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Withodin B

Product Information Sheet

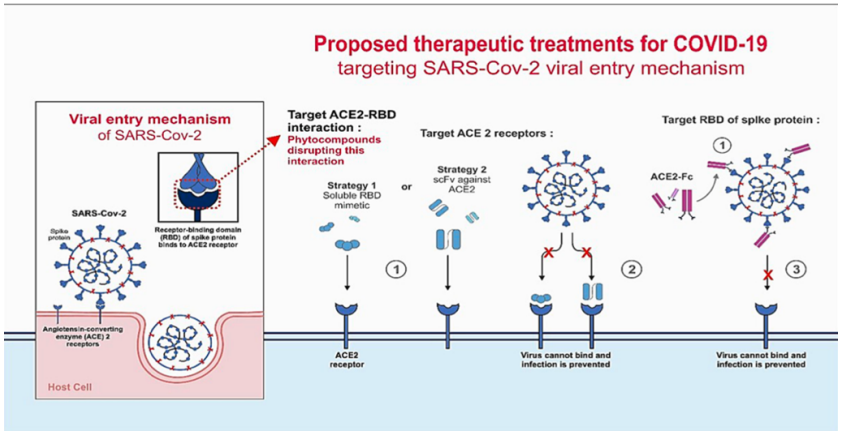
Withodin B™ is a Phytotherapeutic extraction of ***Withania somnifera, Polygonum Multiflorum, Apis mellifera propolis, Camellia sinensis, Monoatomic Au, and Cinnamomum verum***. Using advanced laboratory extraction apparatus & proprietary production protocols, these phytochemicals are known for their S-Spike protein inhibition properties.

SARS-CoV-2 engages human ACE2 through its spike (S) protein receptor binding domain (RBD) to enter the host cell. Recent computational studies have reported that withanone and withaferin A, phytochemicals found in Withania somnifera, target viral main protease. Withanone bound efficiently at the interacting interface of the ACE2-RBD complex and destabilized it energetically. The electrostatic component of binding free energies of the complex was significantly decreased. Withanone as a potent inhibitor of SARS-CoV-2 coronavirus entry into the host cells. Only withanone was found to be docked into the ACE2-RBD complex. It bound at the interface of the ACE2 receptor and RBD, interacted with the residues from both ACE2 and RBD and was thus analyzed further to study its role in weakening or blocking the interactions between the ACE2 receptor and RBD.

SARS-CoV had been shown to exhibit an open reading frame ORF-3a that codes for an ion permeable channel in the infected cells; the activity of the 3a protein may influence virus release. The ORF-3a is also named “New gene” localized between “spike and envelope gene” (SNE) and has been identified in other corona viruses. This includes the SNE of the human coronavirus OC43 (HCoV-OC43) which shows similar ion-channel characteristics as the 3a protein of SARS-CoV. Emodin was identified as an effective component of Polygonaceae to block the interaction of the SARS-coronavirus spike protein (SARS-CoV S protein) with the angiotensin-converting enzyme 2 (ACE2) and to reduce the infection by S protein-pseudo-typed retrovirus. The ACE2 was shown to be a functional receptor for SARS-CoV with a specific binding domain of the S protein. Emodin may contribute to reduced virus release from the SARS-CoV-infected cell through inhibition of the current mediated by 3a protein.

In silico studies have investigated the use of flavonoids in api-compounds as effective therapeutic candidates against COVID-19 by targeting S protein cleavage by host-cell proteases, e.g., TMPRSS2, S protein binding to cell surface receptors such as ACE-II, inhibiting S protein, or S protein binding to the inflammatory B56 unit in PP2A, as well as by interfering with NSPs of SARS-CoV-2, in order to hamper viral replication. Anti-COVID-19 effects of favonoids in Propolis reported by molecular docking studies elucidate efforts directed toward designing anti-COVID-19 drugs focused on impeding viral entry into host cells, interrupting viral replication, and inhibiting viral-host protein interactions, with the aim of aborting the inflammatory responses induced by viral invasion.

