Naringenin as a Possible Candidate Against SARS-CoV-2 Infection and in the Pathogenesis of COVID-19

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Abstract
Naringenin, widely distributed in fruits and vegetables, is endowed with antiviral and other health beneficial activities, such as immune-stimulating and anti-inflammatory actions that could play a role in contributing, to some extent, to either preventing or alleviating coronavirus infection. Several computational studies have identified naringenin as one of the prominent flavonoids that can possibly inhibit internalization of the virus, virus-host interactions that trigger the cytokine storm, and replication of the virus. This review highlights the antiviral potential of naringenin in COVID-19 associated risk factors and its predicted therapeutic targets against SARS-CoV-2 infection.

Keywords
naringenin, anti-inflammatory, covid-19, antiviral, receptor binding

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Introduction
Coronavirus disease 2019 (COVID-19) is an infectious disease caused by an infection with Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2).1,2 The surface spike protein (S protein) of SARS-CoV-2 plays a recognition role, which is directly involved in the infection process.3 For viral endocytosis, cleavage of the S-protein by host serine proteases such as transmembrane protease serine 2 (TMPRSS2) is necessary, and is followed by binding of the receptor-binding domain (RBD) in the S1 subunit of S protein to angiotensin-converting enzyme 2 (ACE2) in host cells,4 and/or cluster of differentiation 147 (CD147), also known as basigin or EMMPRIN.5 The S-protein has two subdivisions named S1 and S2, with S1 as the RBD.6,7 The RBD of the S-protein binds to ACE2 receptors of the host after activation by two host serine proteases: TMPRSS2 and furin.8 ACE2 expression is one of the main explanations for the higher airways infection, as it is highly expressed in the respiratory tract, such as epithelial cells of the alveoli, trachea, and bronchi, some bronchial glands, and alveolar macrophages.9 However, ACE2 is also expressed in the kidney, adipose tissue, heart, brain, blood vessels, stomach, liver, and oral and nasal mucosa,10 which could corroborate the systemic inflammatory profile in COVID-19.

Pathogenesis of SARS-CoV-2
The SARS-CoV-2 spike protein plays an important role in the process of the virus infecting host cells, as it mediates the attachment, fusion, and entering of host cells. The S-protein consists of subunits S1 and S2. While S1 is important for the virus attachment to the ACE2 receptor, S2 allows the fusion of the virus to the cell membranes, followed by internalization of the viral genetic material. Therefore, after attachment to the ACE2 receptor, the S-protein needs to be primed at the S1–S2 site by cellular proteases such as TMPRSS2.11,12 Therefore, the virus is capable of infecting human cells containing both ACE2 receptors and proteases, including lungs, small intestine, heart and kidney cells, as well as the nose, nasopharynx and oral mucosa.13

After the fusion of SARS-CoV-2 with the host cell, the viral RNA is released into the cytosol, which is translated into replicase proteins. The synthesized polyproteins are then processed by a 3C-like protease (3CLpro), also known as the main protease (Mpro), and papain like protease (PLpro) to release non-structural proteins (NSPs), including Nsp13 helicase, responsible for the replication and transcription of the viral genome.14 Thus, these proteins are very crucial for the viral replication cycle and inhibiting them may block the viral replication cycle and thus provide treatment of COVID-19.

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Viral-ACE2 binding is followed by excessive signal rewiring, which alters basic cellular processes (eg, metabolism, antioxidant production, and autophagy) and accelerates processes involved in cell cycle arrest. PLpro, which is involved in viral replication, modulates signaling that alters immune defenses and contributes to the cytokine storm (eg, nuclear factor kappa B and interferon 1) through its deubiquitinating activity and removal of interferon-stimulated gene 15 from cellular proteins. Thus, after cell entry and multiplication, the virus can cause an increase in the natural inflammatory response (defined as a cytokine storm), which can lead to greater immune activation. This cytokine storm has been associated with severe damage to the respiratory tract, blood hypercoagulation, cardiac arrest and lymphocytopenia, among other life-threatening conditions.

