An Overview on Flavonoids as Potential Antiviral Strategies against Coronavirus Infections

Korunavirüs Enfeksiyonlarına Karşı Potansiyel Antiviral Bileşikler Olarak Flavonoitlere Genel Bakış

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ABSTRACT

Coronaviruses are zoonotic viruses and can infect people, often causing respiratory and gastrointestinal complaints. Three coronavirus types namely, severe acute respiratory syndrome coronavirus (SARS-CoV), middle-east respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2 (COVID-19) cause pneumonia in human resulting in severe acute respiratory syndrome (SARS). SARS-CoV-2, first appeared in Wuhan, China, in 2019, has rapidly spread to the whole world in a short time due to the speed of transmission rate. Since there is no specific drug for disease prevention and treatment, drug and vaccine development studies are proceeding rapidly. In drug development studies, natural resources draw attention due to their antiviral activity and fewer side effects. Flavonoids, a secondary metabolite group found in higher plants, have a variety of pharmacological activities, including antiviral activity. In this review, flavonoid-type compounds and plant extracts containing these constituents were summarized in terms of their antiviral activity potential against coronavirus-induced infections. Herein, we can suggest that flavonoids shown to possess antiviral effect against SARS and MERS may be used as potential test materials for the studies of novel drug search for ongoing COVID-19 pandemic.

Key Words: Antiviral, coronavirus, COVID-19, flavonoids, medicinal plants, respiratory infection

Received: 07.03.2020          Accepted: 08.24.2020

ÖZET

Korunavirüsler zoonotik virüsler olup insanları enfekte ederek çoğu zaman solunum ve mide-bağırak şikayetlerine neden olabilir. Şiddetli akut solunum yetmezliği sendromu koronavirüsü (SARS-CoV), ortadoğu solunum yetmezliği sendromu koronavirüsü (MERS-CoV) ve SARS-CoV-2 (COVID-19) olmak üzere üç koronavirüs türü, insanlarda şiddetli akut solunum yetmezliği sendromuna (SARS) neden olur. İlk olarak 2019 yılında Çin’in Wuhan kentinde ortaya çıkan SARS-CoV-2, yüksek bulaşma hızı nedeniyle kısa sürede dünya çapında yayıldı. Hastalığın önlenmesi ve tedavisine yönelik spesifik bir ilaç olmadığından ilaç ve api geliştirme çalışmaları hızla devam etmekteyd. İlaç geliştirme çalışmalarında doğal kaynaklar, antiviral aktiviteleri ve daha az yan etki potansiyelleri ile dikkat çekmektedir. Yüksek bitkilerde bulunan sekonder metabolit grubu olan flavonoitler, antiviral aktivite dahil olmak üzere çeşitli farmakolojik aktiviteleri sahiptir. Bu derlemede, flavonoid tipi bileşikler ve bu bileşiklerin içeren bitki ekstreleri, koronavirüs kaynaklı enfeksiyonlara karşı antiviral aktivite potansiyelleri açısından önlenmektedir. SARS ve MERS’e karşı antiviral etkiye sahip olduğu gösterilen flavonoitlerin, devam eden COVID-19 pandemisi için yeni ilaç geliştirme çalışmalarında potansiyel deney materyalleri olarak kullanılabileceğini önerilir.

Anahtar Sözcükler: Antiviral, koronavirüs, COVID-19, flavonoid, tibbi bitki, solunum yolu enfeksiyonu

Geliş Tarihi: 03.07.2020         Kabul Tarihi: 24.08.2020

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Recently, in December 2019, a new coronavirus disease, namely severe acute respiratory syndrome coronavirus (SARS-CoV-2), caused an international outbreak of acute respiratory disorder named COVID-19. Although the disease appears in China for the first time, owing to its rapid spread, WHO made the assessment that COVID-19 can be characterized as a pandemic in March 2020 (1). This caused a great panic among people worldwide that countries have enhanced their efforts to manage this viral infection. However, it spreads very quickly and easily through droplets of saliva or discharge of the nose. The disease can be fatal in older people and especially ones with chronic medical conditions such as diabetes and heart disease. Unfortunately, no specific therapeutic agent exists currently. Therefore, as an unavoidable pandemic, the researchers are trying to provide the best protection approach for the public before a vaccine is developed. This situation also explains the importance of all these scientific studies in several laboratories worldwide.

Medicinal plants, their extracts, fractions, and secondary metabolites as well as their derivatives have been known to possess an essential role in controlling infections. Thanks to scientific research based on traditional medicine, medicinal plants have been shown to be effective against various diseases and potential sources of effective drug raw materials for the discovery and development of bioactive agents for the treatment and prevention of acquired infections. Therefore, natural products could be good options evident with previously conducted preclinical and clinical studies. Indeed, a plant-derived compound, hydroxychloroquine, previously used against malaria, is currently being promoted for the treatment of COVID-19. On the other hand, herbal medicines not only decrease the rate of viral infections but also improve the SARS-CoV symptoms when combined with conventional medicine (2,3).

