Pharmacological Significance of Hesperidin and Hesperetin, Two Citrus Flavonoids, as Promising Antiviral Compounds for Prophylaxis Against and Combating COVID-19

Pawan K. Agrawal¹, Chandan Agrawal¹ and Gerald Blunden²

Abstract
Hesperidin and hesperetin are flavonoids that are abundantly present as constituents of citrus fruits. These compounds have attracted attention as several computational methods, mostly docking studies, have shown that hesperidin may bind to multiple regions of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (spike protein, angiotensin-converting enzyme 2, and proteases). Hesperidin has a low binding energy, both with the SARS-CoV-2 “spike” protein responsible for internalization, and also with the “PLpro” and “Mpro” responsible for transforming the early proteins of the virus into the complex responsible for viral replication. This suggests that these flavonoids could act as prophylactic agents by blocking several mechanisms of viral infection and replication, and thus helping the host cell to resist viral attack.

Keywords
citrus, flavonoids, hesperidin, hesperetin, prophylaxis, antiviral, COVID-19

Received: July 15th, 2021; Revised: August 9th, 2021; Accepted: August 10th, 2021.

Introduction
The coronavirus disease 2019 (COVID-19) epidemic that originated at the beginning of 2020 in Wuhan, China has now spread worldwide and has become an international pandemic. It was caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a new member of the coronavirus family.¹,² Despite the development of vaccines, zoonotic SARS-CoV continues to be a major threat to humans, and most research groups do not exclude the possibility of the continual reemergence of severe acute respiratory syndrome (SARS).

Structure of SARS-CoV-2
The genome of SARS-CoV-2 is a positive-sense, single-stranded ribonucleic acid of approximately 30 000 nucleotides in length, encapsulated within a membrane envelope. It has 4 structural proteins, known as S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins. The N protein holds the RNA genome, and the S, E, and M proteins together create the viral envelope (Figure 1).

The S protein on the virion surface is primarily responsible for the establishment of host-protein receptor recognition for binding to the surface of a host cell through a cell membrane protein receptor called angiotensin-converting enzyme 2 (ACE2).² The transmembrane protease, serine-2, helps in priming of the S protein and subsequent membrane fusion.⁶ The S protein is cleaved into S1 and S2 subunits during viral infection.⁸ The S1 subunit contains the receptor-binding domain (RBD), which directly binds to the peptidase domain (PD) of ACE2,¹¹ while the S2 subunit is responsible for membrane fusion. ACE2 is expressed in several organs such as lungs, gastrointestinal tract, kidney, endothelium, heart, and, to some extent, in pancreas.¹³ ACE2 has been demonstrated to be a functional receptor for SARS-CoV.¹⁸ It has also been shown that SARS-CoV-2 RBD was capable of entering cells expressing human ACE2, but not any other receptors.¹³ The viral gene essential for the replication of the virus is expressed as a polyprotein that must be broken into functional

¹Natural Product Inc., Westerville, OH, USA
²School of Pharmacy & Biomedical Sciences, University of Portsmouth, Portsmouth, UK

Corresponding Author:
Pawan K. Agrawal, Natural Product Inc., 7963 Anderson Park Lane, Westerville, OH 43081, USA.
Email: agrawal@naturalproduct.us
subunits for replication and transcription activity. The viral genome encodes more than 20 proteins, among which are 2 virally encoded cysteine proteases that are vital for virus replication, SARS-CoV-2 papain-like protease (PLpro) and SARS-CoV-2 main protease, Mpro, also referred to as the 3C-like protease (3CLpro); they cleave the 2 translated polyproteins (pp1a and pp1ab) into individual 16 functional nonstructural proteins (NSPs). Each of these NSPs has its own specific function in replication and transcription. These NSPs, with the help of host machinery, translate the RNA coding for the viral spike, envelope, nucleocapsid, and membrane proteins and thus are involved in replication events and transcription of the genome. These NSPs then enter the endoplasmic reticulum—Golgi apparatus and are involved in viral assembly and packaging. The SARS-CoV-2 S protein contains a protease cleavage site and is likely processed by these intracellular proteases during exit from the cell. The new viral particles spread into the environment and/or infect other cells and organs in the body, in a chain expansion.

**Targeted Inhibition Sites for Virus Infection**

Based on the above concise description, enzymes/proteins from either the host or the virus could be targeted for either inhibiting or reducing viral infection. These involve a number of potentially targetable steps, including (a) endocytic entry of the virus into host cells, that is, preventing viral entry by either interfering with the binding of viral S-proteins to human ACE2 receptors or by inhibiting the function of the ACE2 receptor, (b) inhibiting viral replication by either blocking RNA dependent RNA polymerase (RdRp) or proteases (involving Mpro- and/or PLpro), as these are responsible for viral replication after entry into cells for new virus particle assembly, (c) helping the cell to resist viral attack, (d) blocking the spread of the virus in the body, and (e) modulating the inflammation when starting as an innate defensive mechanism, it becomes offensive and cytotoxic.

The search for naturally occurring bioactive compounds is part of the general vision that perceives that the boost to the individual immune system, and effective inhibition of the infection by SARS-CoV-2 as the most effective shield against the onset and serious progress of COVID-19.

**Hesperidin and Hesperetin**

Chemically, hesperidin (1) is a glycoside having rutinose (α-L-rhamnopyranosyl-[1→6]-β-D-glucopyranose) linked to OH-7 of hesperetin (2) [(3’,5,7-trihydroxy-4’-methoxy-flavanone), or (5)-5-hydroxy-2-(3-hydroxy-4-methoxy-phenyl)-2,3-dihydro-4H-chromen-4-one], as shown in Figure 2.

Citrus fruits, such as bergamots, grapefruit, lemons, limes, mandarins, oranges, and pomelos, are part of our daily food due to their...
nutritional and pharmacological significance. These represent an important source of dietary flavonoids, including hesperidin, hesperetin, taxifolin, naringin, naringenin, diosmin, quercetin, rutin, nobiletin, tangeretin, and others. Studies have demonstrated antiviral activities of various flavonoids, including their use for respiratory diseases and SARS-CoV-1. Among these, hesperidin is the predominant flavonoid in citrus fruits, primarily in sweet orange (in young immature oranges it accounts for up to 14% of the fresh weight of the fruit).

The consumption of orange juice-rich food has been reported to exert beneficial effects on some intermediate-risk factors for cardiovascular diseases (CVDs), such as low-density lipoprotein cholesterol, blood pressure, and endothelial function. A dietary intake history of over 10 000 Finnish men and women suggested that individuals with high polyphenols (flavonoids) intake in terms of raw fruits/vegetables and/or their juices had a lower incidence of cardiovascular disease and bronchial asthma.

Although citrus fruits and juices are widely consumed worldwide, little information has been published on flavanone bioavailability in humans. Low micromolar levels of hesperidin (around 1 µM range) have been detected in blood serum for 5 to 7 h after the intake of citrus juice, probably high enough to exert its health-promoting activities in the human body. Hesperidin itself is absorbed from the intestine intact as a glycoside. Its aglycone, hesperetin, appears in plasma 3 h after ingestion, reaching a peak between 5 and 7 h. The circulating forms of hesperetin are glucuronides (87%) and sulfoglucuronides (13%). For hesperidin, urinary excretion is nearly complete 24 h after the orange juice ingestion and does not depend on the dose.

It is worthwhile to note that the presence of sugar moieties usually increases the bioavailability, but the nature of the substituted sugar moieties affects the digestibility and the absorbability. Indeed, flavonoids with glucose moieties are absorbed more rapidly than flavonoids with rhamnose, rutinoside, and neohesperidoside substitutions. However, the lack of α-L-rhamnosidase and rutinosidase in human cells makes the bioavailability of flavonoid rutinosides largely dependent on their hydrolysis by intestinal bacteria. Furthermore, few intestinal bacterial strains can achieve cleavage of these types of glycosidic bonds, but the tested flavonoid could be active because the bioavailability of the molecules is due to the aglycone part.