Withodin B™ is uniquely extracted from organic herbs using organic cane alcohol and deep ocean mineral water, as the extraction solvent. Utilizing advanced all-glass apparatus **Withodin B™** undergoes hours of reflux extraction that applies heat and ethanol to enhance the bioavailability of the resultant extraction.



Withania somnifera Withanone and withaferin A, phytochemicals found in Withania somnifera, target viral main protease. Withanone bound efficiently at the interacting interface of the ACE2-RBD complex and destabilized it energetically.

Polygonum Multiflorum, Contains Emodin an inhibitor of the SNE-encoded 3a protein as an ion channel. This new observation together with the finding that emodin may disrupt the interaction of S protein and ACE2 support the suggestion that emodin is a potent therapeutic agent in treatment of SARS and other coronavirus-induced diseases.

Bee Propolis, Propolis is a bee- made product exhibiting many biological properties. An overview of viruses, antiviral immunity, propolis safety and its immunomodulatory and antiviral action is reported, as well as perspectives for coronavirus disease 2019 (COVID-19) treatment. Molecular simulations show that flavonoids in propolis and honey (e.g., rutin, naringin, caffeic acid phenyl ester, luteolin, and artepillin C) may inhibit viral spike fusion in host cells, viral-host interactions that trigger the cytokine storm, and viral replication. Similar to the potent antiviral drug remdesivir, rutin, propolis ethanolic extract, and propolis liposomes inhibited non-structural proteins of SARS-CoV-2 in vitro, and these compounds along with naringin inhibited SARS-CoV-2 infection in Vero E6 cells.

Camellia sinensis a major component of green tea, EGCG is famous for its anti-inflammatory and anti-apoptotic properties. EGCG, as a potent inducer of HO-1, can suppress renal injury by reducing oxidative stress and inflammation.

Monoatomic Au, Gold complexes have a long-lasting history in medicine and have been used as disease modifying antirheumatic drugs (DMARDs) for the treatment of rheumatoid arthritis. Auranofin and other gold species has focused on anticancer and anti-infective agents. The application of gold complexes as antiviral drugs shows some promising results use as human immunodeficiency virus (HIV) therapeutics.

Cinnamomum verum Contains antioxidants, including polyphenols, phenolic acid and flavonoids. These compounds work to fight oxidative stress in the body and aid in the prevention of chronic disease.

frontiers in Pharmacology

REVIEW
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Withania somnifera (L.) Dunal: Opportunity for Clinical Repurposing in COVID-19 Management

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As the COVID-19 pandemic is progressing, the therapeutic gaps in conventional management have highlighted the need for the integration of traditional knowledge systems with modern medicine. Ayurvedic medicines, especially Ashwagandha (*Withania somnifera* (L.) Dunal, WS), may be beneficial in the management of COVID-19. WS is a widely prescribed Ayurvedic botanical known as an immunomodulatory, antiviral, anti-inflammatory, and adaptogenic agent. The chemical profile and pharmacological activities of WS have been extensively reported. Several clinical studies have reported its safety for use in humans. This review presents a research synthesis of *in silico*, *in vitro*, *in vivo*, and clinical studies on *Withania somnifera* (L.) Dunal (WS) and discusses its potential for prophylaxis and management of COVID-19. We have collated the data from studies on WS that focused on viral infections (HIV, HSV, H1N1 influenza, etc.) and noncommunicable diseases (hypertension, diabetes, cancer, etc.). The experimental literature indicates that WS has the potential for 1) maintaining immune homeostasis, 2) regulating inflammation, 3) suppressing pro-inflammatory cytokines, 4) organ protection (nervous system, heart, lung, liver, and kidney), and 5) anti-stress, antihypertensive, and antidiabetic activities. Using these trends, the review presents a triangulation of Ayurveda wisdom, pharmacological properties, and COVID-19 pathophysiology ranging from viral entry to end-stage acute respiratory distress syndrome (ARDS). The review proposes WS as a potential therapeutic adjuvant for various stages of COVID-19 management. WS may also have beneficial effects on comorbidities associated with the COVID-19. However, systematic studies are needed to realize the potential of WS for improving clinical outcome of patients with COVID-19.

