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Review Article

Why Ashwagandha for Prevention and Treatment of COVID-19?

Renu Wadhwa[1], Jaspreet Kaur Dhanjal[1], Vipul Kumar[2], Sunil C Kaul[1*], Durai Sundar[2*]

[1]Department of Environmental Research, National Institute of Advanced Industrial Science & Technology (AIST), Tsukuba, Japan; [2]Department of Biochemical Engineering & Biotechnology, Indian Institute of Technology (IIT), New Delhi, India

ABSTRACT

A novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-coronavirus 2; SARS-CoV-2) emerged in December 2019 in Wuhan, China, and caused fatal Coronavirus disease 2019 (COVID-19). It became an international pandemic, disrupted all aspects of normal life and called global health emergency. High infected numbers and deaths world-wide have evoked urgent initiation of new line of drug and vaccine development on one hand, and repurposing of the existing drugs on the other. Because of the immunity enhancing properties of several kinds of traditional home medicine, they have been recommended for the prevention and treatment of COVID-19. Ashwagandha, an Ayurvedic herb that enjoys 5000 years of history was explored for its potential for antiCOVID-19 activity using bioinformatics and experimental technologies. Active with anolides of Ashwagandha (Withaferin-A; Wi-A and Withanone; Wi-N) were tested for their binding to a highly conserved protein (Mpro, an essential protein for virus replication and survival) of SARS-CoV-2. Wi-N, but not Wi-A, showed strong binding to Mpro with the binding energy equivalent to a known Mpro inhibitor (N3) predicting that Wi-N may serve as natural drug for COVID-19. We also examined potential of Wi-A and Wi-N to bind to a cell membrane protein (TPMRSS2) that acts as a gate for entry of virus to the host cell. We found that although both Wi-A and Wi-N could bind and stably interact with TMPRSS2, Wi-N showed stronger interactions. Furthermore, human cells treated with Wi-N showed low level of expression of TMPRSS2 predicting three-way action of Wi-N to deal with SARS-CoV-2 (blocking its entry to the host cells by interaction with TMPRSS2, downregulation of TMPRSS2 expression and diminishing the viral survival through inhibition of viral Mpro protein).

Keywords: Ashwagandha; Withanolides; SARS-CoV-2; TPMRSS2; Blocking; Inhibitor

INTRODUCTION

Coronaviruses (family Coronaviridae; order Nidovirales) are large, enveloped, positive-stranded RNA viruses. They are named as coronaviruses due to their crown like appearance under the electron microscope. Their genome is the largest among all RNA viruses and is packed inside a helical capsid constituted of nucleocapsid protein (N), surrounded by an envelope comprised of three structural proteins; (i) the membrane protein-M, (ii) the envelope protein-E and (iii) the spike protein-S. M and E proteins are involved in virus assembly, the S protein (forms large protrusions from the virus surface, giving it an appearance of crown; hence the name Coronavirus) mediates virus entry into target host cells [1]. They invade the respiratory tract via the nose or mouth. After an incubation period of about 3-7 days, they cause the symptoms of a common cold/bronchitis (sneezing, nasal obstruction, 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. Originated and rapidly spreading (by human to human transmission) from Wuhan city of China to all over the world; infecting ~19 million people and ~700,000 deaths in 7-8 months, it has evoked urgent need to understand the biology of the new strain of coronavirus, and develop prophylactic and therapeutic drugs [2].

Among the several strains of coronaviruses known so far, including HCoV-229E, HCoV-OC43, HCoV-NL63, MERS-CoV, SARS-CoV, 2019-nCoV/SARS-CoV-2, the latter was designated as a novel strain of coronavirus that caused pneumonia outbreak in

Correspondence to: Sunil C Kaul, Department of Environmental Research, National Institute of Advanced Industrial Science & Technology (AIST), Tsukuba, Japan, E-mail: s-kaul@aist.go.jp