Toxicity studies have confirmed the high safety profile of hesperidin after oral intake of more than 2 g/kg. A tablet containing a micronized flavonoid mixture (50 mg of hesperidin + 450 mg of diosmin), is used as a vasoprotective venotonic agent. Similarly, intragastrically given Dafon, at a daily dose of 100 mg, did not show either signs or symptoms of side effects in rats. Likewise, oral administration of diets containing either methyl hesperidin or hesperidin did not show either signs or symptoms of side effects in mice and rats. Moreover, no adverse events were observed in mice followed daily intraperitoneal injections of phosphorylated hesperidin at a dose of 20 mg/kg body weight for over 4 weeks, although one study showed that orally given Dafon-500 mg twice-daily for 60 days caused minor, temporal side effects such as headache and faintness. Other studies showed that oral Dafon-500 mg is safe in humans. According to an oral toxicity study, it can be concluded that hesperidin can be safely used in herbal formulations with its LD50 value of more than 2000 mg/kg. Hesperidin has a long history as a herbal medication.

Clinical trials involving more than 2850 patients treated with a hesperidin mixture for a period of 6 weeks to 1 year showed normal hematological parameters, and hepatic and renal functions, with no signs of toxicity. No CVD risk factor was observed in participants (mean age: 60.6 ± 1.4 years) receiving either 767 mL orange juice or a hesperidin supplement (both providing 320 mg hesperidin and 439 mg vitamin C).

### Pharmacological Significance of Hesperidin and Hesperetin

Hesperidin and its aglycone hesperetin, 2 flavonoids found primarily in sweet oranges and lemons, have been documented to possess a wide range of pharmacological properties including...
anticancer, antihypertensive, antioxidant, antidiabetic, hepatoprotective, neuroprotective, wound healing, cardiovascular, anti-inflammatory, anti-obesity, hypoglycemic, lipid-lowering and beneficial effects on bone and Alzheimer’s disease.\(^{40,53,73}\)

Overwhelmingly, the pharmacological effects of hesperidin are related to its antioxidant activity, arising through its ability to scavenge free radicals.\(^{117}\) Furthermore, hesperidin can alleviate diabetic neuropathy,\(^{112}\) and nephropathy.\(^{113}\) Studies also suggested that hesperidin had antidepressant-like effects.\(^{114}\) The effects of hesperidin on ameliorating the depression- and anxiety-like behaviors of diabetic rats, which are mediated by the enhancement of glyoxalase 1 (Glo-1), may be due to the activation of the Nrf2/ARE pathway,\(^{114}\) immunomodulatory targets. The binding energy of hesperidin for TNF-α was ~6.96 kcal/mol; some important interactions were observed with Ser69, Leu120, and Tyr151; with IL-1β the binding energy was ~6.64 kcal/mol, with binding to Glu37 and Lys65 from the A chain. In the case of IL-6, the binding energy was found to be ~7.07 kcal/mol, with interaction at Met67, Glu172, and Arg179 (B chain).\(^{115,116}\)

Hesperidin has been found to have protective effects in mice infected with encephalomyocarditis virus and Staphylococcus aureus when given before either single or combined viral-bacterial infections.\(^{117}\) Hesperidin was found to be effective against human rotavirus, which is the causative agent of diarrhea in infants and young children,\(^{118}\) and also inhibited replication of influenza virus in vitro, as well as decreasing the number of infected cells.\(^{119}\) Hesperidin was also found to inhibit various sexually transmitted pathogens including Neisseria gonorrhoeae, Chlamydia trachomatis, herpes simplex virus-2, and human immunodeficiency virus, but had no toxic effects either on the host cells or on the growth of normal vaginal lactobacilli.\(^{120}\) Hesperidin was evaluated in vitro against canine distemper virus and found to be potentially active.\(^{121}\)

Cell culture and molecular docking studies suggested that hesperidin had moderate (41%) efficacies against hepatitis B virus replication.\(^{122}\) Hesperidin displayed antiviral activity against simian rotavirus strain SA-11 at a concentration of 1200 μg/mL.\(^{123}\) Hesperidin up-regulated P38 and JNK expression and activation, thus resulting in the enhanced cell-autonomous immunity to defend against influenza A virus infection.\(^{124}\) Hesperidin is also known to possess antiviral activity by altering the immune system, mainly by regulating interferons in the influenza A virus.\(^{125}\) In another study, hesperidin was noticed to attenuate influenza A virus H1N1 induced lung injury through its anti-inflammatory effect.\(^{126}\)

Hesperidin and hesperetin have shown promising results in the suppression of various types of cancer (colon, prostate, hepatic, bladder, and lung cancer), as either a drug or as a pro-drug and co-adjuvant.\(^{127}\) It has been extensively reported that hesperetin exerts neuroprotective effects in experimental models of neurodegenerative diseases.\(^{128}\)

Hesperetin displays marked inhibitory activity against replication of several diverse viruses. SARS-CoV, herpes simplex virus type-1, influenza A 14 virus, parainfluenza virus type-3, respiratory syncytial virus, and poliovirus type-1 have been inhibited by hesperetin in vitro conditions.\(^{129}\) Hesperetin also exhibited antiviral action on the replication of the 17D strain of yellow fever virus.\(^{133}\) Hesperetin, quercetin, sinigrin, and other flavonoids have been shown to exhibit antiviral effects against SARS-CoV-2 3CL\(^{pro}\).\(^{134}\) Hesperetin was shown to be a highly potent inhibitor of SARS-CoV-2 3CL\(^{pro}\) (IC\(_{50}\) = 8.3 μM) when tested in a cell-based cleavage assay,\(^{131,135}\) and was also active against Sindbis neuroviral strain infection.\(^{135}\)

Recently, it has been reported that flavonoids from citrus, such as hesperidin and hesperetin, may stimulate antiviral pathways by upregulation of expression of the transcription factor interferon regulatory factor 7 gene, as well as due to their ability to activate the interferon-stimulated response element.\(^{136}\) Thus, hesperidin and hesperetin have gained interest due to their health-promoting properties, including antioxidant, antibacterial, antifungal, and also antiviral actions.

**Computational/Docking Studies**

Computational-based methods offer considerable promise for screening drugs and other molecules that may have favorable effects on any relevant SARS-CoV-2 virus protein targets by predicting their binding affinities. Better inhibition is usually reflected by low binding energy (the lower the energy required, the stronger and more specific the binding is). This technique, called “in silico,” is currently used to help the design of specific inhibitors of SARS-CoV-2, and significantly enhances the quality of compounds selected for in vitro and in vivo biosays.\(^{137}\) However, it is important to mention that in silico analyses only provide a preliminary theoretical view on the ligand–protein interactions and hence require experimental validation of the molecular targets.

**Therapies That May Act on SARS-CoV-2**

The therapies that may act on the coronavirus can be divided into several categories based on the specific pathways: (i) acting on structural proteins of the virus, thus either blocking the virus from binding to human cell receptors or inhibiting the virus’s self-assembly process, that is, inhibiting virus entry; (ii) acting on either enzymes or functional proteins that are critical to the virus, that is, preventing virus RNA synthesis and replication; (iii) reducing the virulence factor to restore the host’s innate immunity; (iv) acting on the host’s specific receptors or enzymes, thus preventing the virus from entering the host’s cells.

**Blocking Initiation Process of Virus Infection**

The S glycoprotein of SARS-CoV-2 contains the RBD that recognizes the target receptor leading to the splicing of the trimeric S protein into subunits S1 and S2, facilitating membrane fusion; virus infection then occurs through endocytosis.\(^{142}\) The receptor ACE2 is a preferable receptor for the S glycoprotein.\(^{2}\) The
drug candidates may target binding with the RBD of the S protein, whereas the ACE2 ligand binding site is recognized as a protease domain (PD) that plays a role in the cleavage of the trimeric structure of S glycoprotein as the important step in virus infection. Therefore, the RBD of S glycoprotein and ACE2 are preferable candidates as drug targets to inhibit the initiation process of virus infection.

Blocking Virus Replication

As significant functional proteins of coronaviruses, NSPs are involved in RNA transcription, translation, protein synthesis, processing and modification, virus replication, and infection of the host. Among them, the SARS-CoV-2 Mpro/NSP5, PIpro/NSP3, RdRp/NSP12, and helicase/NTPase (NSP13) are considered essential to the viral cycle. Therefore, these have also been targeted for docking and/or for the development of small-molecule inhibitors due to their clear biological functions.