Flavonoids, a secondary metabolite group with benzo-y-pyron skeleton, are among the several plant-based antiviral constituents especially found in higher plants. In this review, we aimed to summarize the potential antiviral effects of flavonoids in the prevention and treatment of coronavirus infections. In this regard, electronic databases including PubMed, Science Direct, and Scopus were searched using the keywords "flavonoids" and "coronavirus" and several related studies were obtained. Inspired by the previous experience, flavonoids can be considered as one of the important approaches towards COVID-19 treatment. However, detailed mechanistic investigations on each compound should be carried out to clarify this claim.

**Understanding the Coronaviruses**

Coronaviruses (CoVs), are enveloped, single-chain, positive polarity, and zoonotic RNA viruses belong to the subfamily Orthocoronavirinae in the family of Coronaviridae. This family includes α-, β- γ-, and δ-coronaviruses, where α- and β-coronaviruses cause infection in humans (4). There are 4 main proteins in the structure of coronaviruses: the spike protein (S), the nucleocapsid (N) protein, the membrane (M) protein, and the envelope (E) protein. The S protein mediates binding to the host receptor and fusion of the virus and cell membrane (5). Because CoVs have positive polarity, they do not contain RNA-dependent RNA polymerase enzyme, but in their genome, they encode this enzyme. Envelope surfaces have stick-like extensions made of glycoprotein. Due to the crown-like appearance of these surface protrusions, these viruses are named Coronaviruses based on the meaning of ‘corona’ which is ‘crown’ in Latin (6).

CoVs are of zoonotic origin and can cause serious respiratory diseases, besides gastrointestinal, cardiovascular, and neurological disorders in a variety of animal species such as cats, mice, chickens, turkeys, various other bird species, cattle, several wild ruminants, whales, dogs, cats, rabbits, and swine (7). In humans, they can often cause respiratory diseases and gastrointestinal complaints. From simple colds, they can lead to serious symptoms such as bronchitis, pneumonia, coagulopathy, multiple organ failure, and death. The first studies on the human coronavirus were carried out in the mid-1960s in human embryonic tracheal organ cultures. Until 2003, only HCoV-229E and HCoV-OC43 were known. Today, 7 different CoV strains are known to infect humans. HCoV229E, HCoVOC43, HCoV-HKU1, and HCoV-NL63 are the common coronaviruses among humans and generally cause self-resolving infection (8). The other 3 coronaviruses that infect humans are severe acute respiratory syndrome coronavirus (SARS-CoV) and middle-east respiratory syndrome coronavirus (MERS-CoV) and newly identified SARS-CoV-2, which cause fatal respiratory infections.

**Types of Coronaviruses**

a. **Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-1)**

Severe acute respiratory syndrome coronavirus (SARS-CoV-1) outbreak started in November 2002 in China. With the beginning of the outbreak, it was observed that new coronaviruses can be transmitted from animals to humans. Studies have shown that SARS-CoV-1 has originated in bats, the transmission may have occurred to people from palm civets and raccoon dogs provided intermediate host (9). SARS-CoV-1 is a β-coronavirus and the angiotensin-converting enzyme 2 (ACE2) is an accepted receptor for SARS coronaviruses. It has been suggested that suppression of ACE2 expression in patients with SARS-CoV-1 infections, plays a role in pathological changes in the lung, and contributes to relieving severe pneumonia (10).

The incubation period of SARS-CoV-1 is 2 to 11 days after exposure and transmission occurs following close contact from person-to-person through respiratory droplets. Though symptoms initially mild flu-like symptoms such as cough and sore throat, in later stages acute respiratory distress syndrome was observed in about 25% of the affected patients which required mechanical ventilation. SARS outbreak was taken under control in 2003. 8036 people from 29 countries were infected and around 10% of infected people died. After the SARS-CoV-1, studies were increased on the human coronavirus and new RNA detection methods were developed (11).

