In Search of Herbal Anti-SARS-Cov2 Compounds

Tatiana Matveeva1*, Galina Khafizova1 and Sofia Sokornova2

1Department of Genetics and Biotechnology, St. Petersburg State University, St. Petersburg, Russia, 2Department of Toxicology and Biotechnology, All-Russian Institute of Plant Protection, St. Petersburg, Russia

On March 11, 2020, the World Health Organization (WHO) announced that the spread of the new coronavirus had reached the stage of a pandemic. To date (23.10.2020), there are more than 40 million confirmed cases of the disease in the world, at the same time there is still no effective treatment for the disease. For management and treatment of SARS-Cov2, the development of an antiviral drug is needed. Since the representatives of all human cultures have used medicinal plants to treat viral diseases throughout their history, plants can be considered as sources of new antiviral drug compounds against emerging viruses. The huge metabolic potential of plants allows us to expect discovery of plant compounds for the prevention and treatment of coronavirus infection. This idea is supported by number of papers on the anti-SARS-Cov2 activity of plant extracts and specific compounds in the experiments in silico, in vitro, and in vivo. Here, we summarize information on methods and approaches aimed to search for anti-SARS-Cov2 compounds including cheminformatics, bioinformatics, genetic engineering of viral targets, interacting with drugs, biochemical approaches etc. Our mini-review may be useful for better planning future experiments (including rapid methods for screening compounds for antiviral activity, the initial assessment of the antiviral potential of various plant species in relation to certain pathogens, etc.) and giving a hand to those who are making first steps in this field.

Keywords: genetic engineering, cheminformatics, secondary metabolites, antiviral drugs, coronavirus, medicinal plants (herbal drugs), biochemical methods

INTRODUCTION

A novel coronavirus strain causing fatal respiratory syndrome was reported in late 2019. In January 2020, it was revealed that it belongs to the beta-coronaviruses, sharing similarity to SARS-coronaviruses, and that its spike protein interacts strongly with the human angiotensin-converting enzyme 2 (ACE2) receptor (Dhama et al., 2020; Xu et al., 2020). On March 11, the World Health Organization (WHO, 2020) announced that the spread of the new coronavirus had reached a pandemic stage. To date (23.10.2020), there are more than 40 million confirmed cases of the disease in the world, while there is still no effective treatment for the disease. In this regard, the search for cures for this disease is undoubtly relevant and significant. Plant-based medicine is attracting a lot of attention today, since medicinal plants are enriched with variety of secondary metabolites including those with antiviral properties (Gurib-Fakim, 2006; Adedeji and Sarafianos, 2014; Dhama et al., 2018; Divya et al., 2020; Vellingiri et al., 2020).
About, a third of FDA-approved drugs over the past 20 years are based on natural products or their derivatives (Carter, 2011).

Natural products can offer safe and inexpensive platforms for discovery of efficient and novel agents for treatment of SARS-CoV-2 with minimizing side effects (Ghildiyal et al., 2020; Huang et al., 2020; Mani et al., 2020). Although structures for 200,000 natural products are known, only 15% of the estimated 350,000 plant species have been investigated for their chemical constituents (Cragg and Newman, 2013). Thus, plants hold great potential as a material for drug development.

**POTENTIAL TARGETS FOR ANTI-SARS-Cov2 DRUG DESIGN**

Coronaviruses are single-stranded (+) RNA viruses, having four structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. The other 25 nonstructural proteins (NSPs) regulate assembling of copies of virus particles and their passing through the host’s immune system (Xu et al., 2020).

Inside the host cell, two polyproteins, pp1a and pp1ab, are directly translated from the viral RNA and then cleaved by two viral proteases, main protease (Mpro, also called 3-chymotrypsin-like cysteine protease, or 3CLpro) and papain-like protease (PLpro). PLpro cleaves junctions of NSP 1, 2, and 3 while Mpro cleaves polyprotein at 11 distinct sites downstream from the NSP4 to generate various NSPs that are important for viral replication (Perlman and Netland, 2009). The Mpro is one of the best-characterized drug targets among the coronaviruses (Anand et al., 2003; Hilgenfeld, 2014). Since no human proteases with similar cleavage specificity are known, the inhibitors of Mpro are unlikely to be toxic (Zhang et al., 2020b). The PLpro is also a target for drug design, because it affects not only coronavirus replication, but also has the additional function of deubiquitination of host cell proteins and ISG15 removal, finally leading to the immune suppression of host cells (Báez-Santos et al., 2015; Lin et al., 2018; Clemente et al., 2020).

The viral S protein is essential for viral attachment, fusion, and entry. It uses host angiotensin-converting enzyme 2 (ACE2) as a receptor to get into the host cells. Since, the structure of S protein of SARS-CoV-2 is revealed (Coutard et al., 2020; Huang et al., 2020; Wrapp et al., 2020) and receptor-binding domain was identified, it can serve as a target for development of inhibitors of S protein and ACE2 interaction (Tai et al., 2020).

Approaches to search for substances interacting with these targets will be described below together with successful examples of their application.

**DRUG SEARCH STRATEGIES IN PHYTOMEDICINE**

Previously, new drugs were discovered mostly by a "trial and error" approach (Butcher et al., 2004). As methods and knowledge in chemistry advance, researchers began to purify the active compounds in herbal extracts known to have medicinal properties and determine their structures (Drews, 2000). The emergence of genomics and proteomics and the development of bioinformatics and cheminformatics made a breakthrough into the drug design, bringing the concept and techniques for large-scale screening. Newly emerging diseases require faster pace of drug development. Worldwide transmission of COVID-19 (van Dorp et al., 2020) and high infectivity (Zhang and Holmes, 2020) of the virus demands rapid development of suitable drugs.

