the viral infection. [7]. The discovery of drugs to treat COVID-19 disease or to improve the condition of patients infected with the SARS-CoV-2 virus should focus on TMPRSS2 due to its crucial role in SARS-CoV-2 infection.

Despite multiple attempts to identify treatments to combat COVID-19, there hasn't been a breakthrough beyond the currently existing antiviral medications. [8]. A molecule or compound typically requires 12 years and more than $1 billion to develop into a therapeutically useful medication [12]. The application of computational techniques in this situation can shorten the time needed [13]. The cost value of developing a novel treatment is further diminished by in-silico screening of compounds, which speeds up the finding of molecules with therapeutic benefits. Moreover, computational modeling offers the chance to

determine the ADME (Absorption, Distribution, Metabolism, and Excretion) and "drug-likeness" of potential herbal compounds, helping to exclude substances that might not be effective throughout the specific stages of drug development [14]. Using computer programmes, interactions between various chemicals and protein targets can be explored in silico to identify potential clinical treatment leads. Hence, the evaluation of phytocompounds in silico using molecular docking and molecular dynamics prediction can help in the fast development of drugs to treat COVID-19.

Several substances have been found to have TMPRSS2 inhibiting properties. Camostat mesylate and Nafamostat are two of the clinically recognised powerful TMPRSS2 inhibitors. [9].

Recent in silico studies have revealed several potent TMPRSS2 inhibitors, including Miers (Menispermaceae), Columbin and Jatrorrhizine from Tinospora cardifolia (Willd.), Baicalein from Scutellaria baicalensis Georgi (Lamiaceae) Myricetin from Torreya nucifera (L.) Siebold & Zucc. (Taxaceae) and Proanthocynidine A2 from Litchi chinensis Sonn. (Sapindaceae) [10]. Moreover, the ability to inhibit TMPRSS2 infection has been observed for the antiviral compounds, carvacrol from Nigella sativa L. (Ranunculaceae), thymol from Trachyspermum ammi (L.) Sprague (Apiaceae) and bisdemethoxycurcumin from Curcuma longa L. (Zingiberaceae) [11].

Morphology

The zoonotic trait of Covid-19 has already shown its unpredictable nature. Recombination, mutator alleles and mutational heartiness area portion of the developmental components make Covid well-equipped for growing their host ranges, including people. The understanding of the Covid virology will help select the suitable target. The SARS-CoV-2 genome is approximately 29.9 kb in size. [15]. Four core proteins—the spike (S) glycoprotein, small envelope (E) glycoprotein, layer (M), and nucleo-capsid (N) proteins—as well as sixteen non-structural proteins (nsp1–16) are present in SARS-CoV-2. These proteins are attached to a single positive-sense viral RNA genome. Nsp1 interferes with both RNA processing and replication. Nsp2 maintains the host cell's endurance flagging pathway in balance. To isolate the decoded protein, Nsp3 is approved. Nsp-4 modifies ER membranes and has transmembrane space 2 (TM2). Nsp5 participates in polyprotein replication. A potential transmembrane space is Nsp6. The mixture of nsp12 and template-primer RNA was

essentially enlarged by the presence of nsp7 and nsp8. Nsp9 has the ability to bind ss-RNA. For the cap methylation of viral mRNAs, Nsp10 is essential. The RNA dependent RNA polymerase (RdRp), a crucial component of Covid replication and record, is found in Nsp12. During the time spent on replication and recording, nsp13 forms a bond with ATP and the zinc-restricting gap in nsp13 plays a role. The modifying exoribonuclease space is Nsp-14. Nsp15 acts as an endoribonuclease with Mn2+-subordinate activity. A 2'-O-ribose methyl transferase is Nsp16. [16].

(a) Spike glycoprotein

The trimeric S proteins, which protrude from the infection membrane and are a crucial component of the system that facilitates infection entry into the host cell, are among these main proteins. [17, 18]. The three components of the S proteins—a massive ectodomain, a single pass transmembrane, and an intracellular tail—all these are clove-shaped type-I transmembrane proteins. The S1 subunit, which contains a receptor-binding domain (RBD), and the film combination subunit make up the ectodomain of S-proteins (S2). The host transmembrane serine protease 2 (TMPRSS2) cuts the spike protein S2 subunit after the viral attachment process, allowing the cell to enter and leading to viral replication endocytosis and the collecting of viral particles. [19].

(b) Envelope Glycoprotein

It is a small protein with an amino acid range of 76 to 109 with a short hydrophilic N-terminal (7–12 amino acids). It possesses a longer hydrophilic carboxyl C-terminal that covers the majority of the protein and a larger hydrophobic transmembrane gap (25 amino acids) [20, 21]. This protein's importance stems from its pivotal role in SARS. It aids in the addition and distribution of the infection to the host cell. The protein also modifies cell processes, illuminating its function in limiting the pathogenicity of the infection. It should be regarded as a crucial pathogenic component of the main SARS-CoV2. [22].

(c) M layer

A crucial role in the infection is played by the M layer protein, especially during the sprouting and gathering stages. It mostly consists of three hydrophobic transmembrane regions, a brief amino acid terminal, and a large carboxyl-terminal location inside the virion. Understanding the structure of the new CoV depends on the M protein, a major structural protein that can interact with other primary proteins including the spike (S) and envelope (E) proteins. [23]. Any modification in the M protein will have a remarkable impact on the

cooperations with the infected cell as it promotes the S protein, which is linked to cell attachment and passage to the host cell. [24].