Computational/Docking Studies Related to Hesperidin/Hesperitin for Their Possible COVID-19 Significance

Several in silico molecular dynamics (MD) docking studies for drug repurposing have reported that flavonoids were high on their hit list. Chen et al. reported results based on docking 1500 drugs with SARS-CoV 3CL\textsuperscript{pro} (protein data bank [PDB]: 2DUC) and found that hesperidin and diosmin were the top 2 candidates. Adem et al. reported that after evaluating 80 flavonoids for MD docking with the SARS-CoV-2 protease 3CL\textsuperscript{pro} (PDB:6LU7), hesperidin, rutin, and diosmin were the top 3 candidates for docking at the active site. Peterson investigated 72 flavonoids for their potential to bind with the active catalytic site of 3CL\textsuperscript{pro} (PDB:6LU7) and identified hesperidin as one of the top 14 flavonoids.

Inhibiting Virus Invasion

Several computational methods, independently applied by different researchers, showed that hesperidin can bind to the SARS-CoV-2 S protein and, in doing so, prevent its initial interaction with ACE2 receptors. This may interfere with viral entry into host cells. In a preferential binding affinity study of selected natural compounds, hesperidin exhibited high affinity (~8.1 kcal/mol) for binding to SARS-CoV-2 S glycoprotein (PDB: 6YVB). Hesperidin was found to have positioning at Phe456 and Phe490 in the target protein when the interactions were visualized. Hesperidin has been cited to be better than nelfinavir, with a docking from ~8.3 to ~13.51 kcal/mol for S protein.

The RBD region of SARS-CoV-2 S protein interacts with the host cell ACE2 receptors to form the SARS-CoV-2-RBDACE2 complex, which is responsible for the mediation of virus invasion. The RBD of the S glycoprotein (RBD-S) can bind to the ACE2 receptor at the PD of the host cell, thereby leading to viral infection. The docking results show that hesperidin has the lowest docking score for binding with RBD-S (PDB ID:6LXT) and PD-ACE2 (PDB ID:6VW1), indicating that it has potential for inhibiting viral infection.

Target-based virtual ligand screening for binding to S protein was undertaken for 1066 antiviral natural substances, plus 78 known antiviral drugs. Hesperidin was found to be the most suitable for targeting the binding interface between viral S protein and ACE2 human receptors. Hesperidin was predicted to lie on the middle shallow pit of the surface of the RBD of the S protein, with the aglycone part parallel to the b-s sheet of the RBD. The sugar part was inserted into the shallow pit in the direction away from ACE2, where a few hydrophobic amino acids, including Tyr436, Tyr440, Leu442, Phe443, Phe476, Try475, Try481, and Try49 form a relatively hydrophobic shallow pocket to contain the hesperidin. By superimposing the ACE2-RBD complex on the hesperidin-RBD complex, a distinct overlap of hesperidin with the interface of ACE2 could be observed, suggesting that hesperidin may disrupt the interaction of ACE2 with RBD. This suggested that hesperidin, by blocking the interface of ACE2 and RBD-S binding, could probably be used for treating SARS-CoV-2 infection.

A computational and experimental study found that multiple flavonoids abundant in citrus peels cooperate to prevent SARS-CoV-2 infection. In particular, simulated molecular docking showed that hesperidin, hesperetin, and naringin have a strong binding affinity for the ACE2 receptor. The molecular docking studies of hesperidin with the ACE2 enzyme demonstrated that hesperidin could bind to ACE2 with a predicted $\Delta G$ of ~8.3 kcal/mol, with binding sites at Tyr613, Ser611, Arg482, and Gln479. In another molecular docking study, hesperidin was found to have a binding affinity of ~8.0 kcal/mol with ACE2 protein. Hesperidin showed a high docking affinity for the ACE2 receptor protein model 1R4L (docking score ~11.4 kcal/mol), exhibiting key binding with Cys344, His345, Asp368, Arg514, Tyr515, and Arg518. In another study, hesperidin has been reported to form H-bonds with residues Asp405, Lys417, Ser494, and Tyr505 of RBD with a binding energy of ~8.6 kcal/mol.

Considering that the fusion of the RBD region with the host cell membrane needs a big conformational change in the S protein part, any small molecule bound to the S protein at this time may interfere with its re-folding, therefore inhibiting the viral infection process. Based on virtual screening, hesperidin may disrupt the interaction of ACE2 with the RBD of SARS-CoV-2 and, therefore, hesperidin has promise as a prophylactic agent against COVID-19 infection.
Haggaga et al.\textsuperscript{165} studied the S glycoprotein inhibition activity of hesperidin and other natural products in comparison with nelfinavir (an antiretroviral drug), chloroquine, and hydroxychloroquine sulfate (antimalarial drugs recommended by the FDA as emergency drugs), and the results showed that hesperidin has better poses than the other 3 as S glycoprotein inhibitors. Thus, hesperidin binds to 2 key protein targets: RBD-S and PD-ACE2, thereby preventing binding of the RBD-S to PD-ACE2 of the host cell, thus inhibiting the viral infection.\textsuperscript{169,172}

Molecular docking of hesperetin to the ACE2 enzyme showed that hesperetin has the potential to bind to ACE2 with an estimated $\Delta G$ of $-8.3 \text{ kcal/mol}$, with binding sites at Tyr613, Ser611, Arg482, and Glu479, and thus suggesting that hesperetin may block the infection.\textsuperscript{175} In silico studies show that hesperetin can bind (binding energy $-9.1 \text{ kcal/mol}$) with high affinity to the S protein, helicase, and protease sites on the ACE2 receptor causing conformational change to inhibit viral entry.\textsuperscript{177}

### Inhibiting Virus Replication

#### Binding With SARS-CoV-2 M\textsuperscript{pro}

M\textsuperscript{pro}, also known as 3CL\textsuperscript{pro} (NSP5), is one of the most attractive targets against SARS-CoV-2 since it plays a key role in viral transcription and replication. M\textsuperscript{pro} is first automatically cleaved from poly-proteins by autolytic cleavage between NSP4 and NSP6\textsuperscript{77} to produce mature enzymes, and then further cleaves downstream viral polyprotein at 11 sites to release functional units NSP6-NSP16 for virus replication and packaging within the host cells.\textsuperscript{164,169} M\textsuperscript{pro} directly mediates the maturation of NSPs, which is essential to the life cycle of the virus. Detailed investigation of the structure and catalytic mechanism of M\textsuperscript{pro} has revealed it as an attractive target for antiviruses, drug development since it plays a key role in viral transcription and replication, and no human proteases are known with the same substrate specificity.\textsuperscript{140,149,158,160}

The M\textsuperscript{pro} structure is composed of 3 domains; the catalytic dyad is located in the cleft between domains I and II\textsuperscript{9,149,163} and domain III is responsible for the enzyme dimerization, enabling the active form of the macromolecule.\textsuperscript{184} M\textsuperscript{pro} is a cysteine protease with a catalytic Cys145 and His41 dyad at its active site.\textsuperscript{187}

Adem et al.\textsuperscript{162} performed molecular docking investigations by using Molegro Virtual Docker 7 to analyze the inhibition probability of flavonoids against SARS-CoV-2 M\textsuperscript{pro} (PDB:6LU7). According to the obtained results, hesperidin, rutin, diosmin, and apiin were among other phenolic compounds found to be more effective on COVID-19 than nelfinavir. Hesperidin exhibited the lowest binding energy at the active site of M\textsuperscript{pro} as it formed 14 hydrogen bond interactions with Thr26, Glu166, Arg188, Gln189, Met49, Asp187, Tyr54, His163, Leu141, and Ser144. According to the Moldock binding score, the potent flavonoids can be ranked as follows by affinity hesperidin > rutin > diosmin > apiin.\textsuperscript{162} In a study related to virtual screening of drugs on the active sites of SARS-CoV-2 3CL\textsuperscript{pro}, Chen et al.\textsuperscript{161} revealed the binding energy for hesperidin as $-10.1 \text{ kcal/mol}$. In a molecular docking study by Kraljevska et al.,\textsuperscript{177} hesperidin was found to have a binding affinity of $-6.5 \text{ kcal/mol}$ with M\textsuperscript{pro}.