**Drug Repurposing and Flavonoids**

Drug repurposing is gaining wider attention in comparison to elucidating new drugs for disease management as it can aid in determining new indications for existing drugs. In this scenario, flavonoids, secondary metabolites found in fruits, vegetables, nuts, seeds, herbs, spices, and flowers, as well as in tea and red wine, represent an important subgroup of naturally occurring nephelic compounds. These have been intensively investigated for their pharmacological properties, such as anti-inflammatory, antilipidemic, antiangiogenic, antihyperglycemic, antiviral, hepatoprotective, anti-gastric ulcer, cardioprotective, neuroprotective, antioxidant and anticancer.

Computational in silico analysis, based on the concept of estimated free energy of binding and the formation of various intermolecular interactions such as hydrogen bonds, hydrophobic interactions and van der Waal’s interactions, predicted that flavonoids can target various essential structural features of Sars-CoV-2 protein required for virus entry and/or replication. Several flavonoids have been identified to be adsorbed to the Spike protein, inhibiting Sars-CoV-2 attachment to ACE2, thus preventing infection, and/or to interact with PLpro/Mpro/RdRp, thus inhibiting the replication process.

**Naringenin: Occurrence and Beneficial Effects**

Naringenin (NAR) [(2S)-4′,5,7-trihydroxyflavan-4-one; (2S)-5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-4H-1-benzo- pyran-4-one, Figure 1], an important natural flavonoid, is present in a wide variety of fruits and vegetables, either in the free state or as glycosides or acylglycosides; the highest concentrations are reported in grapefruit, tangerines, oranges, and tomatoes. NAR is of great interest due to its numerous beneficial activities, such as analgesic, antioxidant, hypolipidemic, hepatoprotective, elastase inhibitor, anti-inflammatory, anti-mutagenic, antitumor, antimicrobial and antiviral effects, and may control neurological, rheumatological, cardiovascular and liver diseases. Emerging evidence has revealed NAR efficacy in treating inflammatory-associated atherosclerosis, arthritis, metabolic syndrome, corneal neovascularization, metabolic diseases, and possibly in treating inflammatory-associated disorders.

**Anti-Inflammatory Activity of Naringenin**

Upon viral entry, the virus induces the host to increase the production and release of inflammatory cytokines, which can lead to greater immune activation and tissue damage. Thus, compounds having antiviral and anti-inflammatory properties could impact and/or restrict COVID-19 development. NAR has been reported to exert anti-inflammatory activity through inhibition of the nuclear factor kappa B (NF-κB) signaling pathway. NF-κB stimulates the expression of several inflammatory proteins, such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), interleukin-1 (IL-1), and inducible nitric oxide synthase (iNOS). In vitro and in vivo animal model studies indicate that NAR can down-regulate the expression of several inflammatory markers, such as toll like receptor 4 (TLR4), TNF-α, IL-1β, IL-6, iNOS, and COX-2 through attenuation of the NF-κB pathway and activation of AMP-activated protein kinase (AMPK), which is associated with the regulation and/or inhibition of multiple pro-inflammatory signaling pathways.

NAR has been reported to inhibit the secretion of IL-6, IL-1 β, and TNF-α in LPS-stimulated acute lung injury in C57/BL6 mice by targeting inhibition of the phosphatidylinositol-3-kinase (PI3K)/Akt pathway. NAR also reduces the levels of IL-6 and TNF-α in LPS-stimulated acute lung injury in rats by inhibition of the NF-κB pathway. In both cases, the required effective dose was found to be 100 mg/kg through the oral route.

NAR inhibits the phosphorylation of MAPKs by reducing NF-κB and AP-1 translocation and DNA binding, which limits the development of pro-inflammatory cytokines such as IL-33, TNF-α, IL-1β, and IL-6. NAR has been reported to have suppressed respiratory overexpression and eosinophilic airway inflammation in asthma and thus reduced acute neutrophilic airway inflammation by blocking the NF-κB pathway. Naringenin may be used against pneumonia associated with the spread of COVID-19 owing to its good anti-inflammatory and antioxidant activity.

