Therapeutic Approach Against 2019-nCoV by Inhibition of the ACE-2 receptor

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ABSTRACT
The continued spread of the 2019 novel coronavirus (2019-nCoV) has prompted global concern. The formal name given to 2019-nCoV by the World Health Organization is COVID-19, while the International Committee on Taxonomy has named it severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Due to this viral attack, nations around the world have issued lockdown restrictions. Presently, there is no effective way to control the spread of 2019-nCoV, except through social distancing and hygienic activities. World-class scientists and researchers are trying to develop vaccines and medicines that will cure this deadly viral disease and control its spread. Our aim in presenting this article is to provide an easy therapeutic approach that effectively combats deadly viral diseases, such as COVID-19, with minimal intervention and effort. Different Ayurvedic therapeutic agents (Curcuma longa L, green tea, and Piper nigrum) inhibit the entry of viruses in the host cell and the transmission of pathogens, while improving immunity. Curcumin and piperine (1-piperoylpiperidine) interact with each other and form a π-π intermolecular complex that enhances the bioavailability of curcumin by inhibition of glucuronidation of curcumin in the liver. Two molecules, curcumin and catechin, bind directly to the receptor-binding domain of the S-protein and the angiotensin-converting enzyme 2 receptor of the host cell, by which these molecules inhibit the entry of viruses in the host cell. As a result, the animal host will survive the infection.

Introduction
In 1912, German veterinarians were puzzled over the case of a feverish cat with extreme superfluous abdomen. That is the first reported case that elucidates the paradigm power of the coronavirus, although veterinarians did not understand the cause at the time. It was nearly two decades later, in 1930, when the coronavirus was causing bronchitis in chickens and intestinal disease in pigs resulting in a high death rate among piglets under two weeks old, the disease was identified for the first time as animal coronavirus. Until the 1960s, the link of these pathogens between animals and humans remained obscure. In 1960, scientists in the United Kingdom and United States isolated two viruses with corona-like (i.e., crown-like) morphology that were responsible for causing common colds in humans. The name coronavirus, which was coined in 1968, was derived from the virus’ resemblance to the solar corona due to its crown-like structure, which could be observed under an electron microscope for the entire group of coronavirus viruses. The sequence of a killer virus may be as follows: dog harms cats, the cat...
targets pig intestines. The emergence of severe acute respiratory syndrome (SARS) in 2003 was caused by a new virus that belongs to the coronavirus family. Since then, SARS has become a lethal disease of humans and due to its worldwide spread, it has been declared a pandemic; however, before the SARS outbreak, coronaviruses were believed to cause only mild symptoms in humans [1]. Today, as mutant strains of the coronavirus family, such as COVID-19, have been produced, whether by natural phenomenon or in a laboratory, and the rapid spread has led to a high death toll. On November 17, 2019, a 55-year-old patient from Hubei province became the first known case of COVID-19, presenting with a novel SARS that was then transmitted worldwide [2]. Dr. Jixian Zhang, who is credited with first identifying the COVID-19 coronavirus, has defended China’s response to the outbreak in an interview with state television on December 27, 2019, amid international accusations that officials initially tried to conceal the crisis. After isolation and identification, this pathogen was originally called the 2019 novel corona virus (2019-nCoV) [3] but has since received the official name severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), given by the World Health Organization (WHO). On February 11, 2020, the WHO announced a new name for the novel coronavirus disease, COVID-19. On the same day, the coronavirus study group of the International Committee on Taxonomy of Viruses named 2019-nCoV as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). From February 2020 to September 25, 2020, there were 32,110,656 confirmed cases and 980,011 deaths reported for COVID-19 globally [4] (A comparative study using a different time interval is reported in ▶ Fig. 1). According to the globally available data, COVID-19 caused by the 2019-nCoV virus is a killing machine [5].

Possible ways to inhibit transmission: Per the discussion above, the urgency of the COVID-19 outbreak has led to empiric use of broad-spectrum antibiotics and antiviral agents, some of which are clinically approved and some that are not, in affected patients in several countries. There are four general approaches to cure and control this pandemic disease caused by SARS-CoV-2: 1. direct antiviral effects, 2. inhibition of viral entry and replication at the cellular level by targeting virus-related processes, 3. enhancement of host immune response, and 4. social distancing, hygienic activities, and complete lockdown [6]. Currently, there are no antiviral agents of SARS-CoV-2 available in the market. Scientists have been facing a big challenge of discovering a possible treatment that would save lives. The discovery of a vaccine for future prevention of the virus, social distancing, and complete lockdown are the only ways to slow down transmission of the virus. Social distancing and lockdown are not effective solutions for defeating the virus, and it is not possible to remain under lockdown for an extended period of time, due to human lifestyle and economical aspects. With this perspective, our aim is to introduce an easy and fast therapeutic approach to cure and control the disease by triple action, i.e., masking/inhibition of the spike protein receptor-binding domain (RBD) of the virus, angiotensin-converting enzyme 2 (ACE-2) receptor, and TMPRSS-2 coenzyme of the host cell against this pandemic disease using an ancient Ayurvedic remedy. Along with a theoretical scientific approach without any failure of inhibition of the viral entry and replication at the cellular level, virus-related processes are targeted and host immunity is enhanced. We strongly recommended a therapy that would cure and control COVID-19, not only in India but all over the globe.