Keywords: Ashwagandha, Ayurveda, *Rasayana*, Immunomodulation, Inflammation, Cytokine, Adjuvant

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INTRODUCTION

The COVID-19 or coronavirus disease 2019 is a contagious disease caused by SARS-CoV-2. The rapidly spreading disease is considered as one of the causes of mortality globally (Zhou P. et al., 2020) ("WHO Announces COVID-19 Outbreak a Pandemic" 2020) ("WHO Coronavirus Disease (COVID-19) Dashboard" 2020).

Understanding the pathophysiology of this disease is rapidly advancing with the availability of new research data. Current evidence suggests that most individuals are asymptomatic or are suffering from mild symptoms. The patients who progress to severity develop pneumonia and ARDS and

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ORIGINAL RESEARCH

Withanone from *Withania somnifera* Attenuates SARS-CoV-2 RBD and Host ACE2 Interactions to Rescue Spike Protein Induced Pathologies in Humanized Zebrafish Model

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Video abstract

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Purpose: SARS-CoV-2 engages human ACE2 through its spike (S) protein receptor binding domain (RBD) to enter the host cell. Recent computational studies have reported that withanone and withaferin A, phytochemicals found in *Withania somnifera*, target viral main protease (M^{Pro}) and host transmembrane TMPRSS2, and glucose related protein 78 (GRP78), respectively, implicating their potential as viral entry inhibitors. Absence of specific treatment against SARS-CoV-2 infection has encouraged exploration of phytochemicals as potential antivirals.

Aim: This study aimed at in silico exploration, along with in vitro and in vivo validation of antiviral efficacy of the phytochemical withanone.

Methods: Through molecular docking, molecular dynamic (MD) simulation and electrostatic energy calculation the plausible biochemical interactions between withanone and the ACE2-RBD complex were investigated. These in silico observations were biochemically validated by ELISA-based assays. Withanone-enriched extract from *W. somnifera* was tested for its ability to ameliorate clinically relevant pathological features, modelled in humanized zebrafish through SARS-CoV-2 recombinant spike (S) protein induction.

Results: Withanone bound efficiently at the interacting interface of the ACE2-RBD complex and destabilized it energetically. The electrostatic component of binding free energies of the complex was significantly decreased. The two intrachain salt bridge interactions (K31-E35) and the interchain long-range ion-pair (K31-E484), at the ACE2-RBD interface were completely abolished by withanone, in the 50 ns simulation. In vitro binding assay experimentally validated that withanone efficiently inhibited (IC_{50}=0.33 ng/mL) the interaction between ACE2 and RBD, in a dose-dependent manner. A withanone-enriched extract, without any co-extracted withaferin A, was prepared from *W. somnifera* leaves. This enriched extract was found to be efficient in ameliorating human-like pathological responses induced in humanized zebrafish by SARS-CoV-2 recombinant spike (S) protein.

Conclusion: In conclusion, this study provided experimental validation for computational insight into the potential of withanone as a potent inhibitor of SARS-CoV-2 coronavirus entry into the host cells.