In phytomedicine, there are several schemes used for this purpose. Let us focus on each of them.

**Classical Approach**

Classical approach represents either the screening based on previously purified natural compounds or on activity-guided screening of crude extract mixture for active compounds. Further, active compounds are purified by activity-guided purification (Szajdak, 2016). This way of identification of antiviral compounds was implemented by Li et al. (2005). More than 200 Chinese medicinal herb extracts were screened for anti-SARS-CoV activities by assay for virus-induced cytopathic effect. The crude extracts of Lycoris radiata, Artemisia annua, Pyrosis lingua, and Linderea showed antiviral activity. The ethanolic extract of L. radiata was the most active. The majority of bioactive components of L. radiata belong to alkaloids that were confirmed by reversed phase high performance liquid chromatography (LC). Finally, lycorine was identified by LC-MS/MS as an effective anti-SARS-CoV component. Now, anti-SARS-CoV2 effect of the lycorine is also shown (Murck, 2020).

Often activity-guided screening indicates that plant fractions have higher antiviral activity than pure substances, because medicinal plants usually contain several biologically active compounds. For example, Kickxia elatine, contain flavonoid pectolinarin (Yuldashev et al., 1996) and iridoid glycosides with anti-inflammatory and antiviral activities (Handjeiva et al., 1993), and therefore, provide some synergistic. Besides, some compounds are able to prevent disease by various means. For example, pectolinarin efficiently blocks the enzymatic activity of Mpro and PLpro (Jo et al., 2020) and demonstrates anti-inflammatory activities (Ho et al., 2020).

**Structure-Activity-Relationship Approach**

Structure-activity-relationship is an approach for finding the relationships between the chemical structure (structural-related properties) and the biological activity of studied compounds. The need to streamline the drug development process has spawned the development of such strategies as “rational drug design,” where large-scale screening can be done through a database of potential candidate molecules to find those of interest (https://www.ebi.ac.uk/chembl, https://pubchem.ncbi.nlm.nih.gov/, Santos et al., 2016; Mumtaz et al., 2017). Medicinal plants contain wide range of secondary metabolites of different activity spectrums. Some of them are known to have antiviral and other properties, for others only structural data is available. The properties of many plants-derived substances remain unclear. These unknown properties can be predicted by computer modeling methods.
Topological indices are able to predict different activities and physicochemical characteristics such as boiling point, entropy, enthalpy, etc. Similarly, based on the topological properties of some chemical structures with antiviral activity, (for example remdesivir, chloroquine, hydroxchloroquine, and theaflavine), the antiviral activity of the new compounds can be predicted (Mondal et al., 2020).

Antiviral features of compounds can be determined by matching them as a ligand to known targets using molecular dynamics and docking simulations (Zhang et al., 2020a), Tallei et al. (2020) made a list of natural components with M3-protease inhibitor effect; among them are hesperidin, morine, rhoifolin, pectolinarin, and nabiximols. Binding interaction and ligand affinity of these compounds to M3 was the same as of nelfinavir, and even better than chloroquine and hydroxchloroquine sulfate, – recommended by the FDA as emergency anti-COVID-treatment anti-malarial drugs (Tallei et al., 2020), Quercetin-3-β-galactoside showed inhibitory activity against SARS-CoV Mpro in silico, via docking simulation, and also in enzymatic inhibition assays. Molecular modeling strongly suggested that the residue Q189 plays a key role, and it was confirmed by site-directed mutagenesis of the M3 (Chen et al., 2006). Molecular docking analysis of M3 and compounds of medicinal plants revealed such inhibiting substances as beta-eudesmol from Laurus nobilis, digitoxigenin from Nerium oleander, crocin from Crocus sativus (Aanouz et al., 2020), paviatannin-C1 and tenuifolin from cinnamon (Prasanth et al., 2020), catechins/polyphenols from green tea (Ghosh et al., 2021), withanoside V from Withania somnifera (Tripathi et al., 2020), and tinosponone from Tinospora cordifolia (Krupanidhi et al., 2020). Inhibitory effects of alkylated chalcones isolated from Angelica keiskei against the SARS-CoV proteases M3 and PL3 were found by Park et al. (2016).

Quercetin showed inhibitory activity against SARS-CoV PL3, although Papyriflavonol A was the most effective inhibitor of PL3 among those studied by Park et al. (2017). Virtual structure-based screening revealed that withanolide A, isocodonocarpine, and calonysterone bind to PL3 (Alamri et al., 2020).

Molecular docking analysis applied on the binding positions with S protein indicated that cannabinoids along with epigallocatechin gallate, herbacetin, hesperidene, pectolinarin, curcumin, and withanoside X hold remarkable binding sites, which could support them to be excellent S protein inhibitors, preventing viral attachment to host cells (Chikhale et al., 2020; Jena et al., 2020; Tallei et al., 2020).

Unfortunately, molecular docking does not consider the effect of selected substances on other key points of the disease and the cumulative effect of several biologically active compounds. However, concept of large-scale screening can be applied to identify new candidates for further analysis, shortening the search process and making it more powerful (Chen et al., 2006; Theerawatanasirikul et al., 2020).

