In this theoretical article, the Australian authors analyzed recent research on natural products (NPs) derived from plants, honey, and marine sponges with properties against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The authors emphasize several potential mechanisms of action for novel antiviral agents. Novel therapies against SARS-CoV-2 may target aspects of the host cell involved in disease progression, such as cell surface proteins that facilitate viral entry, or structural or functional components of the virus. The unique advantages of natural products, including their diverse bioactivity and ability to act synergistically with other drugs, warrant further investigation.

The causative agent of COVID-19 is SARS-CoV-2, which belongs to the *Sarbecovirus* subgenus of betacoronaviruses. The SARS-CoV-2 positive-sense, single-stranded RNA genome consists of 12 open reading frames (ORFs). ORF1ab encodes non-structural proteins. The remaining ORFs encode major spike (S), envelope (E), membrane (M), and nucleocapsid (N) structural proteins and accessory proteins involved in pathogenesis. The S protein is a glycosylated homotrimer with each monomer composed of subunits S1 and S2, separated by host cell proteases. The S1 domain comprises the N-terminal domain (NTD), the receptor binding domain (RBD) with a receptor binding motif (RBM), and two C-terminal domains. The RBD in the S1 subunit recognizes human angiotensin-converting enzyme 2 (ACE2) and is responsible for attachment to host cells.

The first stage of the SARS-CoV-2 life cycle is fusion and entry, which begins with the interaction of the S protein with ACE2. The exact method for SARS-CoV-2 entry depends on the expression of transmembrane serine protease 2 (TMPRSS2), which belongs to the family of type 2 transmembrane serine proteases and is involved in the entry of many pathogenic viruses. If there is sufficient TMPRSS2, this enzyme cleaves the S protein at the S2′ cleavage site and exposes a fusion peptide responsible for facilitating genome release into the cytoplasm through fusion with the cell membrane. In contrast, if there is insufficient TMPRSS2, the virus is endocytosed and the S2′ site is cleaved by cathepsin L following endosomal acidification, enabling fusion between the viral and endosomal membranes.

### *About natural products*

People have used natural products derived from plants, animals, and microbes for thousands of years to treat various diseases. In many countries like China, Japan, Korea, and India, traditional medicines are used along with Western therapeutics, playing an integral role in the treatment of various illnesses.

Although NPs have contributed significantly to pharmaceutical development over the past decades, they have contributed to approximately 6% of all new antivirals produced since 1981. This is due to the constraints on working with NPs, which complicate new bioactive molecule discovery. However, the extensive use of certain classes of NPs in traditional medicines highlights their safety and efficacy in infectious disease therapy. Furthermore, the authors emphasized that many natural products have clinically advantageous bioactivity because they are shaped by natural selection for optimal interaction with biological systems.

Antiviral drugs are developed from purely synthetic compounds or synthetic derivatives of natural products, such as plant secondary metabolites. The discovery of bioactive molecules from NPs follows a pipeline involving the bioactivity-guided fractionation of crude NP extracts. Crude extracts of NPs are obtained through various methods and then separated into fractions and individual compounds. The isolated compounds are further analyzed using *in silico* modeling techniques.

Certain NPs exhibit additive or synergistic effects when combined with other therapies. For example, a study that used a pseudovirus model found that four compounds were capable of inhibiting SARS-CoV-2 entry by blocking membrane fusion, namely oleanonic acid, angeloylgomisin O, schisandrin B, and procyanidin. Angeloylgomisin O was found to act synergistically in combination with remdesivir, significantly reducing viral replication.

The authors emphasize that the pseudovirus assay is particularly useful for *in vitro* screening of drug candidates for viral entry inhibitors and studying highly transmissible viruses such as SARS-CoV-2. This assay allows for a relatively accurate simulation of the viral entry process and reduces the need for the use of a replicating virus. In recent studies, pseudovirus assays have been used to analyze potential inhibitors of SARS-CoV-2 entry derived from natural products, in particular those derived from plants.

### *Inhibitors of SARS-CoV-2 entry derived from plants*

The compounds produced by plants, referred to as phytochemicals, can be categorized either as primary metabolites, which are essential for growth, secondary metabolites, which mediate interactions with the environment, and hormones, which regulate metabolism. There is a lot of overlap between the groups. Their therapeutic benefits are often attributed to secondary metabolites such as polyphenols, alkaloids, and terpenoids. Polyphenols are the largest group of secondary metabolites and include aromatic compounds like flavonoids, catechins, and tannins, while terpenoids and nitrogen-containing alkaloids include

compounds such as sesquiterpenes and quinine.