Keywords: ACE2-RBD complex, *Withania somnifera*, withanone, docking and MD simulation, ELISA, SARS-CoV-2 S-protein, humanized zebrafish model

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Withanone and caffeic acid phenethyl ester are predicted to interact with main protease (M^{pro}) of SARS-CoV-2 and inhibit its activity

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ABSTRACT

The recent novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2/2019-nCoV) has caused a large number of deaths around the globe. There is an urgent need to understand this new virus and develop prophylactic and therapeutic drugs. Since drug development is an expensive, intense and time-consuming path, timely repurposing of the existing drugs is often explored wherein the research avenues including genomics, bioinformatics, molecular modeling approaches offer valuable strengths. Here, we have examined the binding potential of Withaferin-A (Wi-A), Withanone (Wi-N) (active withanolides of Ashwagandha) and Caffeic Acid Phenethyl Ester (CAPE, bioactive ingredient of propolis) to a highly conserved protein, M^{pro} of SARS-CoV-2. We found that Wi-N and CAPE, but not Wi-A, bind to the substrate-binding pocket of SARS-CoV-2 M^{pro} with efficacy and binding energies equivalent to an already claimed N3 protease inhibitor. Similar to N3 inhibitor, Wi-N and CAPE were interacting with the highly conserved residues of the proteases of coronaviruses. The binding stability of these molecules was further analyzed using molecular dynamics simulations. The binding free energies calculated using MM/GBSA for N3 inhibitor, CAPE and Wi-N were also comparable. Data presented here predicted that these natural compounds may possess the potential to inhibit the functional activity of SARS-CoV-2 protease (an essential protein for virus survival), and hence (i) may connect to save time and cost required for designing/development, and initial screening for anti-COVID drugs, (ii) may offer some therapeutic value for the management of novel fatal coronavirus disease, (iii) warrants prioritized further validation in the laboratory and clinical tests.

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SARS-CoV-2 coronavirus; Ashwagandha; Withanone; Withaferin-A; honeybee propolis; caffeic acid phenethyl ester; molecular docking; binding; main protease (M^{pro})

1. Introduction

Coronaviruses, discovered in 1960, are infectious strains of viruses originally named on the basis of their crown like appearance, due to the glycoprotein projections on its envelope, under the electron microscope and grouped into the family Coronaviridae; order Nidovirales. They invade the respiratory tract via the nose. After an incubation period of about 3–7 days, they cause the symptoms of a mild common cold/bronchitis (nasal obstruction, sneezing, runny nose, cough, headache, fever, pneumonia, asthenia and inflammation in airway) in avian and mammalian species. In contrast to animals, wherein they have been shown to infect several tissues causing a large variety of diseases, mainly respiratory infections with mild common cold like symptoms, occasional gastrointestinal and diarrhea have been reported for humans. The infected individuals shed virus in nasal secretions and mucosa resulting in disease transmission that can often be controlled, at least partially, by following hygienic measures. Vaccines for coronaviruses are not available and treatment remains symptomatic.

Designing and development of antiviral medicine requires understanding of the molecular mechanisms of viral replication and packaging into the infectious particles in host cells, their release, selection of antiviral target proteins and development of their inhibitors. Coronaviruses have been shown to invade and replicate in differentiated respiratory epithelial cells resulting in their vacuolation, damaged cilia, local inflammation, swelling, sneezing and fever. Among the several strains of coronaviruses known so far, including HCoV-229E, HCoV-OC43, HCoV-NL63, SARS-CoV, MERS-CoV and 2019-nCoV/SARS-CoV-2 (Graham et al., 2013; van der Hoek et al., 2004; Woo et al., 2010), the latter was designated as a novel strain of coronavirus that caused pneumonia outbreak in Wuhan city of China in December 2019 (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020; Wu et al., 2020; Zhou et al., 2020). As of May 14, 2020, it has infected over 4,258,666 individuals globally with 294,190 deaths, as reported to WHO (WHO, 2020). It has been declared as international public health emergency and advocated rapid research efforts. Genomic characterization of the SARS-CoV-2, its variance, evolution, transmission

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Emodin blocks the SARS coronavirus spike protein and angiotensin-converting enzyme 2 interaction