The Data-Driven Approach

Repurposing of known drugs could significantly accelerate the deployment of novel therapies for COVID-19. The main difference of data-driven approach from the structure-activity-relationship approach is in use of databases of drugs, including phytochemical ones (https://www.drugbank.ca, http://drugcentral.org etc.). The identification of new areas of application for already known drugs saves time for testing their biosafety, but does not provide an opportunity to find fundamentally new substances that can be more effective than previously known drugs. The approach includes three steps:

1. Selection of drugs aimed to treat “Warm diseases,” “Pestilence,” or “Epidemic diseases.”
2. Identification of the active substances of plant origin, responsible for these pharmacology effects.
3. In silico assessment of the activities of selected drugs in relation to key proteins (Mpro, PLpro S protein, and ACE2) involved in the development of the disease.

According to this scheme, among the 96,606 formulations of Traditional Chinese Medicine, the 574 drugs were selected, and only 26 ones remained after the third stage of analysis. These biologically active substances from the licorice (Glycyrrhizae radix) and skullcaps (Scutellariae baicalensis) could also interact with the targets involving in immune and inflammation diseases (Ren et al., 2020).

Similar approach based on the ReFRAME (Repurposing, Focused Rescue, and Accelerated Medchem) library was utilized by Riva et al. (2020).

Immunomodulatory Effects of Herbs

As we can see from the examples mentioned above, extracts of many plants, and even specific compounds, often inhibit several SARS-CoV2 protein targets. Besides, identified compounds can possess a wide range of other pharmacological activities, including immunomodulatory effects. For example, Curcumin inhibits S protein (Jena et al., 2020) and also participates in regulation of immune and inflammatory response associated with coronavirus infections (Chen et al., 2020).

Moreover, plants often contain a number of compounds, with different activities that can complement each other. Thus, Cirsium japonicum, popular in traditional Chinese medicine, contains pectolinarin and pectolinarigenin, which have antiviral and immunomodulatory effects, respectively (Cheriet et al., 2020). Pectolinarigenin shows high suppressive immunomodulatory potency, including inhibitory activity of neutrophil phagocytes respiratory burst as well as T-cells proliferation (Erukainure et al., 2017).

Withania somnifera, a key plant of Ayurveda, contains compounds inhibiting M3 and S protein, (Chikhale et al., 2020; Tripathi et al., 2020) and the extract of the plant provides anti-viral immunity by increasing interferon gamma responses and anti-inflammatory activities by decreasing the quantity of interleukin-1, interleukin-6 and Tumor necrosis factor related to COVID-19 (Niraj and Varsha, 2020).

Tinosponone from Tinospora cordifolia inhibits M3 (Krupanidhi et al., 2020), and the aqueous extracts of the plant affects the cytokine production and activation of immune effector cells (Niraj and Varsha, 2020).

The immune response against coronavirus is vital to control and get rid of the infection. In the ideal situation, the antiviral
drug should check the infection while the immune system prepares to destroy the last virus particles (Mukherjee, 2019). From other hand, maladjusted immune responses may lead to the immunopathology of the disease, resulting in impairment of pulmonary gas exchange (Dhamar et al., 2020). Thus, a fine selection of herbal immunomodulators is required for the treatment of different stages of the diseases and different degrees of its severity.

GENETIC ENGINEERING FOR ANALYSIS OF ANTI-SARS-Cov2 ACTIVITY

Genetic engineering methods allow to optimize the evaluation of anti-SARS activity of compounds at different stages of research. First of all, they include obtaining of recombinant target proteins for searching for antiviral drugs.

The recombinant proteases M<sup>rm</sup> and PL<sup>rm</sup> can be easily expressed in *Escherichia coli* or other organism, purified by routine biochemical methods (Lin et al., 2004, 2005) and used for cell-free cleavage assay (Lin et al., 2005; Chou et al., 2008; Chen et al., 2009; Jo et al., 2020) or cell-based cleavage assay (Lin et al., 2005), as well as for study of x-ray structures of the unliganded SARS-CoV-2 proteins and their complexes with potential drugs (Zhang et al., 2020b).

Cell-free cleavage assay involves the use of a purified enzyme and a substrate, modified at the C and N terminus. Depending on the type of modification (protein tag or fluorescent group), further Enzyme Linked Immunosorbert Assay (ELISA) or protein based fluorogenic assay are used to assess cell-free proteolytic activity and its inhibition by different compounds (Kuo et al., 2004; Lin et al., 2005).

The cell-based cleavage assay does not require purification of the active protease, and represents closely the natural physiological state. Investigating the *Isatis indigotica* phenolic compounds as potential anti-SARS drugs, Lin et al. (2005) have made in-frame construction, containing the M<sup>rm</sup>, the substrate, and the luciferase, and transformed it into Vero cells. Since a more than 30 kDa protein fused at the N-terminus of the luciferase resulted in a dramatic decrease of luciferase activity, the detection of activity of luciferase was considered by authors as a measure for the cis-cleavage by M<sup>rm</sup>. Epigallocatechin gallate abundant in green tea (*Camellia sinensis*), inhibits the proteolytic activity of SARS-CoV M<sup>rm</sup>, expressed in *Pichia pastoris* (Nguyen et al., 2012).

In addition, approaches described above, demonstrated that tanshinones of *Salvia miltiorrhiza* (Park et al., 2012b), diarylheptanoids from *Ahus japonica* (Park et al., 2012a), and geranylated flavonoids from the *Paulownia tomentosa* tree (Cho et al., 2013) are inhibitors of SARS-CoV PL<sup>rm</sup> activity. M<sup>rm</sup> proteases of SARS-CoV and SARS-CoV-2 are inhibited by such compounds as herbacetin, rhoifolin, pectolinarin (Jo et al., 2020) hesperetin, sinigrin (Lin et al., 2005) quercetin-3-β-galactoside (Chen et al., 2006) etc.