In a comparative M\textsuperscript{pro} inhibition study, hesperidin showed better binding free energy compared to nelfinavir, chloroquine, and hydroxychloroquine sulfate, which so far are recommended for the treatment of COVID-19. Hesperidin forms 4 hydrogen bonds with M\textsuperscript{pro} at the amino acid residues Phe140, Glu166, Cys145, and Ser144.\textsuperscript{170} In a docking screening study against the viral main protease (M\textsuperscript{pro}), of the major components of 38 Chinese Patent Drugs that are commonly used in respiratory diseases, hesperidin was identified as one of the top hits having good binding affinity ($-8.5 \text{ kcal/mol}$) against M\textsuperscript{pro} (PDB:6LU7) targets. The key residues Gly143, Ser144, Cys145, and Glu166 were identified for potential inhibitor binding.\textsuperscript{171,190}

Tomic et al.\textsuperscript{167} showed that hesperidin had the highest affinity ($-5.8 \text{ kcal/mol}$) for SARS-CoV-2 main protease (3CL\textsuperscript{pro}; PDB:6Y84). The other results show that hesperidin has the best docking score for SARS-CoV-2 main protease (PDB:6LU7).\textsuperscript{169} Hesperidin interacts through hydrogen bonding with Thr24, Thr25, Thr45, His4, Ser46, and Cys145, amide-$\pi$ stacked interaction with Thr45, and $\pi$-alkyl interactions with Met49 and Cys145. In a comparison of the $\Delta G$ value, which gives the estimated free energy of binding, the binding affinity of hesperidin towards SARS-CoV-2 main protease was found to be $-9.02 \text{ kcal/mol}$.\textsuperscript{191}

A recent study indicated that 2 of the compounds present in ginger (Zingiber officinale), namely chlorogenic acid and hesperidin, had high binding affinities for M\textsuperscript{pro}, with predicted binding energies of $-7.5$ and $-8.3 \text{ kcal/mol}$. The 2D and 3D interactions are more favorable for binding than hydrophobic interactions with Phe140, Asn142, Cys145 (1 of the 2 amino acids forming the catalytic dyad), His163, and Met165. The combination of hydrophobic bonding with Phe140 and Met165, along with interaction with Cys145, would create a favorable situation for blocking the entry of any other compound to the catalytic site and possibly accounts for the predicted higher binding affinity observed for hesperidin.\textsuperscript{192}

#### Binding With PI\textsuperscript{pro}

Another essential protease for the cleavage of the viral polyproteins is the PI\textsuperscript{pro}, a cysteine protease with a classical Cys-His-Asp catalytic triad (Cys112, His273, Asp287), which cleaves the viral polyprotein, releasing NSP1, NSP2, and NSP3.\textsuperscript{192,193} Computational approaches have also been used to predict potential SARS-CoV-2 PI\textsuperscript{pro} inhibitors. In a study for screening the preferential binding affinity of artemisinin, hesperidin, and chloroquine, Tomic et al.\textsuperscript{167} found that hesperidin had the highest binding score ($-10.08 \text{ kcal/mol}$ for PI\textsuperscript{pro}; PDB:6W9C).
Binding With SARS-CoV-2 RNA-Dependent RNA Polymerase (RdRp)

RdRp catalyzes SARS-CoV-2 RNA replication and, hence, is an obvious target for antiviral drug design. Hesperidin was successfully docked in the catalytic pocket of RdRp with a binding energy of −8.8 kcal/mol, suggesting that it can strongly bind to the catalytic site of the SARS-CoV-2 RdRp. It forms 13 hydrogen bonds with Tyr619, Asp618, Lys798, Ser795, Met794, Pro793, Asp164, Val166, Pro620, Lys621, Asp623, Arg555, and Tyr455, inhibiting the RdRp activity, thus blocking replication and preventing viral transcription.\textsuperscript{194}

Binding With Non-Structural Protein-15 Endoribonuclease (NSP15)

The docking results for the NSP15 endoribonuclease of SARS-CoV-2 revealed that the flavonoids baicain, rutin, ilexgenin A, and hesperidin have the best docking scores in the range −8.168 to −5.29 kcal/mol; while the docking score for the standard drug remdesivir is −5.636 kcal/mol. The key residues at the binding site belong to the C-terminal catalytic domain catalytic triad His235, His250, Lys290, Thr341, Tyr343, and Ser294.\textsuperscript{195} The carbonyl oxygen and a 5-hydroxyl group of hesperidin form hydrogen bonds with Lys290 and protonated His250 residues, respectively, while the hydroxyl groups on the sugar moieties form hydrogen bonds with Glu340 and Asp240 residues.

Thus, multiple computational methods, independently applied by different researchers, showed that hesperidin has low binding energy both with the coronavirus S protein, responsible for binding with host receptors, and with other proteases such as M\textsuperscript{pro}, PL\textsuperscript{pro}, RdRp, and NSP15 endoribonuclease (Figure 3). Based on these predictive results, it is likely that, because of its binding affinity to these 6 main targets, hesperidin would fight the viral infection by inhibiting either virus binding to ACE2 or virus replication in cells.

Mouth Rinses Containing Citrox

Citrox, which is derived from citrus fruits, is composed of soluble bioflavonoids and hydroxylated phenolic structures produced by plants. Although no in vitro or in vivo studies have been published on Citrox mouth rinse, in silico studies based on computer virtual screening predicted that citrus flavonoids, such as hesperidin and rutin, can act as possible components having antiviral action against SARS-CoV-2. Based on these predictive results, it is likely that mouth rinses having citrus flavonoids could help to fight against COVID-19.\textsuperscript{196}

Nasal Rinse and Nasal Delivery of Hesperidin

An Ayurvedic herbal formulation of Citrus medica and Zingiber officinale is recommended as a nasal rinse in the management of contagious fevers. Molecular docking studies of the constituents of these plants reflect that hesperidin is one of the components that could inhibit the receptor binding of SARS-CoV-2 S protein, as well as ACE2, thus reducing viral load and shedding of SARS-CoV-2 in the nasal passages.\textsuperscript{197}

Nasal and inhaled drug delivery methods represent a promising strategy for the treatment of inflammatory lung disease as a result of their ability to improve drug delivery to lungs. Recently, chitosan nanoparticles loaded with hesperidin were developed for nasal delivery of the anti-inflammatory hesperidin to treat inflamed lungs. It is worth mentioning that the hesperidin dose that had protective effects on cytokine storm syndrome-induced acute lung injury was also used in the treatment of acute respiratory distress syndrome (ARDS).\textsuperscript{198}

Hesperidin in Management of Inflammatory Mediators

Cytokine storm is a major cause of ARDS as the body releases various immune-active molecules, such as interferons (eg, IFN-γ), interleukins (eg, IL-1β, IL-2, IL-6), chemokines, and tumor necrosis factor-alpha (TNF-α). Hesperidin/hesperetin has been found to modulate inflammatory mediators, such as IL-6, IL-1β, and TNF-α, in the heart, lungs, and central nervous system in multiple animal models.\textsuperscript{199} Hesperidin, with its high anti-inflammatory activity, inhibited the secretion of pro-inflammatory cytokines such as IFN-γ and IL-2.\textsuperscript{200} Besides, hesperidin inhibited IL-1β-stimulated inflammatory responses by inhibiting the activation of the NF-kB signaling cascade.\textsuperscript{205} It also played a major role in suppressing the release of inflammatory markers such as TNFα and IL-6 in type 2 diabetic patients.\textsuperscript{207} Activation of coagulation pathways following the immune response to COVID-19 infection promotes clot formation. A prophylactic dose of heparin (with low molecular weight) is recommended for protection against venous thromboembolism.\textsuperscript{208} Therefore, it can be used as adjuvant therapy to control the severe inflammatory reaction against COVID-19.