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Abstract

Severe acute respiratory syndrome (SARS) is an emerging infectious disease caused by a novel coronavirus (SARS-CoV). SARS-CoV spike (S) protein, a type I membrane-bound protein, is essential for the viral attachment to the host cell receptor angiotensin-converting enzyme 2 (ACE2). By screening 312 controlled Chinese medicinal herbs supervised by Committee on Chinese Medicine and Pharmacy at Taiwan, we identified that three widely used Chinese medicinal herbs of the family *Polygonaceae* inhibited the interaction of SARS-CoV S protein and ACE2. The IC_{50} values for Radix et Rhizoma Rhei (the root tubers of *Rheum officinale* Baill.), Radix Polygoni multiflori (the root tubers of *Polygonum multiflorum* Thunb.), and Caulis Polygoni multiflori (the vines of *P. multiflorum* Thunb.) ranged from 1 to 10 μg/ml. Emodin, an anthraquinone compound derived from genus *Rheum* and *Polygonum*, significantly blocked the S protein and ACE2 interaction in a dose-dependent manner. It also inhibited the infectivity of S protein-pseudotyped retrovirus to Vero E6 cells. These findings suggested that emodin may be considered as a potential lead therapeutic agent in the treatment of SARS.

Keywords: SARS coronavirus; Spike protein; Angiotensin-converting enzyme 2; Emodin

1. Introduction

Severe acute respiratory syndrome (SARS) is a new human disease that results in progressive respiratory failure and death in close to 10% of infected individuals (Ksiazek et al., 2003; Peiris et al., 2003). The etiological agent, SARS coronavirus (SARS-CoV) (Drosten et al., 2003; Fouchier et al., 2003) contains a single-stranded plus-sense RNA genome about 30 kb in length that has a 5′-cap structure and a 3′-polyadenylation tract (Marra et al., 2003; Rota et al., 2003). The genomic organization is typical of coronaviruses, having 14 potential major open reading frames that encode replicase, spike (S), envelope, membrane, and nucleocapsid proteins in the same order as those of other coronaviruses (Tan et al., 2005).

SARS-CoV S protein is a large type I membrane glycoprotein projection from viral envelope (Bosch et al., 2003). SARS-CoV S protein is responsible for binding to cellular receptors and for mediating the fusion of viral and host membranes (Simmons et al., 2004; Tripet et al., 2004). It also contains important virus-neutralizing epitopes that elicit neutralizing antibody in the host species (Hofmann et al., 2004a; Sui et al., 2004). Furthermore, mutations in this gene dramatically affect the virulence, pathogenesis, and host cell tropism (Petit et al., 2005; Yi et al., 2005). Angiotensin-converting enzyme 2 (ACE2) has been identified as a functional receptor for SARS-CoV (Li et al., 2003). Soluble S fragment or ACE2 is able to block S protein-mediated infection (Hofmann et al., 2004b; Moore et al., 2004). Monoclonal antibodies against S protein efficiently neutralize SARS-CoV *in vitro* and *in vivo* (Greenough et al., 2005; Sui et al., 2004). Moreover, vaccines that express the S protein induce T cell and neutralizing antibody responses, and protect animals from SARS-CoV infection (Chen et al., 2005; Yang et

Abbreviations: SARS, severe acute respiratory syndrome; SARS-CoV, SARS coronavirus; S, spike; ACE2, angiotensin-converting enzyme 2; HIV, human immunodeficiency virus; ELISA, enzyme-linked immunosorbent assay; *E. coli*, *Escherichia coli*; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; BSA, bovine serum albumin; IFA, immunofluorescence assay; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; HSV, herpes simplex virus

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Emodin inhibits current through SARS-associated coronavirus 3a protein

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ABSTRACT

The open-reading-frame 3a of SARS coronavirus (SARS-CoV) had been demonstrated previously to form a cation-selective channel that may become expressed in the infected cell and is then involved in virus release. Drugs that inhibit the ion channel formed by the 3a protein can be expected to inhibit virus release, and would be a source for the development of novel therapeutic agents. Here we demonstrate that emodin can inhibit the 3a ion channel of coronavirus SARS-CoV and HCoV-OC43 as well as virus release from HCoV-OC43 with a $K_{1/2}$ value of about 20 μM. We suggest that viral ion channels, in general, may be a good target for the development of antiviral agents.