Thus, at the initial stages, the assessment of the antiviral activity of drugs does not require the use of the virus itself. This makes it possible to significantly expand the number of laboratories where it is permissible to conduct such studies and select the best compounds for the next stages of testing.

Genetic engineering technologies are also applicable at the stage of evaluating the therapeutic effect of drugs using preclinical animal models. Several animal models, from mice to and non-human primates, have been shown to be susceptible to SARS-CoV infection. From one hand, primates are closest to humans; the clinical picture of their disease is almost the same as in humans. From other hand, they are much more expensive than mice. Therefore, drugs are initially tested on mice (Bevernakoppamath et al., 2020; Lutz et al., 2020). Unfortunately, due to structural differences in mouse ACE2 compared to human ACE2 proteins, the SARS coronaviruses exhibit poor tropism characteristics for mouse tissues. So, the wild-type lines are not optimal for studying infections of the newly discovered coronavirus. Several transgenic mice strains, carrying hACE2 under control of different promoters, including human cytokinin 18 (K18) promoter, composite CAG promoter consisting of the cytomegalovirus immediate early enhancer, the chicken β-actin promoter and rabbit globulin splicing and polyadenylation sites, HFH4 lung ciliated epithelial cell-specific promoter, were developed in 2006–2016 to study SARS-CoV infection (McCray et al., 2007; Tseng et al., 2007; Menachery et al., 2016). It was shown that the hACE2 transgenic mice infected with a human SARS-CoV strain via intranasal inoculation demonstrate the symptoms observed in infected human patients. These hACE2 transgenic mice can provide significant findings to support the development of COVID-19 therapeutics (Lutz et al., 2020).

TAXONOMIC STUDIES AND GENOME SEQUENCING SEARCHING FOR BETTER PRODUCERS OF PHYTOCHEMICAL DRUGS

For a more efficient search for better plant producers of specific secondary metabolites and their combinations, it makes sense to use the concept of plant chemotaxonomy – a branch of the science of taxonomy, were plants are classified depending on the similarities and differences in the spectrum of their secondary metabolites. Since some chemicals can be found in nature only in organisms of certain genera, families, or orders, this can be used both for their classification and to search for certain metabolites in the related species (Zidorn, 2019).

Whole genome data in its turn led to insights into biosynthesis pathways (Denoeud et al., 2014). The list of species with sequenced genomes is growing, collaborations are emerging to join efforts and collect more data. The Medicinal Plant Genomic Resource (MPRG)<sup>1</sup> is a domain-specific database created by consortium efforts to collect deep DNA sequencing, RNA sequencing, and metabolomics data. In general, genome sequencing is used to discover new candidates and to elucidate biosynthesis pathways to prepare data sets of new molecules for further analysis. One can predict the presence of a compound

---

<sup>1</sup>http://medicinalplantgenomics.msu.edu
among plant metabolites if gene involved in the biosynthesis of the compound in question was discovered in the plant genome. For example, cardenolides discovered in the Calotropis gigantea genome were historically used to cure pneumonia and as an anti-inflammatory, antitumor, and antimalaria (Hoopes et al., 2018; Boone et al., 2020), and they also can be easily converted to mappicine ketone, an antiviral led compound (Das et al., 1998). Genes to biosynthesize curcumin were found in Oryza sativa genome (Katsuyama et al., 2007). Recently conducted chromosome-scale genome assembly of I. indigotica assisted to reveal new candidate genes for the biosynthesis of several groups of active compounds in this medicinal plant (Kang et al., 2020). Phenolic compounds of I. indigotica have already shown activity against the M' (Lin et al., 2005), and indole alkaloids in this plant demonstrated inhibition of HSV-2 reproduction (Sun et al., 2010). Besides, cultivars of the same species can differ in the amount of the medicinal compounds (Hisashi and Saito, 2013; Kajikawa et al., 2017). Complete genome data allow to identify gene clusters for secondary metabolism, which opens the way to metabolic engineering. For example, whole-genome sequencing can reveal huge number of "silent" gene clusters which can be learned to activate to run currently non-working biosynthetic pathways (Osbourn, 2010).

Thus, in the nearest future chemotaxonomy and genomic data will allow to improve the procedure for obtaining phytochemical drugs.

CONCLUSION

In this short article, we have tried to present various methods of searching for plant compounds with anti-coronavirus activities, as well as approaches that make it possible to accelerate this search. Chemi-informatics methods provide opportunities for primary screening of large amounts of data to find candidate compounds. Genetic engineering methods make it possible to assess the interaction of candidate compounds with their targets in cell-free systems and in cell culture without resorting to the use of viruses, which significantly expands the list of laboratories for such research. Combining these approaches allows for more accurate study of targeting of potential drugs for subsequent trials. Acceleration of the preclinical phase of new drugs testing can be achieved through the use of transgenic animal models.

Analysis of pharmacological databases allows quickly selection of "candidates," already having assessments of their biosafety and stability, but it is unlikely that a "breakthrough" drug will be obtained at the exit. However, these approaches buy time to treat people while longer studies are still pending.

The "hunt" for new substances makes it possible to find substances with new mechanisms of action, reveal new functional groups, etc. The most effective drugs may be among them. However, this path is the longest, since it will require the most complete study of its pharmacological properties, including toxicity, side effects, stability, etc. Therefore, rapid screening systems for target activities are extremely important.

The analysis of a mixture of compounds in plant extracts seems to us the most promising. The development of metabolomics methods based on a small amount of plant material allows to obtain data on the entire spectrum of substances in the sample under study, containing several compounds with antiviral activity.