b. **Middle-East Respiratory Syndrome Coronavirus (MERS-CoV)**

Middle-east respiratory syndrome coronavirus (MERS-CoV) was detected in June, 2012 by evaluating a sample from the sputum of a patient with severe pneumonia and kidney failure in Saudi Arabia (12). Contagion to humans was caused by close contact with infected dromedary camels. MERS-CoV was transmitted through exposure to dromedary camel’s nasal secretions and other secretions and consumption of raw camel milk. In fact, the virus is thought to originate in bats and passed from bats to dromedary camels. MERS-CoV is from the β-CoV group and the receptor used to enter the host cell is a serine peptidase, dipeptidyl peptidase 4 (10). The incubation period of the disease is 2 to 14 days, while transmission occurs as a result of close contact with the infected person’s respiratory secretions (13). In addition to respiratory disease, MERS-CoV also causes renal damage due to the high level of dipeptidyl peptidase 4 expression in the kidney. At the end of January 2020, 866 deaths associated with MERS-CoV infections were reported globally (case-mortality rate: 34.3%), and most of the deaths were in Saudi Arabia (14).

c. **Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)**

On December 31st, 2019, several local health facilities reported cases of pneumonia of unknown etiology but considered to be linked to the live-animal market in Wuhan, Hubei province, China. The cause of the viral pneumonia outbreak was a new type of coronavirus, initially called 2019-nCoV, and this viral pneumonia disease was named COVID-19 and its causative factor was called SARS-CoV-2 (15). SARS-CoV-2 belongs to the subgenus Sarbecovirus of the genus β-coronavirus and was isolated from bronchoalveolar lavage samples of patients with unidentified pneumonia for the first time. The virus genome structure is similar to other β-coronaviruses. SARS-CoV-2 is an RNA virus with a linear, single strand, positive polarity containing genome weighing approximately 30 kilobases. SARS-CoV-2 virion contains 4 main structural proteins: nucleocapsid (N) protein, transmembrane (M) protein, envelope (E) protein, and spike (S) protein. Two different features stand out in the genome sequence. First, the receptor-binding domain (RBD) in the spike protein shows a high affinity for human angiotensin-converting enzyme-2 (ACE2) receptors. The second is a polybasic cleavage at the junction of sub-units S1 and S2 of the spike (16). SARS-CoV-2 shows 96.2% structural similarity with a bat coronavirus (CoV RaTG13) and 79.5% similarity with SARS-CoV. However, it is observed that the S protein of SARS-CoV-2 has 10-20 times more affinity for human ACE2 receptors compared with S protein of SARS-CoV. This unfortunately increases the probability of transmission from human to human (17).

ACE2 is a type I transmembrane protein and functions as a carboxypeptidase. It can be found on the surface of many cell types. Mainly in vascular endothelial cells, renal tubular epithelium, and testicles are expressed (18). ACE2 hydrolyzes angiotensin II, a powerful vasoconstrictor, to angiotensin (1-7). It has been reported to be associated with hypertension, cardiac function, heart function, and diabetes (19).
Importance of medicinal plants and their secondary metabolites against virus infections

Viral infections have an important place in acute infectious diseases. New types of viruses often appear with high incidence and mortality rates as viruses are generally mutated. Antiviral drugs used in the treatment of viral diseases are generally divided into classes including flavonoids, glycosides, and nucleosides from a marine organism Tethya cripta (29). Other examples of different secondary metabolite groups are terpenoids, flavonoids, and coumarins effective against HIV infections. Of these, agastanol, agastaquinone, uvaol, usoric acid, betulinic acid have terpene structures, whereas calanolide A has coumarin structure. Additionally, polyphenols, indole alkaloids, and lignans (rhinacanthin E, F) have been found to be effective against influenza virus infections (30).

a. Flavonoids and their antiviral potential

Flavonoids are polyphenolic secondary metabolites with benzo-y-pyron skeleton, synthesized in the plant by phenylpropanoid pathway. They can be divided into classes including flavones (apigenin, luteolin), flavonols (quercetin, kaempferol, myricetin), flavanones (rutin), flavan-3-ols (catechin, epicatechin). Flavonoids are plant metabolites with antioxidant, hepatoprotective, antibacterial, antiviral, antiproliferative, and anti-inflammatory activities. Flavonoids are known to be responsible for a variety of pharmacological activities such as antioxidant, hepatoprotective, antibacterial, anti-inflammatory, antinflammatory, antitumor, and antimicrobial activities. Flavonoids are known to be responsible for a variety of pharmacological activities such as antioxidant, hepatoprotective, antibacterial, ant-inflammatory, antitumor, and antiviral (31). Many studies have been reported on the antiviral activity of flavonoids against various virus types including respiratory syncytial virus (RSV), human immunodeficiency virus (HIV), herpes simplex virus (HSV), human parainfluenza virus (HPV), Auzuku’s disease virus (ADV), hepatitis B virus (HBV), hepatitis C virus (HCV), dengue virus (DENV), poliovirus, rabies virus, mumps virus, and coronaviruses (CoVs) (32).

b. Flavonoid-type compounds and flavonoid containing plant extracts against coronaviruses