1. Introduction

Several viral genomes encode for transmembrane proteins that may form channels in the membrane of the infected cell (see e.g. (Fischer and Hsu, 2011)) and that play a crucial role in virus life cycle These membrane proteins are considered as new targets for antiviral drugs (Liang and Li, 2010; Wang and Sun, 2011).

Severe acute respiratory syndrome (SARS) first appeared in 2002 in China. In mainland China about 50% of patients were treated with Chinese herbal medicine as an adjunct therapy in addition to Western medicine (see (Zhang et al., 2004)), and some positive effects in SARS patients had been reported (see (Liu et al., 2008)). By screening a large number of Chinese herbs (Ho et al., 2007) emodin was identified as an effective component of *Polygonaceae* to block the interaction of the SARS-coronavirus spike protein (SARS-CoV S protein) with the angiotensin-converting enzyme 2 (ACE2) and to reduce the infection by S protein-pseudo-typed retrovirus. The ACE2 was shown to be a functional receptor for SARS-CoV (Kuhn et al., 2004; Li et al., 2003) with a specific binding domain of the S protein (Babcock et al., 2004; Wong et al., 2004).

SARS-CoV had been shown to exhibit an open reading frame ORF-3a that codes for an ion-permeable channel in the infected cells; the activity of the 3a protein may influence virus release (Lu et al., 2006). The ion channel is permeable for monovalent cations with higher permeability for K^+ than for Na^+. Ba^{2+} in the external solution effectively can block the channel. The ORF-3a is also named "New gene" localized between "spike and envelope gene" (SNE) (Zeng et al., 2004), and has been identified also in other coronaviruses (Lu et al., unpublished, see also (Wang and Sun, 2011)). This includes the SNE of the human coronavirus OC43 (HCoV-OC43) which shows similar ion-channel characteristics as the 3a protein of SARS-CoV.

Here we show that emodin is an inhibitor of the SNE-encoded 3a protein as an ion channel. This new observation together with the finding that emodin may disrupt the interaction of S protein and ACE2 (Ho et al., 2007) support the suggestion that emodin or derivatives may become potent new therapeutic agents in treatment of SARS and other coronavirus-induced diseases. Since the genomes of various other viruses also encode for ion channels, our findings strengthens the view that viral ion chan-

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Review
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OXFORD

Review

Propolis antiviral and immunomodulatory activity: a review and perspectives for COVID-19 treatment

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Abstract

Objectives Viral outbreaks are a frequent concern for humans. A great variety of drugs has been used to treat viral diseases, which are not always safe and effective and may induce adverse effects, indicating the need for new antiviral drugs extracted from natural sources. Propolis is a bee-made product exhibiting many biological properties. An overview of viruses, antiviral immunity, propolis safety and its immunomodulatory and antiviral action is reported, as well as perspectives for coronavirus disease 2019 (COVID-19) treatment. PubMed platform was used for data collection, searching for the keywords "propolis", "virus", "antiviral", "antimicrobial" and "coronavirus".

Key findings Propolis is safe and exerts antiviral and immunomodulatory activity; however, clinical trials should investigate its effects on individuals with viral diseases, in combination or not with antiviral drugs or vaccines.

Summary Regarding COVID-19, the effects of propolis should be investigated directly on the virus *in vitro* or on infected individuals alone or in combination with antiviral drugs, due to its immunomodulatory and anti-inflammatory action. Propolis administration simultaneously with vaccines should be analyzed, due to its adjuvant properties, to enhance the individuals' immune response. The search for therapeutic targets may be useful to find out how propolis can help to control COVID-19.