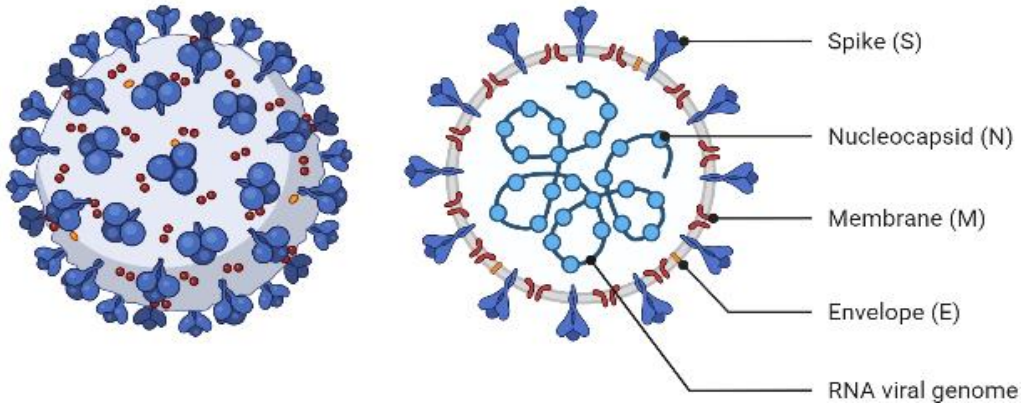


Fig 1: Structure of Covid-19 virus. Made by biorender.com

Transmission of COVID-19

The propagation of SARS-CoV-2 in humans has been extensively documented in the local community, the healthcare system, and among close family and friends. The primary modes of transmission are drips from the respiratory tract, followed by an indirect technique using spores and, less frequently, sprayers. Because that SARS CoV and MERS-CoV may infect the human gastrointestinal tract, it has been hypothesised that SARS-CoV2 may spread through oral waste [25, 26]. Although greater multiplication numbers have been suggested in some reports, the multiplication number (Ro) is typically between 2.0 and 2.8 [27]. The typical incubation period lasts 7 days, ranging from 3 to 11 to 14 days [28]. Those who are suggestive and asymptomatic are the main sources of illness. Indirect touch transmission is another way that the illness can spread. Drops harbouring infections contaminate hands, and when people later come into contact with the mucous films in their eyes, nose, and mouth, they become ill. SARSCoV-2 transmission is not only through the respiratory site. [29, 30].

Replication of CoV-2

The infection offers a fresh approach to replication. On the exterior of the virion, there is an inserted spike protein (S) with a weight of 150 kDa that resembles a crown. The Angiotensin-Converting Enzyme (ACE-2) and Transmembrane Protease Serine 2 (TMPRSS2) receptors on host cells serve as the major components that allow the S protein to enter the host cell.

[31]. Following that, it immediately multiplied at the lower portion of the ciliary epithelium of the parcel for the aircraft route. Via a S protein homodimer structure, it attaches to the host cell receptor there. Two subunits make up the S protein: S1 and S2. S1 associates with the ACE-2 receptor. The host cell transmembrane protease serine 2 (TMPRSS2) or the pH-dependent cysteine protease cathepsin-L operate on combination proteins found in the S2 subunit, allowing the infection to enter the host cell cytoplasm through endosome organisation. Viral RNA enters the system as a result of uncoating. [32,33]. Beginning the process of viral replication, it is converted into PP1a/b and primary proteins, and wrap glycoproteins are enmeshed in the layer of golgi bodies or endoplasmic reticulum. Following the nucleocapsid formation, the viral particles replicate in the ERGIC compartment, which is located halfway between the endoplasmic reticulum and the golgi bodies. In order to release the infection, these viral molecules finally link with the plasma layer. [34].