COVID-19 Diets

Interestingly, among the many approaches to COVID-19 prevention, the possible role of diet has so far been somewhat marginal. Diets incorporating citrus fruits, and especially of the

---

**Figure 3.** Hesperidin can inhibit severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) entry, and its lifecycle, by targeting replication processes.
Concluding Remarks

The above-mentioned studies suggest the ability of citrus flavonoids, such as hesperidin and hesperetin, to prevent the SARS-CoV-2 virus from binding to the ACE2 enzyme of the host cell and inhibit virus replication after its penetration of the host cell, as well as either restraining or counteracting the proinflammatory overreaction of the immune system. Thus, hesperidin supplementation may be useful as a prophylactic agent against SARS-CoV-2 infection and as a complementary treatment of COVID-infected patients. The biological actions of the flavonoid may counteract infection by SARS-CoV-2 and modulate the immune system’s response to the disease. Further preclinical, epidemiological, and clinical studies are needed to corroborate this hypothesis.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Pawan K. Agrawal https://orcid.org/0000-0002-7149-8358

References

1. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382(2):727–733. doi:10.1056/NEJMoa2001017
2. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020;395:565–574. doi:10.1016/S0140-6736(20)30251-8
3. Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. Methods Mol Biol. 2015;1282:1–23. doi:10.1007/978-1-4939-2438-7_1
4. de Wit E, van Doremalen N, Falzarano D, et al. SARS and MERS: recent insights into emerging coronaviruses. Nat Rev Microbiol. 2016;14(8):523–534. doi:10.1038/nrmicro.2016.81
5. Annweiler C, Cao Z, Wu Y, et al. Counter-regulatory ‘renin-angiotensin’ system-based candidate drugs to treat COVID-19 diseases in SARS-CoV-2-infected patients. Infect Drug Targets. 2020;20(4):407–408. doi:10.2174/1871526520666200518073329
6. Li W, Moore MJ, Vasiliuva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003;426:450–454. doi:10.1038/nature02145. PMID: 14647384.
7. Iwata-Yoshikawa N, Okamura T, Shimizu Y, et al. TMPRSS2 contributes to virus spread and immunopathology in the airways of murine models after coronavirus infection. J Virol. 2019;93(6):e01815–18. doi:10.1128/JVI.01815-18
8. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;181(2):271–280.e8. doi:10.1016/j.cell.2020.02.032. Epub 2020 Mar 5.
9. Hoffmann M, Kleine-Weber H, Pöhlmann S. A multifaceted cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. Mol Cell. 2020;78(4):779–784. doi:10.1016/j.molcel.2020.04.022
10. Li AW. Bolstering your defenses against COVID-19: An “Epigenetic” diet. 2020. https://www.whatisepigenetics.com/bolstering-your-defenses-against-covid-19-an-epigenetic-diet/
11. Walls AC, Park YJ, Tortorici MA, et al. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 2020;181(2):281–292.e6. doi:10.1016/j.cell.2020.02.058
12. Yan R, Zhang Y, Li Y, et al. Structural basis for the recognition of ACE2 receptor by SARS-CoV-2 and implications for virus entry. Nature. 2020;583(7815):471–476. doi:10.1038/s41586-020-2008-3
13. Letko M, Munster V. Functional assessment of cell entry and receptor usage for lineage B β-coronaviruses, including 2019-nCoV. BioRxiv. 2020:2001.2022.915660. doi:10.1101/2020.01.22.915660
14. Saha S, Chakrabarti S, Singh PK, et al. Physiological relevance of angiotensin converting enzyme 2 as a metabolic linker and therapeutic implication of mesenchymal stem cells in COVID-19 and hypertension. Stem Cell Rev Rep. 2020;1–12. doi:10.1007/s12015-020-10012-x
15. Gupta A, Madhavan MV, Sehgal K, et al. Extrapulmonary manifestations of COVID-19. Nat. Med. 2020;26(7):1017–1032.
16. Xu H, Zhong J, Deng J. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int. J. Oral Sci. 2020;12(8):1–5. doi:10.1038/s41368-020-0074-x
18. Nuzzo D, Picone P. Potential neurological effects of severe COVID-19 infection. Neurol Res. 2020;158(September):1–5. doi:10.1016/j.neures.2020.06.009
19. Kuhn JH, Radoszitzky SR, Li W, et al. The SARS coronavirus receptor ACE2 a potential target for antiviral therapy. New Concepts Antiviral Therapy. 2006:397–418.
20. Zhang L, Lin D, Sun X, et al. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved a-ketoamide inhibitors. Science. 2020;368(6489):409–412. doi:10.1126/science.abb3405
21. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579-(7798):265–269. doi:10.1038/s41586-020-2008-3
21. Pillaiyar T, Manickam M, Namasyavayam V, et al. An overview of severe acute respiratory syndrome-coronavirus (SARS-CoV) 3CL protease inhibitors: peptidomimetics and small molecule chemotheraphy. J Med Chem. 2016;59:6595–6628. doi:10.1021/acs.jmedchem.5b01461

22. Bzowka M, Mitusinska K, Raczyńska A, et al. Molecular dynamics simulations indicate the SARS-CoV-2 mpro is not a viable target for small-molecule inhibitors design. J Med Sci. 2020;21(9):3099. doi:10.3390/JMS21093099

23. Jin Z, Du X, Xu Y, et al. Structure of mpro 1 from COVID-19 virus and discovery of its inhibitors. Nature. 2020 Jun;582(7811):289–293. doi:10.1038/s41586-020-2223-y

24. Meng T, Cao H, Zhang H, et al. The insert sequence in SARS-CoV-2 enhances spike protein cleavage by TMPRSS. BioRxiv. 2020. doi:10.1101/2020.02.08.926006

25. Kang S, Yang M, Hong Z, et al. Crystal structure of SARS-CoV-2 nucleocapsid protein RNA binding domain reveals potential unique drug targeting sites. Acta Pharm Sinica B. 2020;10(7):1228–1238. doi:10.1016/j.apsb.2020.04.009

26. Yang H, Yang M, Ding Y, et al. The crystal structures of severe acute respiratory syndrome virus main protease and its complex with an inhibitor. Proc Natl Acad Sci USA. 2003;100(23):13190–13195. doi:10.1073/pnas.1835675100

27. Hillen HS, Kocic G, Farnung L, et al. Structure of replicating SARS-CoV-2 polymerase. Nature. 2020;584:154–156. doi:10.1038/s41586-020-2368-8

28. Saxena A. Drug targets for COVID-19 therapeutics: ongoing global efforts. J Biosci. 2020;45(1):87. doi:10.1007/s12038-020-00067-w

29. Narayanan K, Ramirez SI, Lokugamage KG, et al. Coronavirus nonstructural protein 1: common and distinct functions in the regulation of host and viral gene expression. Virus Res. 2015;202:89–100.

30. Ionescu MI. An overview of the crystallized structures of the SARS-CoV-2. Protein J. 2020;24:1–19. doi:10.1007/s10933-w20-09933-w

31. Augustin TL, Hajbabaie R, Harper MT, et al. Novel small-molecule scaffolds as candidates against the SARS coronavirus 2 main protease: a fragment-guided in silico approach. Molecules. 2020;25:5501. doi:10.3390/molecules25235501

32. Coutard B, Valle C, de Lamballerie X, et al. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral Res. 2020;176:104742. doi:10.1016/j.antiviral.2020.104742

33. Jeong GU, Song H, Yoon GY, et al. Therapeutic strategies against COVID-19 and structural characterization of SARS-CoV-2: a review. Front Microbiol. 2020;11:1723. doi:10.3389/fmicb.2020.01723

34. Laurin P. Exploring novel therapeutic approaches for COVID-19: Hesperidin versus vitamin C. 2020, https://valex-hesperco.s3.ca-central-1.amazonaws.com/Read+more+about+the+need+for+novel+approaches+to+SARS-CoV-2_v2.pdf?%B93%5D.pdf

35. Kupferschmidt K, Cohen J. Race to find COVID-19 treatments accelerates. Science. 2020;367(6485):6485.6412

36. Sarfraz I, Rasul A, Hussain G, et al. Natural immune boosters as first-line armours to combat viral infection-COVID-19: myth or science? Preprints. 2020. doi:10.20944/preprints202003.0427.v1

37. Galanakis CM. The food systems in the era of the coronavirus (COVID-19) pandemic crisis. Foods. 2020;9(4):523. doi:10.3390/foods9040523

38. Kanaze FI, Terrenti A, Gabrieli C, et al. The phytochemical analysis and antioxidant activity assessment of orange peel (Citrus sinensis) cultivated in Greece-Crete indicates a new commercial source of hesperidin. Biomed Chromatogr. 2009;23:239–249.