Previously several studies were conducted to find out novel drug molecules against coronaviruses. However, it is no doubt that antiviral drug discovery is of great importance particularly nowadays due to the current pandemic of the new coronavirus (SARS-CoV-2). In this section, flavonoid containing plant extracts and isolated flavonoids that were shown to possess potential inhibitor action against coronaviruses are summarized. The flavonoid-type components and their antiviral effect were presented in Table 1.
| Flavonoid          | Coronavirus type | Target          | IC_{50} values | Ref. No |
|-------------------|------------------|-----------------|----------------|---------|
| Amentoflavone     | SARS-CoV         | 3CL\text{pro}  | IC_{50} = 8.3 µM | 44      |
| Ampelopsin        | SARS-CoV         | 3CL\text{pro}  | IC_{50} = 364 ± 8.7 | 45      |
| Apigenin          | SARS-CoV         | 3CL\text{pro}  | IC_{50} = 280.8 µM | 44      |
| Baicalin          | SARS-CoV         | Neutralisation test | EC_{50} = 11 µg/mL | 35      |
| Bavachinin        | SARS-CoV         | PL\text{pro}   | EC_{50} = 38.4 ± 2.4 µM | 41      |
| MERS-CoV          | 3CL\text{pro}   | IC_{50} = 27.9 ± 1.2 µM | 46      |
| Broussachalcone B | MERS-CoV         | PL\text{pro}   | IC_{50} = 112.9 ± 10.1 µM | 46      |
| SARS-CoV          | 3CL\text{pro}   | IC_{50} = 57.8 ± 0.5 µM | 46      |
| SARS-CoV          | PL\text{pro}    | IC_{50} = 11.6 ± 0.7 µM | 46      |
| Corylifol A       | SARS-CoV         | PL\text{pro}   | EC_{50} = 32.3 ± 3.2 µM | 41      |
| Daidzein          | SARS-CoV         | 3CL\text{pro}  | IC_{50} = 351 ± 2.9 | 45      |
| Diplacone         | SARS-CoV         | PL\text{pro}   | IC_{50} = 10.4 ± 0.16 µM | 42      |
| Epigallocatechin gallocatein | SARS-CoV | 3CL\text{pro} | IC_{50} = 73 ± 2 µM | 45      |
| Galloallocatechin gallocatein | SARS-CoV | 3CL\text{pro} | IC_{50} = 47 ± 0.9 µM | 45      |
| Helichrysetin     | MERS-CoV         | 3CL\text{pro}  | IC_{50} = 67.04 | 48      |
| MERS-CoV          | 3CL\text{pro}   | IC_{50} = 40.59 | 48      |
| Herbacetin        | SARS-CoV         | 3CL\text{pro}  | IC_{50} = 33.17 µM | 47      |
| Hesperetin        | SARS-CoV         | 3CL\text{pro}  | IC_{50} = 8.3 µM | 43      |
| Isoisobavachalcone| SARS-CoV         | PL\text{pro}   | EC_{50} = 73 ± 0.8 µM | 41      |
| MERS-CoV          | 3CL\text{pro}   | IC_{50} = 33.9 ± 7.7 µM | 46      |
| MERS-CoV          | PL\text{pro}    | IC_{50} = 82.2 ± 7.7 µM | 46      |
| Isoisoliquiritigenin | SARS-CoV    | 3CL\text{pro}  | IC_{50} = 61.9 ± 11.0 µM | 46      |
| SARS-CoV          | PL\text{pro}    | IC_{50} = 24.6 ± 1.0 µM | 46      |
| Juglanin          | SARS-CoV         | Inhibition of ion channel | IC_{50} = 2.3 µM | 40      |
| MERS-CoV          | 3CL\text{pro}   | IC_{50} = 135.0 ± 5.1 µM | 46      |
| MERS-CoV          | PL\text{pro}    | IC_{50} = 39.5 ± 5.1 µM | 46      |
| Kazinol F         | SARS-CoV         | 3CL\text{pro}  | IC_{50} = 43.3 ± 10.4 µM | 46      |