In murine macrophages, NAR reduced inflammatory mediators production induced by LPS. In murine macrophages
infected with *Chlamydia trachomatis*, NAR reduced the production of IL-1β, IL-1α, IL-6, TNF, IL-12p70, and IL-10 in a dose-dependent manner. Moreover, NAR’s anti-inflammatory effects have been demonstrated in *ex vivo* human whole-blood models, reducing IL-1β, IL-6, IL-8, and TNF upon LPS stimulus close to that of non-stimulated levels. In an animal model of acute respiratory distress syndrome (ARDS), a syndrome with an increase in IL-6, TNF, and neutrophils in the lungs, NAR supplementation reduced neutrophil infiltration and oxidative stress, greatly reducing airway inflammation and lung injury. In a murine asthma model, treatment with NAR reduced airway hyperactivity and airway inflammation, with a reduction in the levels of IL-4 and IL-13 in bronchoalveolar lavage and serum IgE levels, as well as improvement in lung function assay. NAR has been reported to modulate different inflammation syndromes and at different sites, such as colitis, hepatitis, obesity, cancer, and acute respiratory syndrome. This is particularly important in COVID-19 because SARS-CoV-2 infection induces a systemic inflammation and can infect many different organs including lungs, heart, liver, brain, kidneys, and intestines. In addition, NAR can promote lysosome-dependent cytokine protein degradation, which may be important in COVID-19. Overall, various studies have demonstrated that NAR is a strong candidate as an adjuvant in reducing airway and systemic inflammation.

**Antiviral Significance of Naringenin**

The antiviral effect of NAR has been studied in several viruses, such as dengue, hepatitis B, hepatitis C, zika, chikungunya, Semliki Forest, herpes simplex 1 and 2, Sindbis neuroviral strain (NSV), rotavirus, yellow fever, and human immunodeficiency virus. NAR has been extensively investigated in *in vitro* models of viral infection, but very few results are available for *in vivo* studies. Nevertheless, the *in vitro* and *in vivo* anti-inflammatory potential of NAR has been highlighted in several animal models, including respiratory syndromes. NAR had been recommended as a therapeutic adjunct to Nucleoside-Reverse-Transcriptase-Inhibitors for antiretroviral therapy. In *in silico* docking studies, NAR has demonstrated blocking of the neuraminidase site by NAR and other flavonoids in Influenza *Type A* viruses. Thus, NAR exhibits a broad-spectrum antiviral activity which involves inhibiting a variety of viruses.

Goncalves et al demonstrated that intake of 2.7 mg NAR in patients with hepatitis C resulted in a greatly improved lipid profile and reduction in liver enzyme AST (Aspartate aminotransferase). Therefore, NAR can reduce HCV infection, and, in a dose-dependent manner, its administration can inhibit the post entry stages of CHIKV replication activity by downregulating the production of the viral proteins involved in replication. Moreover, NAR is an inhibitor of endolysosome two-pore channels (TPCs), involved in SARS-CoV-2 and Ebola virus infections, as well as in the ability of HIV-1 protein Tat to escape endolysosomes. NAR can induce cancer cell death by promoting autophagy and downregulating the Akt/mTOR signaling pathway. In *Vero E6* cells infected with SARS-CoV-2, NAR inhibited the cytopathic effect in a time and concentration-dependent manner. This effect was mediated through inhibition of endolysosomal TPCs, a pathway involves in the infectivity of SARS-CoV-2, Ebola, and MERS via facilitating viral entry. These findings suggest a possible use of naringenin against COVID-19 by targeting TPCs and the Akt/mTOR signaling pathway.

Based on existing knowledge, efforts directed toward designing anti-COVID-19 drugs are focused on impeding virus entry into host cells, inhibiting virus-host protein interactions, and interrupting viral replication, with the aim of aborting the inflammatory responses induced by viral invasion. Thus, therapies that may act on the coronavirus can be divided principally into three categories: (i) either blocking the virus from binding to human cell receptors, or acting on the host’s specific receptors, thus preventing the virus from entering the host’s cells ie, inhibiting viral entry; (ii) preventing virus RNA synthesis and replication; and (iii) reducing the virulence factor to restore the host’s innate immunity. As such, in *in silico* studies have investigated the use of flavonoids as effective therapeutic candidates against COVID-19 by targeting S protein cleavage, S protein binding to cell surface receptors such as ACE 2, and binding to viral proteases such as PLpro, Mpro and RdRp, as well as by interfering with NSPs of SARS-CoV-2 in order to hamper viral replication.