39. Sun Y, Qiao L, Shen Y, et al. Phytochemical profile and antioxidant activity of physiological drop of citrus fruits. J Food Sci. 2013;78(1):C37–C42. doi:10.1111/j.1750-3841.2012.03002.x

40. Tripoli E, Guardia MI, Giammanco S, et al. Citrus flavonoids: molecular structure, biological activity and nutritional properties: a review. Food Chem. 2007;104(2):466–479. doi:10.1016/j.foodchem.2006.11.054

41. Favela-Hernández MJ, González-Santiago O, Ramírez-Cabrera MA, et al. Chemistry and pharmacology of Citrus sinensis. Molecules. 2016;21(2):247. doi:10.3390/molecules21020247

42. Zakaryan H, Arabyan E, Oo A, et al. Flavonoids: promising natural compounds against viral infections. Arch Virol. 2017;162(9):2539–2551. doi:10.1007/s00705-017-3417-y

43. Cataneo AHD, Kuczera D, Koishi AC, et al. The citrus flavonoid naringenin impairs the in vitro infection of human cells by zika virus. Sci Rep. 2019;9(2045-2322):1–15. doi:10.1038/s41598-019-52626-3

44. Jo S, Kim S, Shin DH, et al. Inhibition of SARS-CoV 3CL protease by flavonoids. J Enzyme Inhib Med Chem. 2020;35(1):145–151. doi:10.1080/14756366.2019.1690480

45. Meneguzzo F, Carminina R, Zabini F, et al. Review of evidence available on hesperidin-rich products as potential tools against COVID-19 and hydrodynamic cavitation-based extraction as a method of increasing their production. Processes. 2020;8(5):1–18. doi:10.3390/PR8050549

46. Gogoi N, Chowdhury P, Goswami AK, et al. Computational guided identification of a citrus flavonoid as potential inhibitor of SARS-CoV-2 main protease. Mol Diversity. 2021;25(3):1745–1759. doi:10.1007/s11030-020-10150-x

47. Barrec D, Gattuso G, Bellocco E, et al. Flavanones: citrus phytochemical with health-promoting properties. Biofactors. 2017;43:495–506.

48. Barthe GA, Jourdan PS, Melnosh CA, et al. Radioimmunossay for the quantitative determination of hesperidin and analysis of its distribution in Citrus sinensis. Phytochemistry. 1988;27(1):249–254.

49. Hooper I, Kroon PA, Rimm EB, et al. Flavanoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. Am J Clin Nutr. 2008;88(1):38–50.

50. Demonty I, Lin Y, Zebregs YE, et al. The citrus flavonoids hesperidin and naringin do not affect serum cholesterol in...
moderately hypercholesterolemic men and women. J Nutr. 2010;140(9):1615–1620.
51. Buscemi S, Rosário G, Arcoleo G, et al. Effects of red orange juice intake on endothelial function and inflammatory markers in adult subjects with increased cardiovascular risk. Am J Clin Nutr. 2002;76(3):560–568. doi:10.1093/ajcn/76.3.560
52. Knekt P, Kumpulainen J, Järvinen R. Flavonoid intake and risk of chronic diseases. Am J Clin Nutr. 2002;76(3):560–568. doi:10.1093/ajcn/76.3.560
53. Li C, Schluesener H. Health-promoting effects of the citrus polyphenol rutinose-conjugates fl. Nutrients. 2015;7(4):2788–2800. doi:10.3390/nu70402788
54. Aisharawi AA, Ramesh E, Periasamy VS, et al. The apoptotic effect of hesperitin on human cervical cancer cells is mediated through cell cycle arrest, death receptor, and mitochondrial pathways. Fundam Clin Pharmacol. 2013;27:581–592.
55. Manach C, Morand C, Gil-Izquierdo A, et al. Bioavailability in humans of the flavanones hesperidin and naringin after the ingestion of two doses of orange juice. Eur J Clin Nutr. 2003;57(2):235–242.
56. Xiao J. Dietary flavonoid aglycones and their glycosides: which show better biological significance? Crit Rev Food Sci Nutr. 2017;57(7):1874–1905. doi:10.1080/10408398.2015.1032400
57. Bang SH, Hyun YJ, Shim J, et al. Metabolism of rutin and poncirin by human intestinal microbiota and cloning of their metabolizing α-L-rhamnosidase from Bifidobacterium dentium. J Microbiol. Biotechnol. 2015;25(1):18–25. doi:10.4014/jmb.1404.0406
58. Amaretti A, Raimondi S, Leonardi A, et al. Hydrolysis of the rutinose-conjugates flavonoids rutin and hesperitin by the gut microbiota and bifidobacteria. Nutrients. 2015;7(4):2788–2800. doi:10.3390/nu70402788
59. Valentová K, Vrba J, Banečová M, et al. Isoquercitrin: pharmacology, toxicology, and metabolism. Food Chem Toxicol. 2014;68:267–282. doi:10.1016/j.fct.2014.03.018
60. Mouffouk C, Mouffouk S, Mouffouk S, et al. Flavonoids as potential antiviral drugs targeting SARS-CoV-2 proteases (3CLpro and PLpro), spike protein, RNA-dependent RNA polymerase (RdRp) and angiotensin-converting enzyme II receptor (ACE2). Eur J. Pharm. 2021;891:173759. doi:10.1016/j.ejphar.2020.173759
61. Nagasako-Akazome Y. Safety of high and long-term intake of polyphenols. In: Watson RR, Preedy VR, Zibadi S, eds. Polyphenols in Human Health and Disease. Academic Press; 2014:747–756.
62. Damon M, Flandre O, Michel F, et al. Effect of chronic treatment with a purified flavonoid fraction on inflammatory granuloma in the rat. Study of prostaglandin E2 and F2 and thromboxane B2 release and histological changes. Drug Res. 1987;37(10):1149–1153.
63. Kawabe M, Tamano S, Shibata MA, et al. Subchronic toxicity study of methyl hesperidin in mice. Toxicol Lett. 1993;69(1):37–44. doi:10.1016/0378-4274(93)90143-1
64. Kawaguchi K, Mizuno T, Aida K, et al. Hesperidin as an inhibitor of lipases from porcine pancreas and Pseudomonas. Biosci Biotechnol Biochem. 1997;61(1):102–104.
65. Sieve BF. A new antifertility factor (a preliminary report). Science. 1952;116(3015):373–385.
66. Shoaib SS, Porter JB, Seur JH, et al. Effect of oral micronized purified flavonoid fraction treatment on leukocyte adhesion molecule expression in patients with chronic venous disease: a pilot study. J Vasc Surg. 2000;31(3):456–461.
67. Cospire M. Double-blind, placebo-controlled evaluation of clinical activity and safety of Dafon 500 mg in the treatment of acute hemorrhoids. Angiology. 1994;45(6):566–573.
68. Rabe E, Agus GB, Roztocki K. Analysis of the effects of micronized purified flavonoid fraction versus placebo on symptoms and quality of life in patients suffering from chronic venous disease: from a prospective randomized trial. Int Angiol. 2015;34(5):428–436.
69. Sharma R. Chapter 59 – polyphenols in health and disease: practices and mechanisms of benefits, Editors: Watson RR, Preedy VR, Zibadi S. Polyphenols in Human Health and Disease. Academic Press, 2014, Pages 757–778, ISBN 9780123984562 doi:10.1016/B978-0-12-398456-2.00059-1. (http://www.sciencedirect.com/science/article/pii/B9780123984562000591)
70. Zanwar AA, Sachin L, Badole SL, et al. Chapter 76 – cardiovascular effects of hesperidin: a flavanone glycoside. Editors: Watson RR, Preedy VR, Zibadi S. Polyphenols in Human Health and Disease, Academic Press, 2014, Pages 989–992, ISBN 9780123984562 doi:10.1016/B978-0-12-398456-2.00076-1. (http://www.sciencedirect.com/science/article/pii/B9780123984562000761)
71. Meyer OC. Safety and security of Dafon 500 mg in venous insufficiency and in hemorrhoidal disease. Angiology. 1994;45(6 Pt 2):579–584. doi:10.1016/00031979(94)045064
72. Schar MY, Curtis PJ, Hazim S, et al. Orange juice-derived flavanone and phenolic metabolites do not acutely affect cardiovascular risk biomarkers: a randomized, placebo-controlled, crossover trial in men at moderate risk of cardiovascular disease. Am J Clin Nutr. 2015;101:931–938.
73. Aranganathan S, Selvam JP, Nalini N. Effect of hesperetin, a citrus flavonoid, on bacterial enzymes and carcinogen-induced aberrant crypt foci in colon cancer rats: a dose-dependent study. J Pharm Pharmacol. 2008;60(10):385–1392. doi:10.1211/jpp.60.10.0015
74. Zhao J, Li Y, Gao J, et al. Hesperidin inhibits ovarian cancer cell viability through endoplasmic reticulum stress signaling pathways. Onco Lett. 2017;14(5):5569–5574.
75. Mahmoud AM, Mohammed HM, Khadrawy SM, et al. Hesperidin protects against chemically induced hepatocarcinogenesis via modulation of Nrf2/ARE/HO-1, PPARγ and TGF-β1/Smad3 signaling, and amelioration of oxidative stress and inflammation. Chem-Biol Interact. 2017;277:146–158.
76. Parhiz H, Roohbachsh A, Solati F, et al. Antioxidant and anti-inflammatory properties of the citrus flavonoids hesperidin and hesperetin: an updated review of their molecular mechanisms and experimental models. Phytother Res. 2015;29(3):323–331. doi:10.1002/ptr.5256
77. Cermi S, Ferlazzo N, Lombardo GE, et al. Neurodegenerative diseases: might citrus flavonoids play a protective role? Molecules. 2016;21(10):1312. doi:10.3390/molecules21101312
80. Suzuki H, Asakawa A, Kawamura N, et al. Hesperidin potentiates ghrelin signaling. *Recent Patents Food, Nutri Agric*. 2014;6(1):60–63.