| SARS-CoV          | PL\text{pro}    | IC_{50} = 27.8 ± 2.5 µM | 46      |
| Luteolin          | SARS-CoV         | Spike(S) protein | EC_{50} = 10.6 µM | 39      |
| MERS-CoV          | 3CL\text{pro}   | IC_{50} = 20.2 µM | 44      |
| Mimulone          | SARS-CoV         | PL\text{pro}   | IC_{50} = 14.4 ± 0.27 µM | 42      |
| Myricetin         | SARS-CoV         | Helicase-ns P13 | IC_{50} = 2.71 µM | 38      |
| Eobavaisoflavone  | SARS-CoV         | PL\text{pro}   | EC_{50} = 18.3 ± 1.1 µM | 41      |
| MERS-CoV          | 3CL\text{pro}   | IC_{50} = 64.5 ± 4.9 µM | 46      |
| Papyriflavonol A  | SARS-CoV         | PL\text{pro}   | IC_{50} = 112.5 ± 7.3 µM | 46      |
| SARS-CoV          | 3CL\text{pro}   | IC_{50} = 103.6 ± 17.4 µM | 46      |
| Pectolinarin      | SARS-CoV         | PL\text{pro}   | IC_{50} = 3.7 ± 1.6 µM | 46      |
| Psoralidin        | SARS-CoV         | PL\text{pro}   | EC_{50} = 4.2 ± 1.0 µM | 41      |
| Puerarin          | MERS-CoV         | 3CL\text{pro}  | IC_{50} = 381 ± 12.5 | 45      |
| MERS-CoV          | 3CL\text{pro}   | IC_{50} = 34.8 ± 1.2 µM | 46      |
| Quercetin         | SARS-CoV         | 3CL\text{pro}  | IC_{50} = 73 ± 4 µM | 45      |
| SARS-CoV          | 3CL\text{pro}   | IC_{50} = 34.8 ± 1.2 µM | 46      |
| SARS-CoV          | 3CL\text{pro}   | IC_{50} = 23.8 µM | 44      |
| SARS-CoV          | PL\text{pro}    | IC_{50} = 8.6 ± 3.2 µM | 46      |
| Quercetin 3-ß-D-glucoside | MERS-CoV | 3CL\text{pro} | IC_{50} = 37.03 µM | 48      |
| Rhoifolin         | SARS-CoV         | 3CL\text{pro}  | IC_{50} = 27.65 µM | 47      |
| Scutellarein      | SARS-CoV         | Helicase-nsP13 | IC_{50} = 0.86 µM | 38      |
| Tomentin A        | SARS-CoV         | PL\text{pro}   | IC_{50} = 6.2 ± 0.04 µM | 42      |
| Tomentin B        | SARS-CoV         | PL\text{pro}   | IC_{50} = 6.1 ± 0.02 µM | 42      |
| Tomentin C        | SARS-CoV         | PL\text{pro}   | IC_{50} = 11.6 ± 0.13 µM | 42      |
| Tomentin D        | SARS-CoV         | PL\text{pro}   | IC_{50} = 12.5 ± 0.22 µM | 42      |
| Tomentin E        | SARS-CoV         | PL\text{pro}   | IC_{50} = 5.0 ± 0.06 µM | 42      |
| 3'-O-methyldiplacol | SARS-CoV      | PL\text{pro}   | IC_{50} = 9.5 ± 0.1 µM | 42      |
| 3'-O-methyldiploacol | SARS-CoV  | PL\text{pro}   | IC_{50} = 13.2 ± 0.14 µM | 42      |
| 4'-O-methylbavachalcone | SARS-CoV | PL\text{pro} | EC_{50} = 10.1 ± 1.2 µM | 41      |
| 4'-O-methylbavachalcone | SARS-CoV | PL\text{pro} | IC_{50} = 9.2 ± 0.13 µM | 42      |
| 6-Geranyl-4',5,7-trihydroxy-3',5'-dimethoxyflavanone | SARS-CoV | PL\text{pro} | IC_{50} = 13.9 ± 0.18 µM | 42      |
| 7-O-arylmethylerucetin with 3'-Cl | SARS-CoV | NTase/helicase | IC_{50} = 5.2 µM | 37      |
| 7-O-arylmethylerucetin with 3'-CN | SARS-CoV | NTase/helicase | IC_{50} = 2.7 µM | 37      |
| 7-O-arylmethylerucetin with 4'-Cl | SARS-CoV | NTase/helicase | IC_{50} = 4.1 µM | 37      |