In the future, our knowledge of the structure of plant genomes will allow to obtaining the most effective producers of anti-coronavirus compounds by metabolic engineering methods.

AUTHOR CONTRIBUTIONS

TM wrote the basic structure of the paper. GK and SS participated in writing and correcting the paper. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by grant from St. Petersburg State University (id62228593).

REFERENCES

Aanouz, I., Belhassan, A., El-Khatibi, K., Lahlifi, T., El-Ldrissi, M., and Bouachrine, M. (2020). Moroccan medicinal plants as inhibitors against SARS-CoV-2 main protease: computational investigations. J. Biomol. Struct. Dyn. 1-9. doi: 10.1080/07391102.2020.1758790 [Epub ahead of print]

Adejedi, A. O., and Sarafianos, S. (2014). Antiviral drugs specific for coronaviruses in preclinical development. Curr. Opin. Virol. 8, 45–53. doi: 10.1016/j.coviro.2014.06.002

Alamri, M. A., Altharawi, A., Alabbas, A. B., Alassim, M. A., and Alqahtani, S. M. (2020). Structure-based virtual screening and molecular dynamics of phytochemicals derived from Saudi medicinal plants to identify potential COVID-19 therapeutics. ChemRxiv [Preprint]. doi: 10.26434/chemrxiv.12336635.v1

Anand, K., Ziebuhr, J., Wadhwani, P., Mesters, J. R., and Hilgenfeld, R. (2003). Coronavirus main protease (3CLpro) structure: basis for design of anti-SARS drugs. Science 300, 1763–1767. doi: 10.1126/science.1085658

Báez-Santos, Y. M., St John, S. E., and Mesecar, A. D. (2015). The SARS-coronavirus papain-like protease: structure, function and inhibition by designed antiviral compounds. Antivir. Res. 115, 21–38. doi: 10.1016/j.antiviral.2014.12.015

Boone, H. A., Medunjanin, D., and Sijerčić, A. (2020). Review on potential of phytotherapeutics in fight against COVID-19. Int. J. Innov. Sci. Res. Technol. 5, 481–491.

Butcher, E. C., Berg, E. L., and Kunkel, E. J. (2004). Systems biology in drug discovery. Nat. Biotechnol. 22, 1253–1259. doi: 10.1038/nbt1017

Carter, G. T. (2011). Natural products and Pharma 2011: strategic changes spur new opportunities. Nat. Prod. Rep. 28, 1783–1789. doi: 10.1039/c1npr00033k

Chen, X., Chou, C. Y., and Chang, G. G. (2009). Thiopurine analogues inhibitors of severe acute respiratory syndrome-coronavirus papain-like protease, a deubiquitinating and deISGylating enzyme. Antivir. Chem. Chemother. 19, 151–156. doi: 10.1177/095632020901900402

Chen, L., Hu, C., Hood, M., Zhang, X., Zhang, L., Kan, J., et al. (2020). Novel combination of vitamin C, curcumin and glycyr rhiza acid potentially regulates immune and inflammatory response associated with coronavirus infections: a perspective from system biology analysis. Nutrients 12:1193. doi: 10.3390/nu12041193

Bevinakoppamath, S., Ramachandra, S. C., and Akila, P. (2020). An insight into the use of transgenic animal models for conducting research on coronavirus. Int. J. Health Allied Sci. 9, 18–23. doi: 10.4103/ijhas.ijHAS_86_20

Bevinakoppamath, S., Ramachandra, S. C., and Akila, P. (2020). Review on potential of phytotherapeutics in fight against COVID-19. Int. J. Innov. Sci. Res. Technol. 5, 481–491.

Hajek, A., A., and Sarafianos, S. (2014). Antiviral drugs specific for coronaviruses in preclinical development. Curr. Opin. Virol. 8, 45–53. doi: 10.1016/j.coviro.2014.06.002

Kajikawa, M., et al. (2017). Complete genome data allow to identify gene clusters for secondary metabolism, which opens the way to metabolic engineering. For example, whole-genome sequencing can reveal huge number of “silent” gene clusters which can be learned to activate to run currently non-working biosynthetic pathways (Osbourne, 2010).

Matveeva et al. In Search of Herbal Anti-SARS-Cov2 Compounds

November 2020 | Volume 11 | Article 589998

Frontiers in Plant Science | www.frontiersin.org 5

Matveeva et al. In Search of Herbal Anti-SARS-Cov2 Compounds

November 2020 | Volume 11 | Article 589998

Frontiers in Plant Science | www.frontiersin.org 5

Bevinakoppamath, S., Ramachandra, S. C., and Akila, P. (2020). An insight into the use of transgenic animal models for conducting research on coronavirus. Int. J. Health Allied Sci. 9, 18–23. doi: 10.4103/ijhas.ijHAS_86_20

Boone, H. A., Medunjanin, D., and Sijerčić, A. (2020). Review on potential of phytotherapeutics in fight against COVID-19. Int. J. Innov. Sci. Res. Technol. 5, 481–491.