**Coronavirus and Naringenin**

The current COVID-19 pandemic has triggered global efforts for the rapid identification of vaccines and specific antiviral treatments. Based on the known mechanisms of SARS-CoV-2 infection, substances with potentially beneficial effects may act at various stages, such as preventing the binding of the virus to the receptors or inhibiting the function of the receptor, suppressing viral replication, helping cells to resist viral attack via inhibition of cytotoxicity processes, and blocking the virus spread in the body. Coronavirus infection can lead to cytokine storm, progress to septic shock, and cause death, thus, modulating the cytokine storm is a vital process for treating COVID-19. NAR has been used in experimental models to regulate the production of IL-6 and TNF, cytokines that are increased in COVID-19. Also, in an animal model of septic shock, the consumption of NAR has been demonstrated to reduce kidney damage via an increase in antioxidant enzymes.

Various computational or *in silico* techniques, based on the concept of estimated free energy of binding and the formation of various intermolecular interactions such as hydrogen bonds,
hydrophobic interactions and van der Waal’s interactions, have been widely used to gain insights into ligand-protein interactions. Several docking studies have identified naringenin as an important flavonoid which can interact with the host ACE2 receptor, viral spike protein, and proteases, thus inhibiting entry, as well as replication of coronavirus.

Naringenin as a Potential Inhibitor of SARS-CoV-2 Proteins: In Silico Studies

The in silico approach is important to identify antagonist compounds that can specifically target the binding sites of viral proteins through complex molecular interactions responsible for virus inoculation and replication. This goal can be achieved by targeting structurally important binding sites of the host, such as the RBD domain of spike protein, the ACE2 receptor of the host and/or viral proteases, such as Mpro and PIpro. ACE2 and Mpro represent important targets for the development of new antiviral agents. Based on several receptor-ligand interaction studies, NAR has been found to bind effectively with several targets of the virus, as well as the host.

Binding with Spike-Protein

Spike glycoprotein is a type I glycoprotein that extends out of the surface of the virus and is the first component to come into contact with the host cell, and thus it mediates the entry of SARS-CoV-2 into host cells. This glycoprotein protrudes from the surface of mature virions and is critical for SARS-CoV-2 entry into the host cell as it interacts with ACE2, enabling virus penetration into the host. In fact, spike glycoprotein contains a Receptor Binding Domain (RBD) that recognizes the ACE2 receptor leading to cleavage of the trimeric spike protein into S1 and S2 that facilitate membrane fusion and virus infection, followed by endocytosis.

In a study to identify flavonoids in peppermint leaf that prevent RBD/ACE2 (PDB: 6M0J) attachment, NAR was found to have moderate binding affinity regarding the RBD/ACE2 complex (about −6.44 Kcal/mol). Binding site surface analysis showed pocket-like regions on the RBD/ACE2 complex that yield several interactions (mostly hydrogen bonds and π-stacking bonds) between the flavonoid and distinct amino acid residues of both RBD and ACE2 proteins. The target RBD/ACE2 amino acid residues are Arg375, Asn15, and Glu19 from ACE2, and Arg668 from RBD, based on their higher occurrence. In a moleculardocking study by Utomo et al to reveal the antiviral potential of citrus and galangal constituents, NAR was identified to exhibit low energy binding, with a docking score to the spike glycoprotein (PDB: 6LXT) receptor of −7.40 Kcal/mol.

In an another study, NAR was found to have more substantial binding affinity (−9.0 kcal/mol) to viral spike glycoprotein (PDB: 6VSB) than remdesivir. In a docking study by Maurya et al, NAR exhibited significant interactions with spike glycoprotein, PDB: 6VXX (MolDock score - 82.10, interactions - 103.13 kcal/mol), exhibiting binding interactions with Lys304, Arg765, and Thr768.

Among 10 flavonoids docked into S protein, naringin (naringenin-7-O-neohesperidoside) exhibited the highest binding affinity (− 9.8 kcal/mol), even higher than that of dexamethasone (− 7.9 kcal/mol), a standard drug repurposed for treating critically ill COVID-19 patients. Molecular dynamics simulation denoted conformational stability of naringin within the active site of S protein, and decreased viral load and related cytopathic effects in Vero E6 cells. It should be noted that the binding of NAR to the S protein has not been experimentally validated.

Binding with TMPRSS2

Virus infection is initiated by the interaction between the S protein and host cell surface receptors. The S protein would be cleaved by the cellular serine proteases TMPRSS2 into S1 and S2 subunits, which are responsible for receptor recognition and membrane fusion. A computational study involving TMPRSS2 (PDB: 2OQ5) and naringenin reflected that NAR exhibited a strong binding affinity (- 7.3 kcal/mol), and thus may prevent viral entry.