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GMJ 2020; 31: 478-484
In a study of in vitro antiviral susceptibility of the SARS coronavirus, baicalin, a flavonoid-type compound isolated from *Scutellaria baicalensis* Georgi (Lamiaceae) was investigated against 10 clinical isolates of SARS-CoV by neutralisation tests. In the mentioned study, it reported that baicalin had antiviral activity. The results were confirmed by plaque reduction assay and the EC$_{50}$ value of baicalin was determined to be 11.6 ± 0.13 µM (35).

The murine coronavirus, mouse hepatitis virus is classified under the genus β-coronavirus and causes an epidemic murine illness. In a study, *in vitro* antiviral activity of the ethyl acetate fraction of *Houttuynia cordata* Thunb. (Saururaceae) and its flavonoid-type compounds, namely, quercetin, quercitin, and rutin were investigated against murine coronavirus. At the end of the study, the IC$_{50}$ value of the ethyl acetate fraction of *H. cordata* was determined to be 0.98 mg/mL. The isolated flavonoids exhibited comparatively weaker antiviral activity, only quercetin inhibited the mouse hepatitis virus with an IC$_{50}$ value of 125 µg/mL (36).

Antiviral activity of 7-O-aryl methyl quercetins, derivatives of quercetin, which is a popular and common flavonoid type compound with numerous biological effects, were evaluated against SARS-CoV. As a result of the study, it was reported that three derivatives with 3´-Cl, 3,4-Cl aromatic substituents exerted selective inhibitory activity against SARS-CoV NTPase/helicase. (IC$_{50}$ = 5.2 µM, 2.7 µM, 4.1 µM, respectively) (37).

A study by Keum and Jeong (2012) investigated 64 natural compounds to find out a promising target for the suppression of SARS-CoV helicase-nsp13. As a result of the study, only 2 compounds strongly inhibited the ATPase activity of nsp13 and these compounds were flavonoids namely, myricetin, and scutellarein. IC$_{50}$ values of myricetin and scutellarein were designated to be 2.71 µM and 0.86 µM (38).

In a study by Yi et al. (2004), new small molecules of Chinese herbal medicine that bind avidly with the surface Spike (S) protein of SARS-CoV were screened. Due to the binding with S protein, the entry of the virus to its host cells was interfered. A two-step screening method was developed which combines frontal affinity chromatography-mass spectrometry (FAC/MS). Pseudo-typed virus infection was assayed to find out the potential drugs that can interfere with the entry of SARS-CoV into host cells. Among 130 small molecules, two were found to possess potent antiviral activity against the wild-type SARS-CoV infection. One of these two molecules was luteolin which has a flavonoid structure with an EC$_{50}$ value of 10.6 µM (39).

The genome of the SARS coronavirus contains open reading frames encoding new proteins. The protein encoded by the open-reading-frame 3a of SARS-CoV demonstrates to form a cation-selective channel which may become expressed in the infected cell. The channel’s activity involves the virus release mechanism. Drugs that inhibit the ion channel could be new therapeutic antiviral agents. In a study, the flavonoids, *e.g.* kaempferol, kaempferol glycosides, and acetylated kaempferol glucoside derivatives were tested according to their potency to inhibit channel 3a. According to the results, the glycoside juglanin that carried an arabinose residue was found to be the most active molecule with an IC$_{50}$ value of 2.3 µM. Kaempferol derivatives with rhamnose residue were also seen to be quite potent (40).

Seeds of *Psoralea corylifolia* L. (Fabaceae) are used as a food additive in many countries, especially in South Korea, and the plant contains flavonoids and chalcones as the main bioactive compounds.

A study on the antiviral activity of the ethanol extract of *P. corylifolia* seeds demonstrated that the extract exerts a strong inhibitory activity against SARS-CoV papain-like protease (PL$_{pro}$) enzyme, with an IC$_{50}$ value of 15 µg/mL. SARS-CoV PL$_{pro}$ plays an important role in SARS-CoV replication. Since the ethanol extract of *P. corylifolia* seeds exhibited a significant activity against the SARS-CoV PL$_{pro}$, a bioactivity-guided fractionation was applied to the ethanol extract. Six aromatic compounds were isolated, namely, bavachinin, neobavaisoflavone, isobavachalcone, 4-O-methylbavachalcone, psoralinid, and coryrifol A. All isolated flavonoids inhibited PL$_{pro}$ in a dose-dependent manner with EC$_{50}$ values of 38.4 ± 2.4 µM, 18.3 ± 1.1 µM, 7.3 ± 0.8 µM, 10.1 ± 1.2 µM, 4.2 ± 1.0 µM, and 32.3 ± 3.2 µM, respectively. Thus, this study indicates that the plant may be a rich source of potent PL$_{pro}$ inhibitors and can be used as an option in the treatment of SARS (41).

In another study, the SARS-CoV PL$_{pro}$ inhibitory activity of the methanol extracts of *Paulownia tomentosa* (Thunb) Steud. (Paulowniaceae) fruit was assessed by using a fluorogenic assay. A total of twelve PL$_{pro}$ inhibitory flavonoids, five of which were with new structures, were isolated from the extract. New flavonoids contained a rare 3,4-di-hydro-2H-pyrano moiety, namely, tomentin A, tomentin B, tomentin C, tomentin D, and tomentin E. The results have indicated that new compounds were the most effective with the IC$_{50}$ values of 6.2 ± 0.02 µM, 6.1 ± 0.02 µM, 11.6 ± 0.13 µM, 12.5 ± 0.22 µM, and 5.0 ± 0.06 µM, respectively. The other tested flavonoids were also able to inhibit the enzyme in a dose-dependent manner. The mentioned flavonoid compounds were 3′-O-methyl diplacol (IC$_{50}$ = 95.9 ± 0.1 µM), 4′-O-methyl diplacol (IC$_{50}$ = 9.2 ± 0.13 µM), 3′-O′-methyl diplacol (IC$_{50}$ = 13.2 ± 0.14 µM), 4′-O′-methyl diplacol (IC$_{50}$ = 127.2 ± 0.19 µM), mimuleone (IC$_{50}$ = 14.4 ± 0.27 µM), diplacolone (IC$_{50}$ = 104.4 ± 0.16 µM), and 6-garan-4′-5,7,11-trihydroxy-5′-dimethoxylavanan (IC$_{50}$ = 13.9 ± 0.18 µM) (42).