Butcher, E. C., Berg, E. L., and Kunkel, E. J. (2004). Systems biology in drug discovery. Nat. Biotechnol. 22, 1253–1259. doi: 10.1038/nbt1017

Carter, G. T. (2011). Natural products and Pharma 2011: strategic changes spur new opportunities. Nat. Prod. Rep. 28, 1783–1789. doi: 10.1039/c1npr00033k

Chen, X., Chou, C. Y., and Chang, G. G. (2009). Thiopurine analogues inhibitors of severe acute respiratory syndrome-coronavirus papain-like protease, a deubiquitinating and deISGylating enzyme. Antivir. Chem. Chemother. 19, 151–156. doi: 10.1177/095632020901900402

Chen, L., Hu, C., Hood, M., Zhang, X., Zhang, L., Kan, J., et al. (2020). Novel combination of vitamin C, curcumin and glycyr rhiza acid potentially regulates immune and inflammatory response associated with coronavirus infections: a perspective from system biology analysis. Nutrients 12:1193. doi: 10.3390/nu12041193
Chen, L., Li, J., Luo, C., Liu, H., Xu, W., Chen, G., et al. (2006). Binding interaction of quercetin-3-β-galactoside and its synthetic derivatives with SARS-CoV 3CL\textsubscript{pro}: structure–activity relationship studies reveal salient pharmacophore features. *Bioorg. Med. Chem.* 14, 8285–8306. doi: 10.1016/j.bmc.2006.09.014

Cheriet, T., Ben-Bachir, B., Thamri, O., Seghiri, R., and Mancini, I. (2020). Isolation and biological properties of the natural flavonoids pectolinarin and pectolinarigenin—a review. *Antibiotics* 9:417. doi: 10.3390/antibiotics9070417

Chikhale, R. V., Gurav, S. S., Patil, R. B., Sinha, S. K., Prasad, S. K., Shabka, A., et al. (2020). SARS-CoV-2 host entry and replication inhibitors from Indian ginseng: an in-silico approach. *J. Biomol. Struct. Dyn.* 1–12. doi: 10.1080/07391102.2020.1778539 [Epub ahead of print]

Cho, J. K., Curtis-Long, M. J., Lee, K. H., Ryu, H. W., Yuk, H. J., et al. (2013). Geranylated flavonoids displaying SARS-CoV papain-like protease inhibition from the fruits of *Paulownia tomentosa*. *Bioorg. Med. Chem.* 21, 3051–3057. doi: 10.1016/j.bmc.2013.03.027

Chou, C. Y., Chien, C. H., Han, Y. S., Frebhanda, M. T., Hsieh, H. P., Turk, B., et al. (2008). Thiopurine analogues inhibit papain-like protease of severe acute respiratory syndrome coronavirus. *Biochem. Pharmacol.* 75, 1601–1609. doi: 10.1016/j.bcp.2008.01.005

Clemente, V., D’Arcy, P., and Bazzaro, M. (2020). Deubiquitinating enzymes in coronaviruses and possible therapeutic opportunities for COVID-19. *Int. J. Mol. Sci.* 21:3492. doi: 10.3390/ijms2103492

Coutard, B., Valle, C., De Lamballerie, X., Canard, B., Seidah, N., and Decroly, E. (2004). Characterization of trans- and cis-cleavage activity of the SARS coronavirus *S* protein and ACE2 of human cell membrane: insights from computational study and implication for research. *Sci. Rep.* 4:20. doi: 10.1186/s13007-014-0191-7

Denoeud, F., Carretero-Paulet, L., Dereeper, A., Droc, G., Guyot, R., Pietrella, M., et al. (2004). Identification of natural compounds with antiviral activities against SARS-associated coronavirus. *Antivir. Res.* 67, 18–23. doi: 10.1016/j.antiviral.2005.02.007

Dhama, K., Karthik, K., Khandia, R., Munjal, A., Tiwari, R., Rana, R., et al. (2020). Inhibition of SARS-CoV-2 host entry and replication inhibitors from Indian medicinal plants. *FEBS Lett.* 594, 4284–4290. doi: 10.1016/j.febslet.2020.05.017

Dhama, K., Kaur, G., and Newman, D. J. (2013). Natural products: a continuing source of novel drug leads. *Biochim. Biophys. Acta* 1830, 3670–3695. doi: 10.1016/j.bbadeng.2013.02.008

Das, B., Madhusudhan, P., and Kashinatham, A. (1998). Two efficient methods for the conversion of camptothecin to mappicine ketone, an antiviral lead compound. *Tetrahedron Lett.* 39, 431–432. doi: 10.1016/S0040-4039(97)01539-1

Demou, F., Carretero-Paulet, L., Dereeper, A., Droc, G., Guyot, R., Pietrella, M., et al. (2014). The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antivir. Res.* 176:104742. doi: 10.1016/j.antiviral.2020.104742

Ghosh, R., Chakraborty, A., Biswas, A., and Chowdhuri, S. (2020). Evaluation of green tea polyphenols as novel corona virus (SARS CoV-2) main protease inhibitors—an in silico docking and molecular dynamics simulation study. *J. Biomol. Struct. Dyn.* 1–10. doi: 10.1080/07391102.2020.1779818 [Epub ahead of print]

Ghilgenfeld, R. (2014). From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design. *FEBS J.* 281, 4085–4096. doi: 10.1111/feb.12936

Hisashi, Y., and Saito, K. (2013). Network analysis for gene discovery in plant-identified metabolism. *Plant Cell Environ.* 36, 1597–1606. doi: 10.1111/pce.12069

Ho, K. V., Schreiber, K. L., Park, J., Vo, P. H., Lei, Z., Sumner, L. W., et al. (2020). Identification and quantification of bioactive molecules inhibiting pro-inflammatory cytokine production in spent coffee grounds using metabolomics analyses. *Front. Pharmacol.* 11:229. doi: 10.3389/fphar.2020.00229