Binding with ACE2

ACE2, which is located on the surface of the host cells, has been identified as a key preferable receptor for the binding of the spike protein of COVID-19. This enzyme acts as the receptor that the virus uses to enter the cell. The S protein is activated by the host TMPRSS2 or cathepsin L. Previous studies showed that certain flavonoids exhibit angiotensin-converting enzyme inhibition activity.

The invasion of SARS-CoV-2 involves binding of S protein to the ACE2 receptor on the host’s cell surface, thus substances that may either compete with the ACE2 receptor or reduce the ACE2 expression may represent an alternative or adjuvant therapy. In fact, NAR consumption has been associated with a reduction in ACE2 expression in the kidneys of rats. However, nutritional interventions aiming to regulate SARS-CoV-2 entry receptor ACE2 need to be carefully evaluated, as downregulation of ACE2 could also lead to greater inflammation and lung damage. Oral consumption of NAR reduced acute lung injury in a mouse model and reduced the production of pro-inflammatory cytokines.

ACE2, the major receptor for SARS-CoV-2 viruses in humans, represents the gateway for entry. In fact, ACE2 is abundantly expressed in the lungs, heart, kidney, vasculature, and cardiorespiratory neurons within the brainstem. An issue regarding ACE2 and coronavirus infections is that most of the chronic treatment of hypertension and diabetes involves the use of ACE inhibitors (ACEIn). Molecular docking studies have been performed to predict the binding affinity of various flavonoids, including NAR, to ACE2. Cheng et al
showed that NAR could bind to ACE2 with an estimated docking energy of −6.05 kcal/mol, with binding sites to Pro146, Leu143, and Lys131. In another study, Alzaabi et al have shown that NAR binds to ACE2 (PDB: 1R4L) having a binding energy of −8.5 kcal/mol.\(^{11}\) NAR exhibited low energy binding with a docking score of −7.69 Kcal/mol to the PD-ACE2 (PDB: 6VW1) receptor.\(^{66}\)

Naringin, which is the 7-O-rutinoside of NAR, exhibits the highest binding activity to the ACE2 enzyme with an estimated docking energy of −6.85 kcal/mol, with potential binding sites at Tyr515, Glu402, Glu398, and Asn394.\(^{179}\) NAR interacts with Ala348, Asp350, His378, Asp382, Tyr385, Arg393, Asn394, and His401 at the active site of ACE2 (PDB: 1R42), with a generated MolDock score of − 83.42 kcal/mol, and interaction energy of − 102.46.\(^{167}\) Thus, NAR has significant binding affinity towards the ACE2 receptor and, therefore, may be used for ACE2-mediated attachment inhibition of SARS-CoV-2

**Binding with PLpro**

The papain-like protease (PLpro) is an essential coronavirus enzyme that is required for processing viral polyproteins to generate a functional replicase complex, and thus is a validated antiviral drug target.\(^{180,181}\) A study by Cho et al found that *Paulownia tomentosa* Steud flavonoids (quercetin, catechin, naringenin and geranylated flavonoids) inhibit SARS-CoV-PLpro and reduce the concentration of pro-inflammatory cytokines (IL-1β) and TNFα.\(^{182,183}\) It should also be noted that NAR has not been tested against PLpro in the enzymatic assay.

**COVID-19 Main Protease (Mpro) Inhibition**

Among the coronaviral targets that have been studied in the past, the main proteases (Mpro, 3CLpro, nsps) received major attention as being responsible for processing CoV-encoded polyproteins that facilitate viral transcription and replication. They are responsible for the proteolytic cleavage of virus polyprotein in 11 non-structural proteins responsible for its replication. Thus, Mpro plays an indispensable role in the maturation of NSPs and promotes the biosynthesis of the virus, and so can be considered as a dominant target against SARS-CoV-2.\(^{184}\) Mpro exclusively cleaves polypeptide sequences after a glutamine residue, positioning the main protease as an ideal drug target.\(^{185-189}\) Mpro has 3 domains, and the substrate-active site of binding is located in the cleft of domain I and domain II, consisting of His41 and Cys145.\(^{190}\) Thus, Mpro is a cysteine protease with a catalytic dyad (cysteine and histidine) in its active center and is surrounded by other residues which confer substrate specificity. Thus, the occurrence of Mpro only within SARS-CoV-2 and not in the host cell has focused attention on this as a possible inhibition site for COVID-19 treatment.\(^{190-195}\)