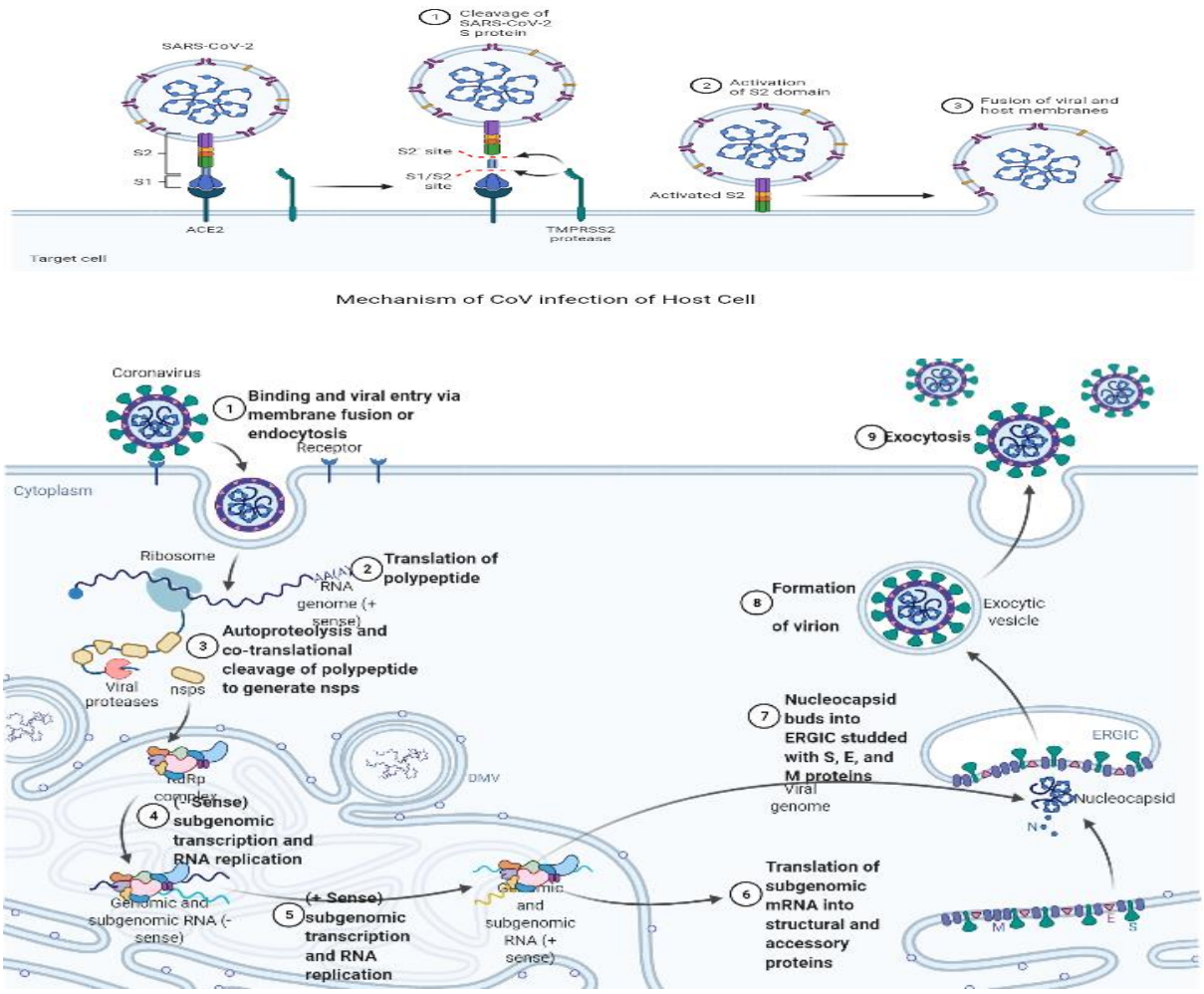


Fig 2: Mechanism of Covid-19 replication.Made by biorender.com

IN-SILICO APPROACH FOR PHYTOCONSTITUENTS

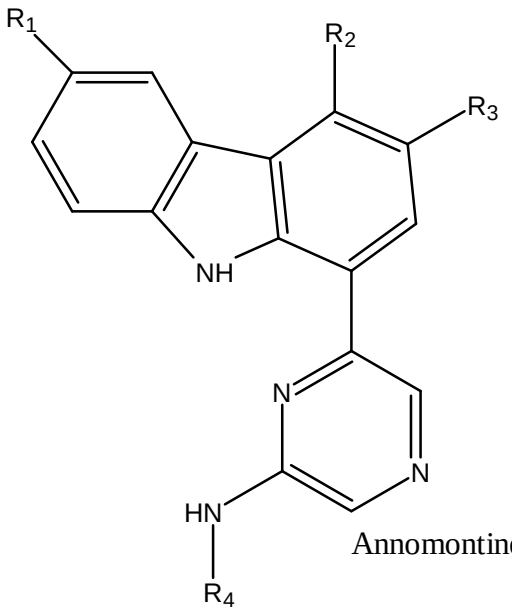
1. Annomontine Alkaloids

A class of heterocyclic alkaloids known as annomontines is found in the trunk and root bark of plants in the Annonaceae family. [35, 36]. In an in-silico study, 23 novel annomontine analogues (P3A, P3B, P3C, P3D, P3E, P3F, P3G, P3H, P3I, P3J, P3K, P3L, P3M, P3N, P3O, P3P, P3Q, P3R, P3S, P3T, P3U, P3V, P3W) have been designed and were docked against the different sub-atomic targets for SARS-CoV-2 and humans like MPRO, PLPRO, spike protein, ACE2, and TMPRSS2 separately to approve them as possible atoms for future investigation against SARS-Cov-2. The total of each molecule's docking score against each target, shown as a Lig E score versus hydroxycholoquine, was used to analyse the efficiency of each molecule. The majority of the annomontine derivatives displayed higher binding affinity with targets than hydroxycholoquine, according to the docking score. Any ligand with a score below that was considered to have a weak interaction because the cut-off value for a significant binding interaction was established at greater than -5.5 kcal/mol. The formula LigE Score=1/4 P Dock Score (ACE2 + NSP9 + MPro + PL-Pro + SP + TMPRSS2) was used to determine the most potent ligand against COVID-19. [37, 38].

Table 1: Molecular Docking results of annomontine based derivatives (ΔKcal/mol)

S.No.	Molecule	Docking score TMPRSS2 (kcal/mol)
1	P3U	-4.8345
2	P3V	-4.797
3	P3A	-3.31
4	P3C	-4.5285
5	P3W	-4.795
6	P3E	-4.215
7	P3R	-4.6255
8	P3L	-4.942
9	P3I	-5.2345
10	P3B	-4.465
11	P3O	-4.4965
12	P3A	-4.49
13	P3K	-4.892

14	P3F	-4.374
15	P3M	-4.702
16	P3Q	-4.2655
17	P3D	-4.296
18	P3N	-4.172
19	P3J	-4.497
20	P3P	-3.99
21	P3G	-3.96
22	P3S	-4.2895
23	P3Q	-4.45
24	P3T	-4.6275
25	P3H	-3.7915