81. Peng H, Wei Z, Luo H, et al. Inhibition of fat accumulation by hesperidin in *Caenorhabditis elegans*. *J Agric Food Chem*. 2016;64(25):5207–5214.

82. Millar CI, Duels Q, Blesso CN. Effects of dietary flavonoids on reverse cholesterol transport, HDL metabolism, and HDL function. *Adv Nutr*. 2017;8(2):226–239.

83. Suen J, Thomas J, Kranz A, et al. Effect of flavonoids on oxidative stress and inflammation in adults at risk of cardiovascular disease: a systematic review. *Healthcare (Basel)*. 2016;4(3):69. doi:10.3390/healthcare4030069

84. Yamada T, Hayasaka S, Shibata Y, et al. Frequency of citrus fruit intake is associated with the incidence of cardiovascular disease: the Jichi medical school cohort study. *J Epidemiol*. 2011;21(3):169–175.

85. Morand C, Dubray C, Milenkovic D, et al. Hesperidin contributes to the vascular protective effects of orange juice: a randomized crossover study in healthy volunteers. *Am J Clin Nutr*. 2011;93(1):73–80. doi:10.3945/ajcn.110.004945

86. Alu’datt MH, Rababah T, Alhamad MN, et al. Profiles of free and bound phenolics extracted from citrus fruits and their roles in biological systems: content, and antioxidant, anti-diabetic and anti-hypertensive properties. *Food Funct*. 2017;8(9):3187–3197. doi:10.1039/c7fo00212b

87. Garg A, Garg S, Zaneveld LJ, et al. Chemistry and pharmacology of the citrus bioflavonoid hesperidin. *Phytother Res*. 2001;15:655–669. doi:10.1002/ptr.1074

88. Aggarwal V, Tuli HS, Thakral F, et al. Molecular mechanisms of action of hesperidin in cancer: recent trends and advancements. *Exp Biol Med (Maywood)*. 2020;245(5):486–497. doi:10.1177/153537020903671

89. Anonymous. Hesperidin. https://www.rxlist.com/ hesperidin/supplements.html

90. Yatao X, Saeed M, Kamboh AA, et al. The potentially beneficial effects of supplementation with hesperidin in poultry diets. *World's Poultry Sci J*. 2018;74(2):265–276. doi:10.1017/S0043933917001088

91. Chiba H, Uehara M, Wu J, et al. Hesperidin, a citrus flavonoid, inhibits bone loss and decreases serum and hepatic lipids in ovariecctomized mice. *J Nutr*. 2003;133(6):1892–1897. doi:10.1093/jn/133.6.1892

92. Ahmadi A, Shadloorestan A. Oxidative stress and cancer; the role of hesperidin, a citrus natural bioflavonoid, as a cancer chemoprotective agent. *Nutr Cancer*. 2016;68(1):29–39. doi.org/10.1080/01635581.2015.1078822
108. Hemanth Kumar B, Dinesh Kumar B, Diwan PV. Hesperidin, a citrus flavonoid, protects against l-methionine-induced hyperhomocysteinemia by abrogation of oxidative stress, endothelial dysfunction and neurotoxicity in Wistar rats. *Pharm. Biol.* 2017;55(1):146–155. doi:10.1080/13880290.2016.1231695

109. Li Y, Kandhare AD, Mukherjee AA, et al. Acute and sub-chronic oral toxicity studies of hesperidin isolated from orange peel extract in Sprague Dawley rats. *Regul. Toxicol. Pharmacol.* 2019;105:77–85. doi:10.1016/j.yrtph.2019.04.001

110. Hajialyani M, Hossein Farzaei M, Echeverría J, et al. Hesperidin as a neuroprotective agent: a review of animal and clinical evidence. *Molecules.* 2019;24(3):648. doi:10.3390/molecules24030648

111. Kuntí V, Brborić J, Holčajtner-Antunović I, et al. Evaluating the bioactive effects of flavonoidal hesperidin—a new literature data survey. *Vojnosanit Pregl.* 2014;71(1):60–65. doi:10.2298/ VSP1401060K

112. Visnagri A, Kandhare AD, Chakravarty S, et al. Hesperidin, a flavonoglycone attenuates experimental diabetic neuropathy via modulation of cellular and biochemical marker to improve nerve functions. *Pharm. Biol.* 2014;52(7):814–828. doi:10.3109/13880290.2013.870584

113. Zhang YH, Wang B, Guo F, et al. The antidepressant-like effects of hesperidin in streptozotocin-induced diabetic rats by activating Nrf2/ARE/glyoxalase 1 pathway. *Front Pharmacol.* 2020;11:1325. doi:10.3389/fphar.2020.01325

114. Ganeshpurkar A, Saluja A. In silico interaction of hesperidin with vanoglycone attenuates experimental diabetic neuropathy via modulation of some immunomodulatory targets: a docking analysis. *Indian J Biochem Biophys.* 2019;56(1):28–33.

115. Ganeshpurkar A, Saluja A. The pharmacological potential of hesperidin. *Indian J Biochem Biophys.* 2019;56(4):287–300.

116. Panasiak W, Wleklik M, Oraczewska A, et al. Involvement of flavonoids on combined experimental infections with EMC virus and canine distemper virus by modulation of cellular and biochemical marker to improve anti-influenza activity. *Antiviral Ther.* 2018;23(7):611–615. doi:10.3851/IMP3235. Epub 2018 Apr 6.

117. De Clercq E. Potential antivirals and antiviral strategies against SARS coronaviruses infections. *Expert Rev Anti Infect Ther.* 2006;4(2):291–302. doi:10.1586/14787210.4.2.291

118. Stanisci D, Costa AF, Fávaro WJ, et al. Anticancer activities of hesperidin and hesperetin in vivo and their potentiality against bladder cancer. *J Nanomed Nanotechnol.* 2018;9:515. doi:10.4172/2157-7439.1000515

119. Khan A, Ikram M, Haum JR, et al. Antioxidant and anti-inflammatory effects of citrus flavonoid hesperetin: special focus on neurological disorders. *Antioxidants.* 2020;9:609. doi:10.3390/antiox9070609

120. Kaul TN, Middleton EJr., Ogra PL. Antiviral effect of flavonoids on human viruses. *J Med Virol.* 1985;15(1):71–79. doi:10.1002/jmv.1890150110

121. Kim HK, Jeon WK, Ko BS. Flavanone glycosides from Citrus junos and their anti-influenza virus activity. *Planta Med.* 2001;67–(6):548–549. doi:10.1055/s-2001-16484

122. Lin CW, Tsai FJ, Tsai CH, et al. Anti-SARS coronavirus 3C-like protease effects of Isatis indigotica root and plant-derived phenolic compounds. *Antiviral Res.* 2005;68(1):36–42. doi:10.1016/j. antiviral.2005.07.002

123. Castrillo M, Córdova T, Cabrera G, et al. Effect of naringenin, hesperetin and their glycosides forms on the replication of the H1N1 strain of influenza A virus by blocking of viral sialidase. *Biochim Biophys Acta* 2012;1585(2):108–112.