In a molecular docking study, aimed at identifying compounds found in medicinal plants with the potential to be COVID-19 Mpro inhibitors, NAR exhibited a binding energy of −7.99 kcal/mol when docking with Mpro (PDB: 6LU7), suggesting NAR as a potential COVID-19 Mpro inhibitor. NAR forms H-bonds with the 6LU7 amino acid His164, Glu166, Asp187, and Thr190.\(^{191}\) In another computer-aided virtual screening approach to identify potential Mpro inhibitors, more than 8000 natural products were screened and eighteen showing promising in silico studies were selected for further in vitro screening, which resulted in five potential hits (naringenin, 2,3',4,5',6-pentahydroxybenzophenone, apigenin-7-O-glucoside, sennoside B, and acetoside). Naringenin displayed high activity (98% inhibition of enzyme activity at 100 µM concentration) against the viral protein.\(^{196}\) The most potent compounds were tested in vitro on SARS-CoV-2 Egyptian strain. Of the compounds tested, only naringenin showed moderate anti-SARS-CoV-2 activity at non-cytotoxic micromolar concentrations, with a promising selectivity index (CC50/IC50 = 178.748/28.347 = 6.3). The IC50 value for naringenin was 92 nM, compared to 44 nM of the positive control (GC376). Docking studies for NAR with SARS-CoV-2 MPRO active site (PDB entry: 6w63) showed that naringenin was able to form hydrogen bonds with the carbonyl of Thr190 and the phe- nolic group of Tyr54, in addition to van der Waal’s interactions with His41, Met49, Met165, and Pro168, and having an XP GScore of −7.083.\(^{192}\) In another study, NAR’s docking with Mpro (PDB: 6LU7) showed a binding energy of −6.8 Kcal/mol.\(^{31}\) In another report, NAR exhibited low energy binding with a docking score of −12.44 Kcal/mol to the Mpro (PDB: 6LU7) receptor.\(^{68}\)

A virtual docking screening study related to 49 bioactive phytochemicals from several medicinal plants used in Jamu (Indonesian traditional herbal medicine) and 3CLpro (PDB: 6LU7) showed that eleven compounds exhibited good binding affinity with 3CLpro (-7.2 to −8.5 Kcal/mol), with an energy binding of 7.7 Kcal/mol for naringenin. Naringenin interacted with three hydrogen bonds (HBs) with residues Cys145, Glu166, and Asp187, van der Waal’s interaction with residues His41, Pro54, Tyr54, Leu141, Asn142, Ser144, His164, Met165, Arg188, and Glu189, unfavorable donor-donor interaction with residue Gly143, cation-π interaction with residue His163, and pi-alkyl interaction with Met49. Thus, naringenin has interactions with the active site and substrate-binding pocket located between the clefts of domain I and II of 3CLpro.\(^{193}\) NAR, having a log p value of 2.46, followed the Lipinski rule of five for high drug ability, with no violation.\(^{168}\)

Additionally, pharmacokinetic studies showed that NAR possesses favorable drug-likeness properties. NAR showed low GI absorption and a high volume of distribution, and, therefore, it should attain therapeutic effects upon oral administration.\(^{169}\) In a study by Nguyen et al, related to the inhibitory effects of plant polyphenols on SARS-CoV-2 Mpro, it was found that NAR exhibited 50% inhibition and an IC50 of 150 ± 10 µM.\(^{194}\) Using an in silico method, via AutoDock Vina 1.1.2., for selected components of grapefruit seed extract (narrirutin, naringin, naringenin, limonin, ascorbic acid and citric acid) against SARS-CoV-2 main protease (PDB ID: 6Y84), naringenin was identified as one of the components, having a
binding affinity of −8.2 kcal/mol. In a docking study involving Mpro (PDB:6lu7) and Mpro (PDB: 6y2f), NAR displayed HB interactions with Glu166, Gly143, and Leu141 and two HBs with Thr25, respectively. In addition to HB interactions, the 4′-hydroxyl group of NAR occupied the amino acid clefs through hydrophobic–hydrophobic interactions. In order to ensure the effect of NAR’s absolute configuration in docking mode (enantioselective docking relationship) and subsequently explore the structure activity relationship (SAR), the NAR-(R) isomer demonstrated a different binding pose and mode with the receptor. Docking results clarify that the two enantiomers of NAR display dissimilarity in their binding pose inside the active site. These findings suggest that naringenin shows potential for inhibition of SARS-CoV-2 Mpro, but further studies are needed, as well as preclinical and clinical trials for final confirmation of its inhibitory functionality.