Hoopes, G. M., Hamilton, J. P., Kim, J., Zhao, D., Wiegert-Rinegge, K., Crisovan, E., et al. (2018). Genome assembly and annotation of the medicinal plant *Calotropis gigantea*, a producer of anticancer and antimalarial cardenolides. *G3: GENES, GENOMICS, GENETICS* 8, 385–391. doi: 10.1534/g3.117.300331

Huang, J., Song, W., Huang, H., and Sun, Q. (2020). Pharmacological therapeutics targeting RNA-dependent RNA polymerase, proteinase and spike protein: from mechanistic studies to clinical trials for COVID-19. *J. Clin. Med.* 9:1131. doi: 10.3390/jcm9041131

Ijikawa, M., Kawaguchi, H., Bakaher, N., Ivanov, N. V., Hashimoto, T., et al. (2017). Identification of natural compounds with antiviral activities against SARS-associated coronavirus. *Antivir. Res.* 140, 117–125. doi: 10.1016/j.antiviral.2017.02.011

Islam, M. S. (2017). Pectolinarigenin from the leaves of Clerodendrum trichotomum—an immunomodulatory compound. *Fitoterapia* 114, 39–43. doi: 10.1016/j.fitote.2017.01.008

Krupanidhi, S., Abraham Peele, K., Venkateswarulu, T. C., Ayyagari, V. S., and Islam, M. S. (2017). Pectolinarigenin from the leaves of Clerodendrum trichotomum—an immunomodulatory compound. *Fitoterapia* 114, 39–43. doi: 10.1016/j.fitote.2017.01.008

Kuo, C. J., Chiu, Y. H., Hsu, J. T., and Liang, P. H. (2004). Characterization of SARS main protease and inhibitor assay using a fluorogenic substrate. *Biochem. Biophys. Res. Commun.* 318, 862–867. doi: 10.1016/j.bbrc.2004.04.098

Lai, C. -W., Tsai, C. -H., Tsai, F. -J., Chen, P. J., Lai, C. -C., Wan, L., et al. (2004). Identification of natural compounds with antiviral activities against SARS-associated coronavirus. *Antivir. Res.* 67, 18–23. doi: 10.1016/j.antiviral.2005.02.007

Liu, M. H., Moses, D. C., Hsieh, C. H., Cheng, S. C., Chen, Y. H., Sun, C. Y., et al. (2018). Disulfram can inhibit MERS and SARS coronavirus papain-like proteases via different modes. *Antivir. Res.* 150, 155–163. doi: 10.1016/j.antiviral.2017.12.015

Lin, C.-W., Tsai, C.-H., Tsai, F.-J., Chen, P.-I., Lai, C.-C., Wan, L., et al. (2004). Identification of natural compounds with antiviral activities against SARS-associated coronavirus. *Antivir. Res.* 67, 18–23. doi: 10.1016/j.antiviral.2005.02.007

Lutz, C., Maher, L., Lee, C., and Kang, W. (2020). COVID-19 preclinical models: human angiotensin-converting enzyme 2 transgenic mice. *Hum. Genomics* 14:20. doi: 10.1186/s40246-020-00272-6

Mani, J. S., Johnson, J. B., Steel, J. C., Broszczak, D. A., Neilsen, P. M., Walsh, K. B., et al. (2020). Natural product-derived phytochemicals as potential agents against coronaviruses: a review. *Viruses* 8, 197989. doi: 10.3390/viruses820197989

McCray, P. B. Jr., Pewe, L., Wohlford-Lenane, C., Hickey, M., Manzel, L., Shi, L., et al. (2007). Lethal infection of K18-HACE2 mice infected with...
severe acute respiratory syndrome coronavirus. *J. Virol.* 81, 813–821. doi: 10.1128/JVI.02012-06

Menachery, V. D., Yount, B. L. Jr., Sims, A. C., Debink, K., Agnihothram, S. S., Gralinski, L. E., et al. (2016). SARS-like WIV1-CoV poised for human emergence. *Proc. Natl. Acad. Sci. U. S. A.* 113, 3048–3053. doi: 10.1073/pnas.1517191113

Mondal, S., De, N., and Pal, A. (2020). Topological indices of some chemical compounds applied for the treatment of COVID-19 patients. *Pycycol. Aromat. Compd.* 1–15. doi: 10.1007/s10466.2020.1770306

Mukherjee, P. K. (2019). “Antiviral evaluation of herbal drugs” in Quality control and evaluation of herbal drugs. ed. K. Morrissey (Cambridge, MA, USA: Elsevier), 599–628.

Muntaz, A., Ashfaq, U. A., ul Qamar, M. T., Anwar, F., Gulzar, F., Ali, M. A., et al. (2017). MPD3: a useful medicinal plants database for drug design. *Nat. Prod. Res.* 31, 1228–1236. doi: 10.1080/14786419.2016.1233409

Murck, H. (2020). Symptomatic protective action of glycyrrhizin (Licorice) in COVID-19 infection? *Front. Immunol.* 11:1239. doi: 10.3389/fimmu.2020.01239

Nguyen, T. T. H., Woo, H. -J., Kang, H. -K., Nguyen, V. D., Kim, D. -W., Ahn, S. -A., et al. (2012). Flavonon-mediated inhibition of SARS CoV 3C-like protease expressed in *Pichia pastoris*. *Biotechnol. Lett.* 34, 831–838. doi: 10.1007/s10529-011-0845-8

Niraj, S., and Varsha, S. (2020). A review on scope of immuno-modulatory drugs in Ayurveda for prevention and treatment of COVID-19. *Plant Sci.* Today 7, 417–423. doi: 10.14719/pst.2020.7.3.831