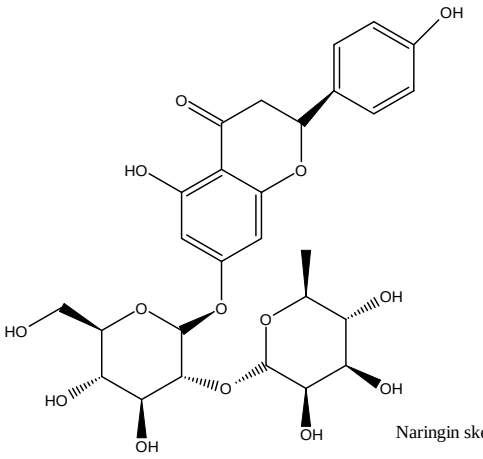


Annomontine skeleton

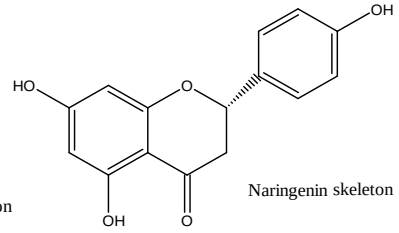
2. Natural Metabolites

Glycosylated flavonols, flavanones, terpenoids, and alkaloids such as naringin, naringenin, baicalin, hesperitin, eugenol, pseudojervine, 6-gingerol, quercetin, and myricetin are among the most remarkable in silico antagonists to SARS-CoV-2. Compared to most repurposed medication candidates, these molecules demonstrated improved control in a computer simulation. Their primary strategy against SARS-CoV-2 at the moment is to impede ACE2 and TMPRSS.

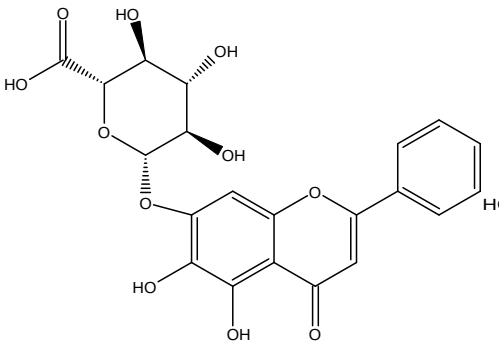
Terpenoids, for instance, have a lower restricting energy with 3CLpro, which lowers their ability to bind to cell films functioning directly in the infection structure and increase infection capacity. As RNA polymerase inhibitors for SARS-CoV-2, terpenic acids, such as oleanolic acid, have demonstrated encouraging effects. Because it is specific to the infection rather than host proteins, RNA polymerase interference is an extra element (ACE2 andTMPRSS2) [39].