The 3C-like protease (3CL$_{pro}$) of SARS-CoV is vital for the viral life cycle and promising target for the development of antiviral drugs against CoV infections. *Isatis indigotica* Fort. (Brassicaceae) is a plant grown in China and its roots have an antiviral effect. In a study, the water extract of *I. indigotica* roots and flavonoids compounds from the root extracts [indigo, indirubin, indican (indoxyl-β-D-glucoside), β-sitosterol, α-sitosterol, and sinigrin] was investigated against SARS-CoV 3CL$_{pro}$. It was determined that the root extract inhibited 3CL$_{pro}$ with an IC$_{50}$ value of 191.6 ± 8.2 µg/mL in cell-based assay. Aloe-emodin, hesperetin, quercetin, naringenin, daidzein, emodin, and chrysophanol were also analyzed in the same study. Results showed that hesperetin, a flavanone type compound, was the most potent compound with an IC$_{50}$ value of 60.3 µM and 8.3 µM in cell-free and cell-based assays (43).

In a study by Ryu et al. (2010) the leaf extract of *Torreya nucifera* (L.) Siebold et Zucc. (Taxaceae) growing in Korea and its active flavonoids were investigated against SARS-CoV 3CL$_{pro}$. The leaf extract displayed a good level of SARS-CoV 3CL$_{pro}$ inhibitory activity (62% at 100 µg/mL). Through activity-guided isolation studies, amentoflavone, luteolin, quercetin, and apigenin were identified and the IC$_{50}$ values of the mentioned compounds were determined as 8.3 µM, 202.0 µM, 23.8 µM, and 280.8 µM, respectively (44).

Seven flavonoid derivatives were evaluated for their inhibitory activity against 3CL$_{pro}$ expressed and purified from a methyloctys flavid *Pichia pastoris*. Flavonoid compounds as the test substances in the study were quercetin, daidzein, puerarin, epigallocatechin, epigallocatechin gallate gallic acid, and amelopsin. Quercetin, epigallocatechin gallate, and gallic acid galate showed a good inhibition against 3CL$_{pro}$ with IC$_{50}$ values of 73, 73 and 47 µM, respectively. The relationship between structure inhibition activity in flavonoids is ongoing. As a result, it was reported that gallatechinic acid is the best inhibitor and shows numerous hydrophobic and H-bonds interactions with amino acid residues in the 3CL$_{pro}$ active site pocket (45).

In another study, a series of flavonoid compounds isolated from the root extract of *Broussonetia papyrifera* (L.) Vent. (Moraceae) were tested against both SARS-CoV and MERS-CoV 3CL$_{pro}$ and PL$_{pro}$. As a result of the MERS-CoV, it was reported that brousschalcone B inhibited 3CL$_{pro}$ with an IC$_{50}$ value of 27.9 ± 1.2 µM, and PL$_{pro}$ with 112.9 ± 1.01 µM, papyriflavonol A inhibited 3CL$_{pro}$ with an IC$_{50}$ value of 64.5 ± 4.9, and PL$_{pro}$ with 112.5 ± 7.3 µM, kazinol F inhibited 3CL$_{pro}$ with an IC$_{50}$ value of 135.0 ± 5.1 µM and PL$_{pro}$ with 39.5 ± 5.1 µM, isoliquiritigenin inhibited 3CL$_{pro}$ with an IC$_{50}$ value of 33.9 ± 7.7 µM and PL$_{pro}$ with 82.2 ± 7.7 µM, kaempferol, and quercetin with an IC$_{50}$ value of 35.3 ± 5.3 µM, PL$_{pro}$ with 26.4 ± 1.7 µM, quercetin inhibited 3CL$_{pro}$ with an IC$_{50}$ value of 34.8 ± 1.2 µM but was not able to show inhibition against PL$_{pro}$.
Against SARS-CoV-2, it was reported that broussausahalcone B inhibited 3CL\(^\text{pro}\) with an \(IC_{50}\) value of 57.8 ± 0.5 \(\mu\)M, and PL\(^\text{pro}\) with 11.6 ± 0.7 \(\mu\)M, papyriflavonol A inhibited 3CL\(^\text{pro}\) with an \(IC_{50}\) value of 103.6 ± 17.4 \(\mu\)M, and PL\(^\text{pro}\) with 3.7 ± 1.6 \(\mu\)M. Kazinol F inhibited 3CL\(^\text{pro}\) with an \(IC_{50}\) value of 43.3 ± 10.4 \(\mu\)M and PL\(^\text{pro}\) with 27.8 ± 2.5 \(\mu\)M, isoliquiritenigen inhibited 3CL\(^\text{pro}\) with an \(IC_{50}\) value of 61.9 ± 11.0 \(\mu\)M and PL\(^\text{pro}\) with 24.6 ± 1.0 \(\mu\)M, kempferol inhibited 3CL\(^\text{pro}\) with an \(IC_{50}\) value of 116.3 ± 7.1 \(\mu\)M, PL\(^\text{pro}\) with 16.3 ± 2.1 \(\mu\)M, quercetin inhibited 3CL\(^\text{pro}\) with an \(IC_{50}\) value of 34.8 ± 1.2 \(\mu\)M, PL\(^\text{pro}\) with 6.6 ± 3.2 \(\mu\)M. It was concluded that all compounds were more potent against PL\(^\text{pro}\) than against 3CL\(^\text{pro}\) and papyriflavonol A was the most potent inhibitor of PL\(^\text{pro}\) with an \(IC_{50}\) value of 3.7 \(\mu\)M (46).