**Binding with RNA-Dependent RNA Polymerase (RdRp)**

RNA-dependent RNA polymerase (RdRp or NSP12) is an essential enzyme required for viral replication and transcription. In a virtual docking screening study with RdRp (PDB: 7BV2), NAR exhibited significant binding, showing a binding energy of −7.7 Kcal/mol.

**Targeting the Endo-Lysosomal Two-Pore Channels (TPCs)**

It has been shown that CoV infection depends on trafficking of the virus to lysosomal compartments and processing of the S protein by lysosomal proteases. The role played by endo-lysosomal Two-Pore Channels (TPCs) on CoV biology and the feasibility of blocking the intracellular pathway of the virus by inhibiting these channels can be of significance to impede virus trafficking. Interestingly, NAR can inhibit the activity of TPC1 and TPC2, both in humans and plants. NAR is a hydrophilic substance with a higher affinity for the cytoplasmic membrane, generating intracellular accumulation of NAR. It has recently been demonstrated that the activity of human TPC channels can be inhibited by NAR. Naringenin exhibited a partial inhibition of SARS-CoV-2 replication observed at 24 h post-infection (hpi) in cells upon Two-pore channel 2 (TPC2) silencing, while stronger inhibition was observed at 48 and 72 hpi. Therefore, the TPC’s modulation by NAR should be further investigated as a possible anti-coronavirus intervention.

**Nasal Spray, Containing Xylitol Plus GSE (Grapefruit Seed Extract)**

Grapefruit seed extract (GSE), a commercial product made from grapefruit seeds and pulp, is often used as a dietary supplement. The secondary metabolites of grapefruit seeds are predominately limonoids and flavonoids, such as limonin, naringin, narirutin, naringenin and hesperidin. GSE showed anti-viral activity against enveloped viruses, but not against non-enveloped viruses. A study by Go et al showed that GSE plus xylitol as a nasal spray solution, commercially available as Xlear nasal spray, can be used as a potential adjunct treatment of COVID-19. In silico analysis showed that several components of grapefruit seed extract, including naringenin, bind with Mpro with a binding affinity of −8.2 kcal/mol.

**Traditional Chinese Medicine (TCM)**

Traditional Chinese Medicines (TCM) or Chinese herbal medicines (CHM) have often been used in treating epidemic diseases and in the management of COVID-19. Molecular docking was found to be a method of choice, which has often been complemented with drug target prediction and network pharmacology analyses to evaluate the potential of TCM as it allows identification of molecules and formulations that have the highest potential to either affect host-pathogen interaction or treat already infected patients.

By using molecular docking and network pharmacology analyses, Deng et al screened out naringenin, robinin, kaempferol, quercetin, isorhamnetin and iridoidene from Huoxiang Zhengqi as potential 3CLpro inhibitors, by targeting PIK3CG and E2F1 through the PI3K-Akt signaling pathway. Wu et al, Zhao et al, Xu et al and Fan et al applied network pharmacology to analyze Qingfei PaiDou decoction and found that quercetin, luteolin, kaempferol, naringenin, β-sitosterol, isorhamnetin, baicalein, and tussilagone could be the main active ingredients. Ren et al showed that kaempferol, naringenin, and wogonin in Jinhua Qinggan granules docked well with specific target proteins of SARS-CoV-2. Ling et al reported that 18β-glycyrrhetinic acid, stigmasterol, indigo, β-sitosterol, luteolin, quercetin and naringenin in Lianhua Qingwen Prescription might target ACE2 and protect the target organs of COVID-19 through the renin-angiotensin pathway. Network pharmacology combined with molecular docking was used to rationalize the mechanism of action for other TCM formulations, such as Qing-Fei-Da-Yuan (QFDY) granules; quercetin, luteolin, and naringenin showed strong binding abilities with COVID-19 3CL hydrolase.