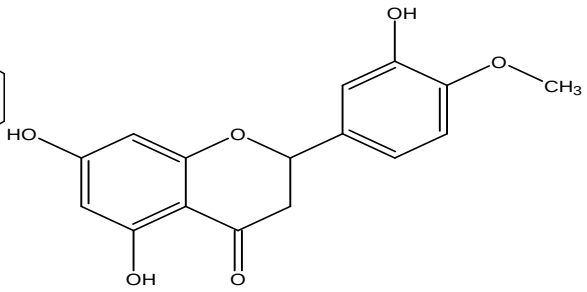
Naringin skeleton



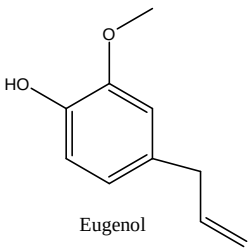
Naringenin skeleton



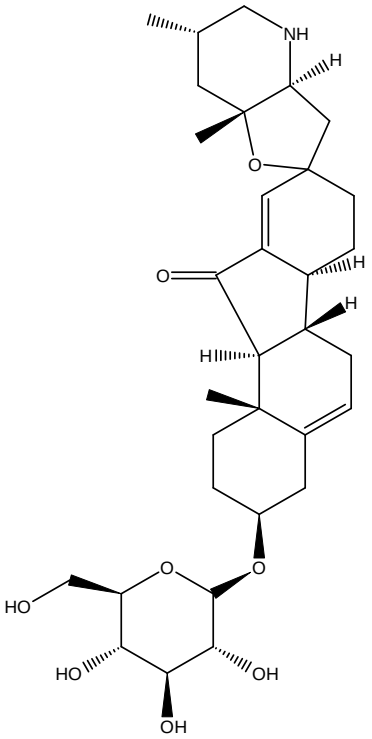
Baicalin skeleton



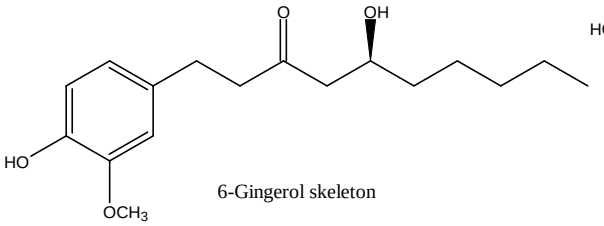
Hesperitin



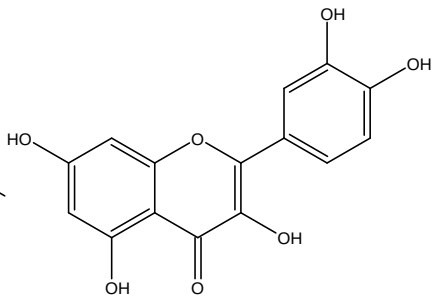
Eugenol



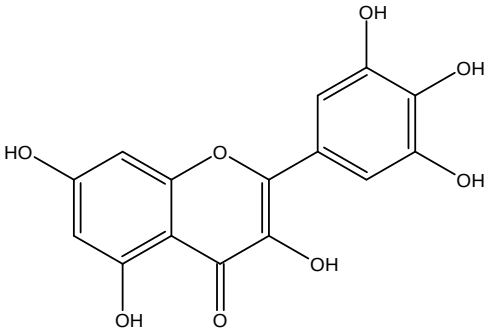
Pseudojervine skeleton



6-Gingerol skeleton



Quercetin skeleton

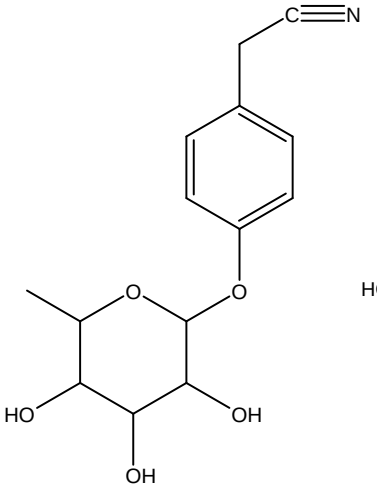


Myricetin skeleton

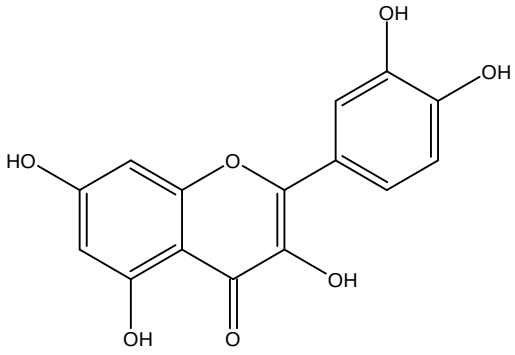
3. Phytochemicals from Zingiber officinalis Roscoe (Zingiberaceae), Artemisia annua L. (Asteraceae), and Moringa oleifera Lam. (Moringaceae)

As possible TMPRSS2 inhibitors, substances from the plants Z. officinalis, A. annua, and M. oleifera were evaluated. The actions of human TMPRSS2 were reported to be inhibited by

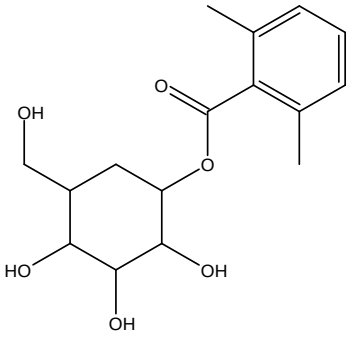
niazirin, quercetin, and moringyne by homology showing, atomic docking, and sub-atomic element reproduction. The three drugs showed good ADMET characteristics, however quercetin use necessitates caution. Almost, the subatomic element reproduction result advised moringyne as an amazing phytochemical to be taken into consideration as a possible TMPRSS2 inhibitor. The PubChem database's SDF files for the 2D chemical structures of the 132 phytochemicals were taken out and used for molecular docking. The control substance was camostat mesylate, an approved serine inhibitor with promising anti-SARS-CoV-2 viral treatment efficacy. Phytochemicals were molecularly docked with a transmembrane protease serine 2 (TMPRSS2) model in the PyRx virtual screening tool using the Open babel and Autodock vina wizard. [40].



Niazirin skeleton



Quercetin skeleton

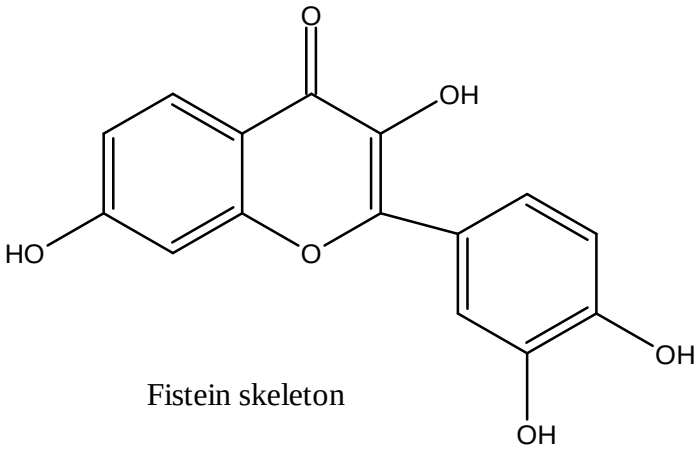


Moringyne

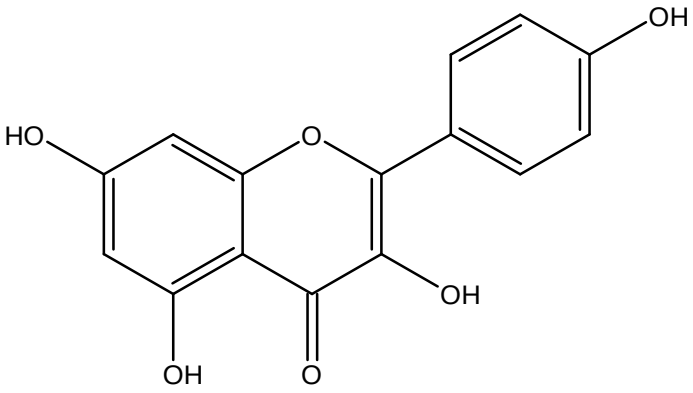
4. Flavonoid& non-flavonoid phytochemicals vs Hydrochloroquine