Keywords: propolis; virus; antiviral action; antimicrobial action; coronavirus

Introduction

Viral outbreaks are a frequent concern for humans. Despite the most famous epidemics known to mankind, there have been outbreaks in the last decade of dengue virus (DENV) in Nepal and Hawaii,[1, 2] hepatitis viruses in India,[3] yellow fever virus (YFV) in Brazil,[4] norovirus (NV) in industrialized countries[5] and the pandemics of Influenza H1N1[6] ebola virus (EBOV)[7] and the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[8]

A great variety of molecules and drugs have been used to treat viral diseases, such as interferon-α, ribavirin, cidofovir, acyclovir, ganciclovir and others that may control viral epidemics.[9, 10] However, these drugs are not always safe and effective and may induce adverse effects on humans such as kidney injury,[11] neurological damages[12] and others. Moreover, they may lead to antiviral drug resistance.[13] In addition, it has been reported that the misuse of antibiotics to treat viral respiratory diseases can lead to more problems, mainly bacterial drug-resistance.[14]

molecules

MDPI

Review

Propolis, Bee Honey, and Their Components Protect against Coronavirus Disease 2019 (COVID-19): A Review of In Silico, In Vitro, and Clinical Studies

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Abstract: Despite the virulence and high fatality of coronavirus disease 2019 (COVID-19), no specific antiviral treatment exists until the current moment. Natural agents with immune-promoting potentials such as bee products are being explored as possible treatments. Bee honey and propolis are rich in bioactive compounds that express strong antimicrobial, bactericidal, antiviral, anti-inflammatory, immunomodulatory, and antioxidant activities. This review examined the literature for the anti-COVID-19 effects of bee honey and propolis, with the aim of optimizing the use of these handy products as prophylactic or adjuvant treatments for people infected with severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2). Molecular simulations show that flavonoids in propolis and honey (e.g., rutin, naringin, caffeic acid phenyl ester, luteolin, and artepillin C) may inhibit viral spike fusion in host cells, viral-host interactions that trigger the cytokine storm, and viral replication. Similar to the potent antiviral drug remdesivir, rutin, propolis ethanolic extract, and propolis liposomes inhibited non-structural proteins of SARS-CoV-2 in vitro, and these compounds along with naringin inhibited SARS-CoV-2 infection in Vero E6 cells. Propolis extracts delivered by nanocarriers exhibit better antiviral effects against SARS-CoV-2 than ethanolic extracts. In line, hospitalized COVID-19 patients receiving green Brazilian propolis or a combination of honey and *Nigella sativa* exhibited earlier viral clearance, symptom recovery, discharge from the hospital as well as less mortality than counterparts receiving standard care alone. Thus, the use of bee products as an adjuvant treatment for COVID-19 may produce beneficial effects. Implications for treatment outcomes and issues to be considered in future studies are discussed.

Keywords: Coronaviruses; coronavirus disease 2019; COVID -19; severe acute respiratory syndrome; SARS-CoV-2; cytokine storm; propolis; bee honey; bee products; flavonoids; ACE-II; non-structural proteins; spike glycoprotein; main protease; in silico; in vitro; randomized clinical trials; molecular docking/biochemical modeling

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1. Introduction

Coronavirus disease 2019 (COVID-19), announced by the World Health Organization as a global pandemic in March 2020, is a highly contagious viral infection caused by the newly discovered severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) [1,2]. SARS-CoV-2 gets access to the human body through the respiratory tract causing severe acute pneumonia-associated respiratory syndrome (ARDS). It is rapidly transmitted among humans through droplet and direct contact [3–6]. To date, 23 February 2021, at least 111,419,939 confirmed cases of COVID-19 have been reported while global deaths reached 2,470,772 [7].