These studies suggest that quercetin, kaempferol, luteolin, isorhamnetin, baicalein, naringenin, and wogonin are important ingredients having potentially high affinity for druggable targets; AEC2 and 3CL protein could be the potential direct targets for anti-SARS-CoV-2; COX-2, CASP3, IL-6, MAPK1, MAPK14, MAPK8 and RELA are the top seven targets; and IL-17, arachidonic acid metabolic, HIF-1, NF-kB, Ras, and TNF are the top six signaling pathways. These ingredients exert beneficial effects in the management of COVID-19 via inhibition of viral adsorption and replication, as well as the regulation of inflammatory mediators, and anti-inflammatory, and immune-regulatory
effects to prevent cytokine storm and to protect the target organs. It was suggested that various known TCM formulations could be effective due to the multicomponent, multitarget, synergistic action of their constituents. However, there is a lack of knowledge of other interactions, including drug-natural products and off-target interactions, that could cause adverse effects. Studies that include molecular dynamics simulations of multicomponent systems and experimental validation of theoretical studies should be considered as important components of an integrated approach for drug development.

Consumption, Pharmacokinetics and Safety

The consumption of NAR via citrus fruits or supplementation can rapidly increase circulating and intracellular levels of NAR. An increase in the concentration of NAR in plasma samples can be observed around 4 h post-consumption. In addition, in vitro models have also demonstrated a long-term anti-viral benefit, even after discontinuation of supplementation with NAR. The consumption of 500 mL/day for 8 weeks of orange juice, rich in NAR, has demonstrated an adjuvant effect in antiviral therapy. The consumption of 340 mL of grapefruit juice per day (containing approximately 210 mg of NAR) also improved cardiac-related measurements. NAR is mostly absorbed in the small intestine and differences in microbiota may also be an important inter-individual variable.

From a clinical point of view, the therapeutic potential and safety, as well as pharmacokinetics and metabolism of NAR indicated its safety. A dose of 600 mg in healthy volunteers resulted in a serum Cmax of about 50 µM, without relevant toxicity. In addition, the hydrophobic nature of NAR facilitates its crossing of biological membranes and reaching the cell in a suitable concentration.

TPSA (Topological polar surface area) is used as a good measure for prediction of drug transport properties. It correlated efficiently with human intestinal absorption. Naringenin revealed a good TPSA value of 86.9 Å, indicating its possible intestinal permeability and 68% absorption.

Furthermore, NAR interactions with the cytochrome P450 (CYP) system need to be evaluated, as NAR can affect drug-metabolizing enzymes and pharmacokinetics of important drugs that may be of either regular use or specific to COVID-19 patients.

Concluding Remarks

In silico analysis demonstrated that NAR can prevent the entry of the virus into host cells by inhibiting the binding capacity of ACE2 receptors and by lowering Mpro and PLpro activities. It consequently inhibits viral transcription and replication (Figure 2). NAR’s potential as an anti-inflammatory nutritional intervention has been demonstrated in many different diseases. Therefore, NAR might provide a promising treatment strategy, and consumption of foods enriched with flavonoids can be of importance for the prevention and treatment of SARS-CoV-2, while providing enough safety for the human body. However, further investigations and clinical trials are necessary before the use of NAR can be recommended as part of antiviral therapy.

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| Abbreviation | Definition |
|--------------|------------|
| ACE          | Angiotensin-converting enzyme |
| ARDS         | Acute respiratory distress syndrome |
| AST          | Aspartate aminotransferase |
| 3CL\textsuperscript{pro} | 3-chymotrypsin-like main protease |
| COVID-19     | Coronavirus Disease 2019 |
| HBs          | Hydrogen bonds |
| MERS-CoV     | Middle East Respiratory Syndrome Coronavirus |
| M\textsuperscript{pro} | Main protease |
| NSP          | Non-structural protein |
| PL\textsuperscript{pro} | Papain-like protease |
| PD           | Peptidase domain |
| SARS         | Severe Acute Respiratory Syndrome |
| RBD          | Receptor binding domain |
| S protein    | Spike protein |
| RdRp         | RNA-dependent RNA polymerase |
| SARS-CoV-2   | Severe Acute Respiratory Syndrome Coronavirus 2 |
| TMPRSS2      | Transmembrane protease serine 2. |