TMPRSS2 was created and docked with kaempferol, curcumin, pterostilbene, fisetin, quercetin, isorhamnetin, genistein, luteolin, resveratrol, and apigenin; the activity of these compounds was compared to that of hydrochloroquine. The docking analysis showed that

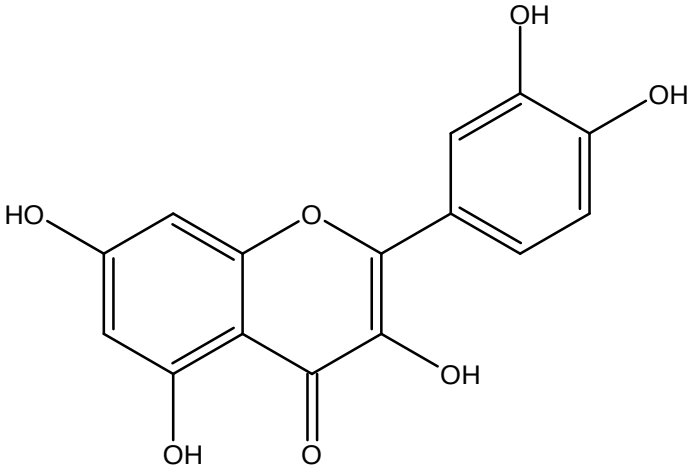
these phytochemicals and HQC displayed targeting for two of the three distinct spaces—S1-C terminal and S2 of the spike protein of SARS-CoV2—out of the three distinct spaces, namely S1-N terminal, S1-C terminal, and S2. Fisetin and quercetin both exhibit comparable restricting inclinations for the S2 region of the spike protein and have lowest binding energies of 8.5 kcal/mol. Three phytochemicals, fistein, kamferol, and quercetin, were identified by molecular docking studies as having promising activity against the TMPRSS2-S protein complex. [41].



Fistein skeleton



Kamferol



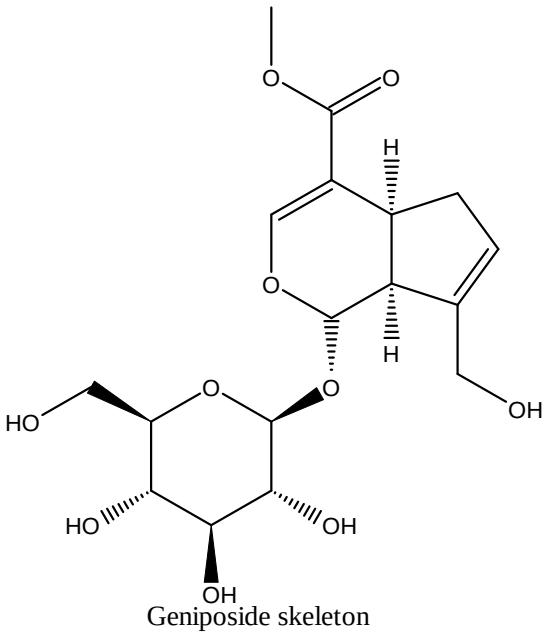
Quercetin skeleton

5. Virtual screening of phytochemical

Two approaches for compound screening, namely a pharmacophore-based approach and a sub-atomic docking score-based methodology, are used to search for active phytochemicals. The known inhibitor of serine protease 2 camostat mesylate's ten pharmacophoric components—anionic and cationic atoms, an H-bond donor and acceptor, an aromatic centre, a Pi ring focus, and a hydrophobic centroid—were chosen for the main compound screening method. In light of these components of the known inhibitor, 2140 compounds out of 30,927 were noted. These 2140 combinations were docked against TMPRSS2 in the subsequent chemical screening method to search for potent inhibitors. The 3D structure of TMPRSS2 was made using the SWISS MODEL programme. The molecular docking analysis found 85 substances with a docking score comparable to or lower than the FDA-approved drug camostat mesylate, a conventional inhibitor. Among these drug-like compounds, Compound 1 (NPC306344) got the highest docking score, at -14.69. The docking score of each of the found drug-like compounds with TMPRSS2 was (-13), which was greater than the docking score of the usual inhibitor (-11.06). The active site was identified using the MOE software. Methyl is the substance with the highest docking score (1S,4aS,7aS) -7-(hydroxymethyl) -1-[3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxy-1,4a,5,7a-tetrahydrocyclopenta[c] geniposide, also known as pyran-4-carboxylate, is one of the main iridoid glycosides found in gardenia fruit. [42].

Table 2: Docking scores of the top compounds with the lowest binding energies.

S.NO.	COMPOUND ID	DOCKING SCORE
1	NPC306344	-14.69
2	NPC473877	-14.38
3	NPC470916	-14.27
4	NPC66108	-14.02
5	NPC328914	13.96
6	NPC476270	-13.92
7	NPC84324	-13.59
8	NPC163169	-13.55
9	NPC155015	-13.38
10	NPC19631	-13.31
11	NPC53889	-13.10
12	NPC19622	-13.07
13	Camostat mesylate	-11.06