▶ Fig. 1 Comparative study of the total number of patients, total number of deaths, increase in the number of patients, and increase in the number of deaths at different time intervals.
Ancient Ayurvedic Therapeutic Agents

Curcuma longa L. (Turmeric)

Indian traditions hold the belief that turmeric is a divine plant that was given to human beings by God. Therefore, turmeric has always been part of Vedic rituals in India. Sun-dried powder of Curcuma longa L. (Indian name halide) is a good source of curcumin [7]. Curcumin and its derivatives have been demonstrated to be effective inhibitors of triple target receptors that arrest the infection of SARS-CoV-2, ACE-2 receptor and TMPRSS-2 of host cells, and the spike protein of the virus. Curcumin and dimethoxy curcumin potently inhibited the activity of aminopeptidase N (APN)/CD13. Curcumin has enough binding energy for each receptor and strong inhibitory activities against COVID-19. Therefore, it can be used as a potential therapeutic agent against COVID-19 patients without adverse side effects.

Black pepper (Piper nigrum)

Black pepper is used as a medicine to treat digestive tract and respiratory-tract-related diseases caused by viral infections such as acute respiratory infections, asthma, chronic indigestion, and fever [8]. Black pepper is a good source of piperine (1-piperoylpiperidine). Piperine via oral supplementation increases the plasma levels of coenzyme Q10. Black pepper increases enzymatic activity, decreases lipid peroxidation, antioxidants, provides enhanced bioavailability, exerts an immunomodulatory effect, improves WBC count, and inhibits adipogenesis [9]. The bioavailability effects of piperine increase the levels of curcumin and catechin in plasma and play an efficient role in inhibiting the entry of 2019-nCoV in cells.

Green tea

Green tea contains a large number of compounds that are said to be beneficial to human health, such as catechins, caffeine, theanine, vitamins (vitamin C, vitamin B6, folic acid, β-carotene, vitamin E), saponins, fluorene, γ-aminobutyric acid, and chlorophyll. Catechins are a type of polyphenol and are the main astringency components in green tea. Catechin was first isolated from the Indian plant extract catechu. Catechin has a dual binding affinity, i.e., the spike protein of the RBD of the virus and the ACE-2 receptor of the target cell. Catechin shows a potential inhibitory effect on COVID-19. The piperine component of black pepper has shown a bioavailability enhancer effect and increases the catechin concentration in the human body and tissues, achieving distribution with a concomitant effect by inhibiting glucuronidation and gastrointestinal transit. Hence, both of them in combined form can serve as a potential therapeutic agent against COVID-19 disease.

Recipe for Treating COVID-19

COVID-19 patients may be treated with an Ayurvedic recipe that shows promising results along with fast recovery. This recipe is beneficial for preventing and combating 2019-nCoV infection, while enhancing the immune response.

The Ayurvedic recipe consists of 3 gm fresh turmeric paste mixed with 0.5 gm fresh black pepper powder and equally divided into 3 parts. Each part of freshly prepared Ayurvedic medicine is administered to the patient orally with lukewarm cow milk thrice in a day, and 100 ml green tea twice in a day (2 gm dry leaf of green tea with 0.25 gm of black pepper boiled in 100 ml water). After administering the dose to the patient, 2.5 gm freshly prepared sunflower seed powder mixed in lukewarm cow milk is administered to the COVID-19 patient once a day. This aforesaid therapy shows promising results in COVID-19 patients.