The cytokine storm is the main cause of fatalities of SARS-CoV-2 (around 15% of cases). It frequently occurs in old people, obese, diabetics, hypertensive, and those with

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Metallodrugs

Gold Metallodrugs to Target Coronavirus Proteins: Inhibitory Effects on the Spike-ACE2 Interaction and on PLpro Protease Activity by Auranofin and Gold Organometallics**

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Abstract: Gold complexes have a long tradition in medicine and for many examples antirheumatic, anticancer or anti-infective effects have been confirmed. Herein, we evaluated the lead compound Auranofin and five selected gold organometallics as inhibitors of two relevant drug targets of severe acute respiratory syndrome coronaviruses (SARS-CoV). The gold metallodrugs were effective inhibitors of the interaction of the SARS-CoV-2 spike protein with the angiotensin converting enzyme 2 (ACE2) host receptor and might thus interfere with the viral entry process. The gold metallodrugs were also efficient inhibitors of the papain-like protease (PLpro) of SARS-CoV-1 and SARS-CoV-2, which is a key enzyme in the viral replication. Regarding PLpro from SARS-CoV-2, the here reported inhibitors are among the very first experimentally confirmed examples with activity against this target enzyme. Importantly, the activity of the complexes against both PLpro enzymes correlated with the ability of the inhibitors to remove zinc ions from the labile zinc center of the enzyme. Taken together, the results of this pilot study suggest further evaluation of gold complexes as SARS-CoV antiviral drugs.

The current pandemic outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused an unprecedented global health crisis with to date more than 29 million infected individuals.[1,2] While the world struggles with the control of the fast outspread of this coronavirus and it's enormous impact on healthcare, economy and society, efforts to develop vaccines and therapeutics have been undertaken worldwide at a rate, which modern drug discovery has not witnessed ever. The lack of an effective antiviral drug for the treatment of the Coronavirus disease-2019 (COVID-19) has triggered major drug repurposing efforts; however, to this date no approved therapeutic has proven to have sufficient efficacy in the many ongoing clinical trials. The urgent development of new innovative drug candidates against SARS-CoV-2 is the most important mission that medicinal chemists are currently facing.

Regarding drug activity evaluation, several molecular pathways have been in the focus of the search for a possible COVID-19 treatment based on strategies that had already been considered for the SARS-CoV and Middle East respiratory syndrome MERS-CoV outbreaks.[3] Amongst others these include the entry of the coronavirus into the host cell (e.g. the interaction of TMPRSS2[4] or ACE2 with spike proteins of the coronavirus[5]), the viral replication process in the host cell (e.g. the proteases 3CLpro[6] and PLpro[3,7,8]), transcription, the nucleocapsid protein, or exocytosis of the new virion.[3,7,9]

Gold complexes have a long lasting history in medicine and have been used as disease modifying antirheumatic drugs (DMARDs) for the treatment of rheumatoid arthritis. Intensive research on other possible therapeutic applications of the lead compound Auranofin and other gold species has focused on anticancer and anti-infective agents. The application of gold complexes as antiviral drugs has not been studied very intensively, although some promising results suggest a possible future use as human immunodeficiency virus (HIV) therapeutics.[10]

Here we report the results of a pilot study, in which we investigated the effects of Auranofin and selected experimental gold metallodrugs (see Figure 1) on two relevant coronavirus targets (spike protein, papain like protease, PLpro). Whereas **Au-1**,[11] **Au-3**[12,13] and **Au-5**[14] were selected from our previous works on organometallic gold metallodrugs, **Au-2** and **Au-4** have not been reported before and their synthesis and characterization are described here. Complexes **Au-1** to **Au-5** are organometallics containing either a *N*-heterocyclic carbene (NHC) or an alkynyl ligand. Complexes of these types have demonstrated promising activities in a fast increasing number of recent reports.[15]

The entry of SARS-CoV-2 into target cells is facilitated by the spike (S) protein of coronaviruses and mediated by the angiotensin-converting enzyme 2 (ACE2) as the entry receptor.[1,4] The S1 subunit of the SARS-CoV-2 spike protein contains the

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