Geniposide skeleton

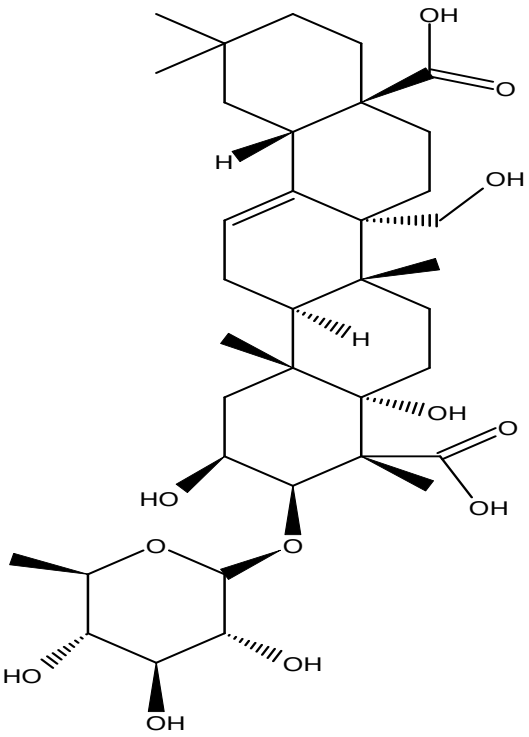
6. Cinnamon against main protease and spike glycoprotein of SARS CoV-2

Cinnamon, which is a rich source of antiviral agents. Since many years, cinnamon has been used as a traditional Indian medicine to treat a variety of lung-related problems, including

pneumonia, pulmonary disease, as well as life-threatening pleural effusion. Recent studies also provided rational evidence to support and reveal its antiparasitic, antihypertensive, antidiabetic, hostile to hyperlipemic, against oxidant, calming, pain relieving, antimicrobial, antiviral, antitumor, hostile to hypertension, against hyperlipemic, gastro-defensive, and immunomodulation activities. Molecular docking was carried out for 48 phytoconstituents obtained from various varieties of cinnamon on the limiting domain of catalyst COVID-19 in order to establish a potent candidate for regulating the coronavirus. The graphical user interface programme "Auto-Dock Tools" was used to prepare, run, and analyse the docking simulations. The 48 compounds were positioned based on their dock score and docked against the COVID-19 targeting enzyme. Out of 48 compounds, a total of 7 compounds were chosen based on dock score. The active substances were those with a dock score of 7.0 or less. Tenufolin demonstrated the desired dock score (8.8 Kcal/mol) against the primary protease and Pavetannin C1 got the highest docking score (11.11 Kcal/mol) with SARS-CoV2 spike protein. [43].

Table 3: Interactions of COVID-19 Main Protease amino acid residues with ligands at receptor sites.

Ligands	Binding affinity, ΔG (Kcal/mol)
N-[(5-methylisoxazol-3-yl)carbonyl]alanyl-l-valyl-n~1~-((1r,2z)-4-(benzyloxy)-4-oxo-1-{[(3r)-2-oxopyrrolidin-3-yl]methyl}but-2-enyl)-l-leucinamide	-7.4
Tenuifolin	-8.8
Cinnamtannin-B1	-8.4
Procyanidin-B7	-8.2
Kaempferol 3-alpha-L-arabinofuranoside-7-rhamnoside	-8.1
Proanthocyanidin-A2	-8
6-Glucopyranosyl procyanidin B1	-7.6
Pavetannin-C1	-7.3