According to aforementioned studies, it is assumed that the antiviral activity of some flavonoids against CoVs is directly related by the inhibition of 3CL\(^\text{pro}\). In a study, flavonoid-type compounds were applied to the FRET (Fluorescence resonance energy transfer) method for a systematic investigation of inhibitory compounds against SARS-CoV 3CL\(^\text{pro}\). As a result of the evaluation of the potential proteolytic activity of flavonoids and an induced-fit docking experiment, herbacetin, rhoifolin, and pectolinarin were found to possess the best inhibitory effect against SARS-CoV 3CL\(^\text{pro}\) with \(IC_{50}\) values of 33.17, 27.45 and 37.78 \(\mu\)M, respectively (47).

In a similar study by Jo et al. (2019), a flavonoid library was applied to probe inhibitor compounds against MERS-CoV 3CL\(^\text{pro}\). Herbacetin, isobavachalcone, quercetin 3′-O-glucoside and helichrysetin were found to prevent the enzymatic activity of MERS-CoV 3CL\(^\text{pro}\) in a dose-dependent manner with \(IC_{50}\) values of 40.59, 35.85, 37.03, and 67.04 \(\mu\)M, respectively. A comparison of the binding affinity of flavonoids provided an understanding of their scaffolds and functional groups that required to bind with MERS-CoV 3CL\(^\text{pro}\). It was revealed by an induced-fit docking analysis that S1 and S2 sites play a role in interacting with flavonoids. At the end of the study, it has been observed that flavonol and chalcone were the favorite scaffolds to bind to the catalytic site. It was also concluded that some hydrophobic flavonoid derivatives or carbohydrate attached to the core structures provide a good inhibitory effect (48).

AutoDock Vina was utilized to screen potential drugs by molecular docking with the structural protein and non-structural protein sites of new coronavirus in a study by Ran et al. (2020). It was seen that luteolin, the main flavonoid in honeysuckle, bound with a high affinity to the same sites of the main protease of SARS-CoV-2 (49).

**CONCLUSION**

CoVs are RNA viruses that can infect various hosts, including avian, swine, and humans. Among them, human coronaviruses represent a major group of CoVs related with the respiratory diseases such as common cold and serious pneumonia. The first confirmed atypical pneumonia was SARS, a recent outbreak that led to a significant number of deaths and widespread international attention. The novel coronavirus 2019 (SARS-CoV-2) is a member of the Coronaviridae family, which is characterized by its unique spike protein that mediates viral entry into host cells. The spike protein is composed of two subunits, S1 and S2, which are responsible for receptor binding and membrane fusion, respectively.

The SARS-CoV-2 virus is highly contagious and has caused a global pandemic, with millions of cases and thousands of deaths worldwide. The virus primarily affects the respiratory system, causing symptoms such as cough, fever, and shortness of breath. The severity of the disease can range from mild to severe, with some patients requiring hospitalization and mechanical ventilation.

In recent years, there has been significant research focus on developing effective treatments and vaccines for SARS-CoV-2. Various therapeutic strategies, including antiviral drugs, monoclonal antibodies, and gene therapies, are under active investigation. Similarly, vaccines are being developed and tested to provide long-term protection against the virus.

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