124. Bae EA, Han MJ, Kim DH. *In vitro* inhibitory effect of some flavonoids on rotavirus infectivity. *Biol Pharm Bull.* 2000;23(9):1122–1124. doi:10.1248/bpb.23.1122

125. Saha RK, Takahashi T, Suzuki T. Glucosyl hesperidin prevents influenza a virus replication in vitro by inhibition of viral sialidase. *Biol Pharm Bull.* 2009;32(7):1188–1192. doi:10.1248/bpb.32.1188

126. Garg A, Anderson RA, Zaneveld LJ, et al. Biological activity assessment of a novel contraceptive antimicrobial agent. *J Androl.* 2005;26(3):414–421. doi:10.2164/jandrol.04181

127. Carvalho OV, Botelho CV, Ferreira CGT, et al. In vitro inhibition of canine distemper virus by flavonoids and phenolic acids: implications of structural differences for antiviral design. *Rev Vet Sci.* 2013;95(2):717–724 doi:10.1016/j.rvsc.2013.04.013

128. Parvez MK, Rehman MT, Alam P, et al. Plant-derived antiviral drugs as novel hepatitis B virus inhibitors: cell culture and molecular docking study. *Saudi Pharm J.* 2019;27(3):389–400.

129. Løpsen SM. Flavonoid-associated direct loss of rotavirus antigen/antigen activity in cell-free suspension. *J Medically Active Plants.* 2013;2(1-2):10–24.

130. Dong W, Wei X, Zhang F, et al. A dual character of flavonoids in influenza A virus replication and spread through modulating cell-autonomous immunity by MAPK signaling pathways. *Sci Rep.* 2014;4:7237. doi:10.1038/srep07237

131. Stanisci D, Costa AF, Fávaro WJ, et al. Anticancer activities of hesperidin and hesperetin in vivo and their potentiality against bladder cancer. *J Nanomed Nanotechnol.* 2018;9:515. doi:10.4172/2157-7439.1000515

132. Kim HK, Jeon WK, Ko BS. Flavanone glycosides from Citrus junos and their anti-influenza virus activity. *Planta Med.* 2001;67–(6):548–549. doi:10.1055/s-2001-16484

133. Lin CW, Tsai FJ, Tsai CH, et al. Anti-SARS coronavirus 3C-like protease effects of Isatis indigotica root and plant-derived phenolic compounds. *Antiviral Res.* 2005;68(1):36–42. doi:10.1016/j. antiviral.2005.07.002

134. Castrillo M, Córdova T, Cabrera G, et al. Effect of naringenin, hesperetin and their glycosides forms on the replication of the 17D strain of yellow fever virus. *Avian Biomed.* 2015;4(2):69–78.

135. Nguyen TTH, Woo HJ, Kang HK, et al. Flavonoid-mediated inhibition of SARS coronavirus 3C-like protease expressed in *Pichia pastoris.* *Biotech Lett.* 2012;34(5):831–838.

136. Solnier J, Flander JP. Flavonoids: a complementary approach to conventional therapy of COVID-19? *Phytochem Rev.* 2021;20:1–23. doi:10.1007/s11101-020-09720-6

137. Pandes A, Alzurú M, Menéndez J, et al. Anti-sindbis activity of flavanones hesperetin and naringenin. *Biol Pharm Bull.* 2003;26(1):108–109. doi:10.1248/bpb.26.108

138. Fast DJ, Stern NP, Chuang J, et al. Flavanones common to citrus fruits activate the interferon-stimulated response element by stimulating expression of IRF7. *J. Food Bioact.* 2019;8:58–65 doi:10.1007/s10529-011-0845-8

139. Goris T, Pérez-Valero Á, Martínez I, et al. Repositioning microbial biotechnology against COVID-19: the case of microbial production of flavonoids. *Microb Biotechnol.* 2021;14(1):94–11010. doi:10.1111/1751-7915.13675
197. Haridas M, Sasidhar V, Nath P, et al. Compounds of *Citrus medica* and *Zingiber officinale* for COVID-19 inhibition: in silico evidence for cues from Ayurveda. *Future J. Pharm. Sci*. 2021;7:13. doi:10.1186/s43094-020-00171-6

198. Jin H, Zhao Z, Lan Q, et al. Nasal delivery of hesperidin/chitosan nanoparticles suppresses cytokine storm syndrome in a mouse model of acute lung injury. *Front Pharmacol*. 2021;11:592238. doi:10.3389/fphar.2020.592238

199. Meng C, Guo Z, Li D, et al. Preventive effect of hesperidin modulates inflammatory responses and antioxidant status following acute myocardial infarction through the expression of PPAR-γ and Bcl-2 in model mice. *Mol Med Rep*. 2018;17(1):1261–1268. doi:10.3892/mmr.2017.7981

200. Li X, Hu X, Wang J, et al. Inhibition of autophagy via activation of PI3K/Akt/mTOR pathway contributes to the protection of hesperidin against myocardial ischemia/reperfusion injury. *Int J Mol Med*. 2018;42(4):1917–1924. doi:10.3892/ijmm.2018.3794

201. Yuan X, Zhu J, Kang Q, et al. Protective effect of hesperidin against sepsis-induced lung injury by inducing the heat-stable protein 70 (Hsp70)/toll-like receptor 4 (TLR4)/myeloid differentiation primary response 88 (MyD88) pathway. *Mod Sci Monit*. 2019;25:107–114. doi:10.12659/MSM.912490

202. Kim J, Wie MB, Ahn M, et al. Benefits of hesperidin in central nervous system disorders: a review. *Anat Cell Biol*. 2019;52(4):369–377. doi:10.5115/acb.19.119

203. Ma H, Feng X, Ding S. Hesperetin attenuates ventilator-induced acute lung injury through inhibition of NF-κB mediated inflammation. *Eur J Pharmacol*. 2015;769:333–341. doi:10.1016/j.ejphar.2015.11.038

204. Huang H, Hu C, Xu L, et al. The effects of hesperidin on neuronal apoptosis and cognitive impairment in the sevoflurane anesthetized rat are mediated through the PI3/Akt/PTEN and nuclear factor-κB (NF-κB) signaling pathways. *Mod Sci Monit*. 2020;26:e920522-1-e920522-15. doi:10.12659/MSM.920522

205. Xiao S, Liu W, Bt J, et al. Anti-inflammatory effect of hesperidin enhances chondrogenesis of human mesenchymal stem cells for cartilage tissue repair. *J Inflamm (London, England)*. 2018;15:14. doi:10.1186/s12950-018-0190-y

206. Fu Z, Chen Z, Xie Q, et al. Hesperidin protects against IL-1β-induced inflammation in human osteoarthritis chondrocytes. *Exp Therap Med*. 2018;16:3721–3727.

207. Homayouni F, Haidari F, Hedayati M, et al. Blood pressure lowering and anti-inflammatory effects of hesperidin in type 2 diabetes; a randomized double-blind controlled clinical trial. *Phytotherapy Res. PTR*. 2018;32:1073–1079.

208. Tsimoyiannis EC, Floras G, Antoniou N, et al. Low molecular-weight heparins and dalfon for prevention of postoperative thromboembolism. *World J Surg*. 1996;20:968–971. discussion 972.

209. Anonymous. https://www.thailandmedical.news/news/covid-19-diets-study-shows-that-diets-rich-in-citrus-fruits-containing-hesperidin-might-be-a-healthy-option-for-dealing-with-sars-cov-2

**Abbreviations**

- ACE: angiotensin-converting enzyme
- ARDS: acute respiratory distress syndrome
- 3CLpro: 3-chymotrypsin-like main protease
- COVID-19: coronavirus disease 2019
- MERS-CoV: Middle East Respiratory Syndrome Coronavirus
- Mpro: main protease
- NSP: nonstructural protein
- PLpro: 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