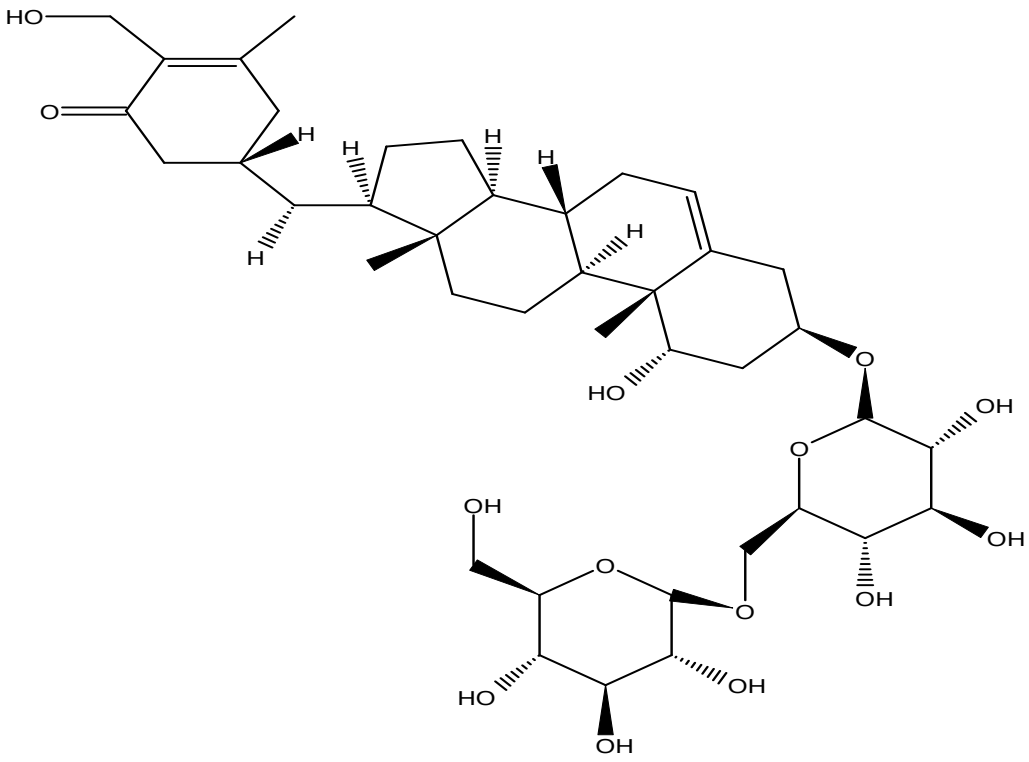
Tenufolin skeleton

8. Ashwagandha against main protease enzyme.

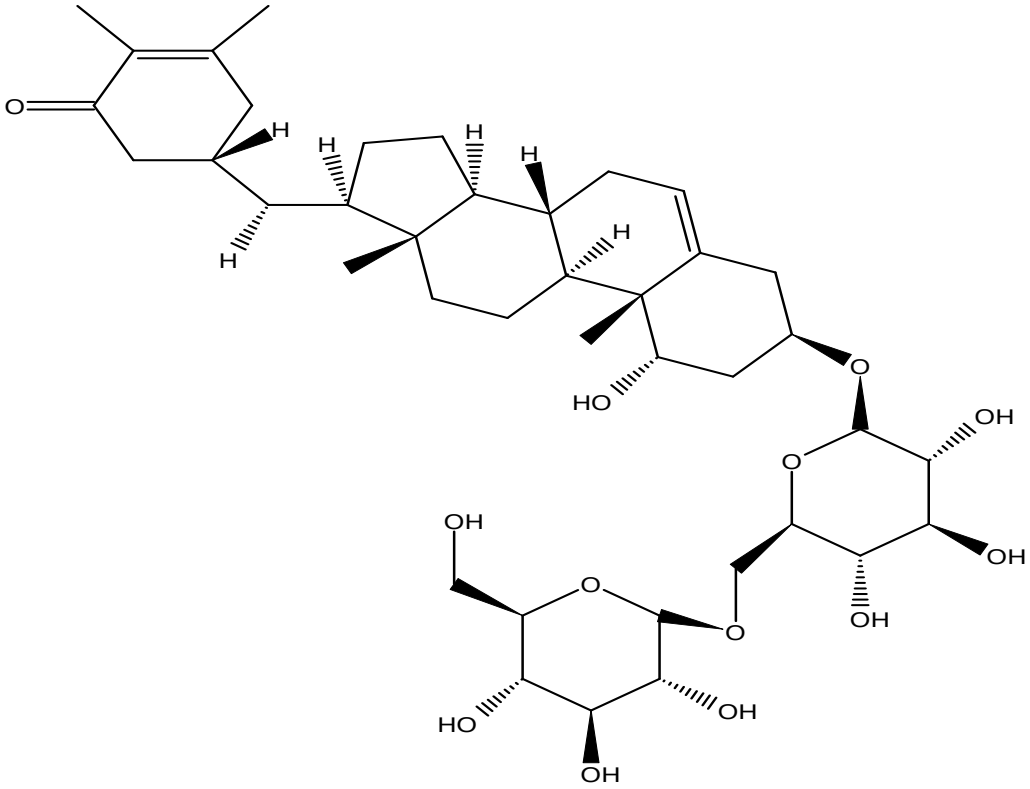
The Indian Ayurveda herb ashwagandha (Withania somnifera) is well-known for its medicinal benefits and capacity to boost the body's resilience. According to reports, the TMPRSS2 inhibitor camostat mesylate. As a result, it became a reference molecule for molecular docking. At the TMPRSS2 catalytic site, the binding affinity of Camostat mesylate was estimated, and the docking score in the ideal binding pose was 5.90 kcal/mol. At both the transcriptional and translational levels, the six withanolides withaferin-A, withanone, withanolide-A, withanoside-IV, and withanoside-V greatly reduced the outflow of TMPRSS2. Withanoside-IV and Withanoside-V outperformed Camosat mesylate in terms of docking scores. Moreover, computational analyses and molecular docking predicted that the majority of these withanolides might also suppress the Mpro of SARS-CoV-2. Withanoside-IV and Withanoside-V were predicted by Mpro to be the top scoring compounds after docking in the computational inquiry to determine the interaction of various withanolides with TMPRSS2. When the docked complexes were subjected to a 100 ns molecular docking simulation, the interaction between Withanoside-V and both molecular targets was unexpectedly stable. The binding score for the withanoside-IV and TMPRSS2 complex was 6.92 kcal/mol. The TMPRSS2-Withanoside-V complex received a docking score of 7.96 kcal/mol in the optimal binding pose. [44].

Table 4: Residues of TMPRSS2 interacting with the ligands during the course of MD simulations along with the free binding energy of each protein-ligand complex.

Ligands	**Free binding energy/Docking score**
TMPRSS2- Camostat mesylate	-54.98
TMPRSS2-Withaferin A	-37.80
TMPRSS2-Withanone	-46.80
TMPRSS2-Withanoside V	-36.19
TMPRSS2-Withanoside IV	-42.80
TMPRSS2-Methoxy withaferin A	-39.40
TMPRSS2-Withanolide B	-51.69



Withanoside IV



Withanoside V

CONCLUSION

This review paper on the computational research of phytochemicals tries to anticipate potential phytochemical inhibitors of human proteases, specifically TMPRSS2, which is in charge of the covid-19 virus's attachment to the host cell. The information acquired in this publication adds a substantial and crucial component to the mining of phytochemicals against COVID-19 and has generated some compelling hypotheses that would be confirmed with additional study and experimentation. Prior to being used in clinical trial investigations, the identified phytochemicals in this review need to undergo additional in vitro and in vivo testing.

DECLARATION

Conflict of Interest All authors have participated in conception and design, or analysis and interpretation of the data; drafting the article or revising it critically for important intellectual content; and approval of the final version. This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue. The authors have no affiliation

with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

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