The extensive use of NPs derived from plants in traditional medicines led to significant efforts to characterize their anti-SARS-CoV-2 properties, including anti-spike (S) protein activity. One previous study that screened 125 compounds from *Glycyrrhiza uralensis*, a flowering plant used in traditional Chinese medicine, for their ability to block SARS-CoV-2 viral entry, found numerous triterpenoid saponins with strong affinity for the S protein RBD. Another study employed a variety of techniques, including a lentivirus-based pseudovirus assay, to screen 31 flavonoids for their ability to block the interaction between the S protein and ACE2. Myricetin was the most potent inhibitor of SARS-CoV-2 pseudovirus entry into a HEK293T cell line expressing ACE2, with an IC50 of 10.27 ± 2.32 µM. In addition, the interaction of S protein with ACE2 was significantly inhibited (65%, 100%) by both water and ethanol extracts of honeysuckle (*Lonicera japonicae*).

*Glycyrrhiza uralensis*

Furthermore, a molecular docking analysis of flavonoids with antiviral properties against wild-type, Delta, Omicron BA.1 and BA.2 SARS-CoV-2 variants identified a binding pocket within the RBD of the S protein capable of forming stable interactions with myricetin, quercetin, and kaempferol. It is believed that the flavonoids such as myricetin are among the most promising natural drug candidates for antiviral development. Numerous *in vitro*

and *in silico* studies have also demonstrated that compounds such as epicatechin, luteolin, curcumin, and quercetin inhibit the S protein/ACE2 interaction. https://discovermednews.com/beneficial-effects-of-quercetin-in-covid-19/

### *Inhibitors of SARS-CoV-2 entry derived from honey*

Like plants, honey has been used in traditional medicine due to its inherent antioxidative, anti-inflammatory, immunomodulatory, and antimicrobial properties. Manuka honey, collected by monofloral honeybees in New Zealand, displayed *in vitro* activity against viruses like influenza A, HIV-1, and varicella-zoster virus. Despite limited research on the potential anti-SARS-CoV-2 activity of honey, the phenolic content of honey has been associated with a decreased expression of proinflammatory cytokines. A placebo-controlled randomized study, which evaluated the combined use of honey and *Nigella sativa* seeds in the treatment of COVID-19, found that participants who received this treatment had a shorter duration of viral load and lower mortality rate. A study that screened 12 honey samples produced by Indonesian stingless honeybees found that 10 had a 50% or greater inhibitory effect against the S protein/ACE2 interaction. Among these samples, the most potent activity displayed a bitter variant of the honey produced by the honeybee *Wallacetrigona incisia.* These and other studies have shown that honey is a promising alternative source of bioactive compounds with antiviral properties. Further research is needed to better evaluate its potential contribution to the development of antivirals, such as entry inhibitors.

### *Inhibitors of SARS-CoV-2 entry derived from marine sponges*

Marine sponges represent another source of natural compounds with antiviral properties. Nucleosides, quinones, and alkaloids from marine sponges displayed antiviral activity, particularly against HIV-1. Recent studies have highlighted the potential use of such molecules in the SARS-CoV-2 antiviral development. Recent *in silico* studies have revealed a strong affinity for several viral proteins of the marine sponge metabolite ilimaquinone, as well as the potential antiviral properties of the marine sponge metabolite 8-hydroxymanzamine. Some marine sponge metabolites such as thorectidiol A, a novel terpenoid isolated from the marine sponge *Dactylospongia elegans,* and its acetylated derivative thorectidiol A diacetate have significant inhibitory activities against the S protein/ACE2 interaction with respective IC50 values of 1.0 ± 0.7 µM and 7.3 ± 2.6 µM.

Further i*n vitro* and *in silico* analyses are needed to characterize these compounds and validate their activity against SARS-CoV-2 variants.

*Dactylospongia elegans*

### *Conclusion*

The authors emphasized the significance of studies on the bioactivity and composition of crude extracts as crucial preliminary steps in drug discovery from natural products. Identifying the key components of crude extracts with strong bioactivity and validating their potential should be the focus of further investigation.

Given the urgent need to develop coronavirus entry inhibitors in anticipation of future outbreaks of coronaviruses, the authors concluded that natural products with significant potential in antiviral development should be thoroughly investigated.

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