Mechanism of the Pathogen

Coronaviruses (CoVs) are relatively large viruses that contain a single-stranded positive-sense RNA genome encapsulated within a nucleocapsid protein and glycoprotein membrane envelope. Coronavirus genomes encode four types of structural proteins—nucleocapsid protein, membrane glycoprotein, envelope protein, and spike protein—in which the spike protein is a major functional inducer of host immune responses [10]. SARS caused by coronavirus is covered by enveloped spike glycoproteins, which give the virus its crown-like appearance [11] and are essential for viral entry into the host cell containing a variable RBD [6]. The RBD of coronavirus binds to the ACE-2 receptor [12] and APN, which is also known as CD13, dipetidyl peptidase 4 (DPP4) [13, 14], carboxyembryonic antigen-related cell adhesion molecule 1 (CEACAM1), and cellular serine protease found in the heart, lungs, kidney, and gastrointestinal tract, thus facilitating viral entry into the host cells [15, 16]. Coronavirus are also enveloped positive-strand RNA viruses possessing large genomes of RNA viruses characterized by 3–4 envelop-binding and subsequent membrane-fusion processes of coronavirus mediated by the spike membrane glycoprotein (spike protein). The S protein membrane contains three segments: a large ectodomain, single-pass transmembrane anchor, and short intracellular tail [15]. The ectodomain is a distinct functional RBD near the subunit amino (S1 or N) and a membrane-fusion subunit carboxy (S2 or C) terminal [17]. These spikes function to define viral tropism with their receptor specificity S1 or N terminal binds on the host cell surface by ACE-2 receptor for viral attachment, and perhaps also by their membrane fusion via the S2 or C terminal subunit activity virus genomes as they enter into the host cell by complex proteolytically processing by the type-2 transmembrane serine protease enzyme encoded by the TMPRSS-2 gene leading to cleavage of ACE-2 and the activation of the spike protein. Viral entry and cell infection trigger the host’s immune system, and the inflammatory cascade is initiated by antigen-presenting cells (APCs). The process starts with the APC performing two functions: first, the foreign antigen is presented to CD4 + T-helper cells; then, interleukin-12 is released to further stimulate the T-helper cells. The T-helper cells stimulate CD8 + T-killer cells, which will target any cells containing foreign antigens. In addition, activated T-helper cells stimulate beta cells to produce antigen-specific antibodies [18–20].

Concomitant Effects of Curcumin and Catechin with Piperine

Curcumin has poor absorption bioavailability due to rapid metabolism in the liver and small intestine wall. The major component of black pepper is alkaloid piperine (1-piperoylpiperidine), which enhances the bioavailability of drugs by inhibiting glucuronidation in
the liver and small intestine. Concomitant administration of turmeric powder with piperine and catechin with piperine increases the absorption bioavailability 2000%, with no adverse side effects [21]. Due to increased bioavailability, this treatment has promising potential to cure and control COVID-19 outbreaks globally, while enhancing individual immune response.

**Molecular Bonding**

Ligand (curcumin or catechin) molecule links to the site of ACE2 receptor, which provides the way to virus entry in host cell. Similarly, the ligand molecule binds to the RDB amino acid (S protein) that is involved in host-cell binding. A carbon–hydrogen bond, conventional hydrogen bond, and Van der Waals force are formed between the curcumin or catechin (ligand) and the S-protein (amino acid). This demonstrates the strong bonding affinity of ligands with ACE-2 as well as with the S-protein, although the binding affinity of catechin was greater than that of curcumin. Hence, curcumin and catechin create an inhibition environment between the RBD and ACE-2 receptor, which blocks the virus from entering the host cell (> Fig. 2).


▶ Fig. 2 Binding representation. **a** binding of ACE2 with curcumin, **b** binding of the S-protein with curcumin, **c** binding of ACE2 with catechin, **d** binding of the S-protein with catechin.
significant component of viral freedom [22–24]. Along these lines, it is evident that transmission of viral disease can be prevented by using Curcuma longa L, Green tea, and Piper nigrum in daily life.

Intercalation of Piperine with Curcumin
To survey the intercalation affinity of piperine with curcumin as a transporter of the curcumin particle by way of metabolism, we considered the idea of an intermolecular complex. A low binding energy ~ 3.1 kcal/mol complex is formed via the π–π linkage between the benzene ring of curcumin and piperine (▶Fig. 3). As we discovered, the separation between the enolic proton of curcumin to piperine was smaller than the phenolic protons of curcumin and piperine; thus, we recommend that the essential method of intercalation is through enolic intermolecular hydrogen holding and the sandwich π–π linkage between the benzene rings of curcumin and piperine [25]. Subsequently, these collaborations between piperine and curcumin vie for intercalation between adjoining layers of curcumin, thus accommodating a channel for piperine to tie to curcumin in support of its transportation through metabolic pathways. Subsequently, piperine helps to move curcumin and hinder uridine 5′-diphospho (UDP)-glucuronosyltransferase, thereby delaying glucuronosylation of curcumin into a high water-soluble substance. When glucuronosylation of curcumin is hindered by piperine, curcumin acquires a larger molecular size and remains expanded, thus providing longer absorption time and upgrading the bioavailability by 20-fold through this dual activity.

Conclusion
The COVID-19 pandemic has caused a severe blow to social life, health, and economic status for many individuals. The malignancy of the virus can be detected only by millions of deaths reported so far. Hence, there is a need to mitigate the spread of COVID-19 comprehensively. Our research suggests that daily routine use of Curcuma longa L (curcumin), Piper nigrum (piperine), and green tea (catechin) can prevent COVID-19 outbreaks and cure 2019-nCoV infection.

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Conflicts of Interest
The authors declare that they have no conflicts of interest associated with this work.

References