Osbourne, A. (2010). Secondary metabolic gene clusters: evolutionary tools for chemical innovation. *Trends Genet.* 26, 449–457. doi: 10.1016/j.tig.2010.07.001

Park, J. Y., Jeong, H. I., Kim, J. H., Kim, Y. M., Park, S. J., Kim, D., et al. (2012a). Diarylheptanoids from *Alnus japonica* inhibit papain-like protease of severe acute respiratory syndrome coronavirus. *Biol. Pharm. Bull.* 35, 2036–2042. doi: 10.1248/bpb.b12-00623

Park, J. Y., Kim, J. H., Kim, Y. M., Jeong, H. I., Kim, D. W., Park, K. H., et al. (2012b). Tanshinones as selective and slow-binding inhibitors for SARS-CoV cysteine proteases. *Bioorg. Med. Chem.* 20, 5928–5935. doi: 10.1016/j.bmc.2012.07.038

Prasanth, D. S. N. B. K., Murahari, M., Chandramohan, V., Panda, S. P., Niraj, S., and Varsha, S. (2020). A review on scope of immuno-modulatory drugs in Ayurveda for prevention and treatment of COVID-19. *Plant Sci.* Today 7, 417–423. doi: 10.14719/pst.2020.7.3.831

Ren, X., Shao, X. X., Li, X. H., Song, T., Zhou, W. Y., et al. (2020). Identification of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. *Cell. Mol. Immunol.* 17, 613–620. doi: 10.1038/s41423-020-0400-4

Tai, W., He, L., Zhang, X., Pu, J., Voronin, D., Jiang, S., et al. (2020). Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. *Cell. Mol. Immunol.* 17, 613–620. doi: 10.1038/s41423-020-0400-4

Tallei, T. E., Tumlaza, S. G., Niede, N. J., Fatimawati, F., Kepel, B. J., Idroes, R., et al. (2020). Potential of plant bioactive compounds as SARS-CoV-2 main protease (Mpro) and spike (S) glycoprotein inhibitors: a molecular docking study. Preprints [Preprint], 202004102. doi: 10.20944/preprints202004.0102v1

Thereawatanasirisuk, S., Kuo, C. J., Phetcharata, N., and Lekcharoensuk, P. (2020). In silico and in vitro analysis of small molecules and natural compounds targeting the 3CL protease of feline infectiousperitonitis virus. *Antivir. Res.* 174:104697. doi: 10.1016/j.antiviral.2019.104697

Tripathi, M. K., Singh, P., Sharma, S., Singh, T. P., Ethyathulla, A. S., and Kaur, P. (2020). Identification of bioactive molecule from *Withania somnifera* (Ashwagandha) as SARS-CoV-2 main protease inhibitor. *J. Biomol. Struct. Dyn.* 1–14. doi: 10.1080/07391102.2020.1790425. [Epub ahead of print]

Tseng, C. T., Huang, C., Newman, P., Wang, N., Narayanan, K. W., Watts, D. M., et al. (2007). Severe acute respiratory syndrome coronavirus infection of mice transgenic for the human angiotensin-converting enzyme 2 virus receptor. *J. Virol.* 81, 1162–1173. doi: 10.1128/JVI.01702-06

Vakh, D., Dorp, L., Acrman, M., Richard, D., Shaw, L. P., Ford, C. E., Ormond, L., et al. (2020). Emergence of genomic diversity and recurrent mutations in SARS-CoV-2. *Infect. Genet. Evol.* 83:104351. doi: 10.1016/j.meegid.2020.104351

Vellingiri, B., Jayaramayya, K., Iyer, M., Narayanasamy, A., Govindasamy, V., Girdharan, B., et al. (2020). COVID-19: a promising cure for the global panic. *Sci. Total Environ.* 725:138277. doi: 10.1016/j.scitotenv.2020.138277

World Health Organization (WHO) (2020). Coronavirus disease (COVID-19) situation reports. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports (Accessed October 19, 2020).

Wang, T., Zhang, X., Wang, L., Wang, Y., et al. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 367, 1260–1263. doi: 10.1126/science.abb2507

Xu, X., Chen, P., Wang, J., Feng, J., Zhou, H., Li, X., et al. (2020). Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci. China Life Sci.* 63, 457–460. doi: 10.1007/s11427-020-1637-5

Yildashev, M. P., Malikov, V. M., and Batirov, É. K. (1996). Flavonoids of the epigonal part of *Kuckisia elatine*. *Chem. Nat. Compd.* 32, 30–52. doi: 10.1007/BF01373784

Zhang, Y. -Z., and Holmes, E. C. (2020). Commentary a genomic perspective on the origin and emergence of SARS-CoV-2. *Cell* 181, 223–227. doi: 10.1016/j.cell.2020.03.035

Zhang, L., Lin, D., Sun, X., Curth, U., Drosten, C., Sauerhering, L., et al. (2020b). Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved o-ketoidamide inhibitors. *Science* 368, 409–412. doi: 10.1126/science.abb3405

Zhang, D. H., Wu, K. L., Zhang, X., Deng, S. Q., and Peng, B. (2020a). In silico screening of Chinese herbal medicines with the potential to directly inhibit novel 2019 novel coronavirus. *J. Integr. Med.* 18, 152–158. doi: 10.1007/j.1679-6954.2020.02.005

Zidorn, C. (2019). Plant chemophenetics—a new term for plant chemosystematics/plant chemotaxonomy in the macro-molecular era. *Phytochemistry* 163, 147–148. doi: 10.1016/j.phytochem.2019.02.013

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.