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Wuhan city of China in December 2019. Genomic characterization of the SARS-CoV-2, its variance, evolution, transmission dynamics (human to human transmission by droplets or direct contact) was reported [3,4]. It was confirmed to be the enveloped single-stranded RNA β-coronavirus similar to the SARS and MERS viruses and closely related to two bat-derived SARS-like coronaviruses (CoVZC45 and bat-SL-CoVZXC21). The entry of SARS-CoV-2 into the host cell was shown to be dependent on the interaction with the host cell surface protein ACE-2. Upon attachment with ACE-2, the S protein of the virus gets cleaved and activated for its fusion into the host cell membrane, which is facilitated by another cell membrane protein, transmembrane protease serine 2 (TMPRSS-2). Once inside the host cells, virus uses host cell protein synthesis machinery to decode it's genetic information to proteins and generate infectious particles that infect the neighboring cells and carry the cycle forward to multiply in high numbers in short duration of time. It has been confirmed that the host cell membrane proteins ACE-2 and TMPRSS2 act as gates for entry of SARS-CoV-2 to host cells. It is initiated with the binding of viral surface glycoprotein (spike protein-S) to ACE-2 that is also shown to determine the severity of SARS-CoV-2 infection. The viral spike protein-S is synthesized as a precursor protein. Its cleavage by host cell proteases including TMPRSS2, endosomal cathepsin L protease or Human Airway Trypsin-like (HAT) is essential for its entry to host cells. Hence, these host cell proteins regulate virus infectivity and propagation. In these premises, protease inhibitors are considered as drugs for treatment/prevention of viral infections. Indeed, over expression of TMPRSS2 and HAT was shown to promote the growth of different subtypes of human and avian influenza viruses. On the other hand, serine protease inhibitors abrogated the virus proliferation. Camostat mesylate (an inhibitor of serine protease, clinically used to treat chronic pancreatitis) has also been suggested as a candidate antiviral drug to prevent or suppress TMPRSS2-dependent infection by SARS-CoV, at least in part. The simultaneous treatment with inhibitors of cathepsin L and TMPRSS2 was shown to completely block virus entry into cells. Cysteine protease inhibitor K11777 (used for the treatment of tropical parasitic disease, American trypanosomiasis) has been shown to target cathepsin-mediated cell entry and act as broad-spectrum antivirals. It was shown to inhibit SARS-CoV and Ebola virus entry into the host cells. SARS-CoV-2 was shown to use the ACE2 receptor for entry and the serine protease TMPRSS2 for S protein priming. Camostat mesylate blocked entry of SARS-CoV-2 virus into the cells [5].

In the absence of effective drug and vaccine for SARS-CoV-2, repurposing of drugs is largely implemented using bioinformatics tools. Indinavir and Remdesivir were investigated for their binding potential to SARS-CoV-2 protease. While neither of these docked on any active sites of the protease, the active form of Remdesivir (CHEMBL2016761) showed perfect docking in the overlapping region of the NTP binding motif urging its validation through clinical trials for COVID-19 infection [6]. The U.S. Food and Drug Administration has now issued an emergency use authorization for Remdesivir for the treatment of suspected or laboratory-confirmed adults and children hospitalized with severe symptoms of SARS-CoV-2 disease [7]. In another recent study employing an in silico approach, it was reported that Belachinal, Macaflavanone E, and Vibsanol B phytochemicals may inhibit the functional activity of SARS-CoV-2 E protein [8]. In line with this, several studies have recently explored the repurposing of drugs to find an immediate therapeutic strategy for the deadly COVID-19, by mainly targeting SARS-Cov-2 main protease, NSP15 and prefusion 2019-nCoV spike glycoprotein, RNA-dependent RNA polymerase, N and E protein, and cell surface receptors (ACE-2 and TMPRSS2) of host cells.

Ayurveda, a traditional medicine system, is being practiced for 5000 years in the Indian subcontinent. In Sanskrit, Ayurveda means "Science of life" as it deals with the health and wellbeing of people using holist approaches to have balanced energies in body, mind and soul. Ayurvedic principals describe the use of herbs for prevention and treatment of stress and ailments that range from common cold, arthritis, neuro-dysfunctions, insomnia, amnesia, diabetes and cancer, and are often claimed to extended lifespan and QOL. Ashwagandha (Withania somnifera; referred to as the Queen of Ayurveda for its popular use and trusted for its therapeutic properties, including antioxidant, anti-inflammatory, neuroprotective, anticancer and anti-metastatic activity) has been tested in several laboratory studies that have also provided the molecular basis of its activities [9]. Categorized as GRAS (Generally Regarded As Safe) herb and proved to be nontoxic in its reasonable doses, its major constituents from various parts have been defined as steroidal alkaloids and steroidal lactones, a class of chemicals known as withanolides. The small molecules have C28 steroidal nucleus with C9 side chain, having six-membered lactone ring. So far, 12 alkaloids, 35 withanolides and several sitoindosides have been isolated, and their structures have been elucidated. Various alkaloids include Withanine, Somniferine, Somnine, Somniferinine, Withananine, Tropine, Psuedotropine, Choline, Cuscohygrine, Isopelletierine, Anaferine and Anahydrine. Among withanolides, Withaferin A, Withanone, Withanolide A, Withanolide B, Withanolide IV, Withanolide V, Beta-methoxy-Withaferin A have been use in laboratory studies. Withaferin A has emerged as a potential anticancer compound [9]. On the other hand, Withanone was shown to be nontoxic to normal cells. It instead was shown to possess antistress activity and was proved to be good for stressed brain functions [10,11]. Ashwagandha is trusted as immunity enhancer and possesses a variety of prophylactic and therapeutic activities. Several studies have provided evidence to its anti-microbial activities. Wi-A was shown to possess inhibitory activity for HPV and influenza viruses [12]. In these premises and our ongoing research focus on exploration on the molecular basis of Ashwagandha bioactivities, we embarked on testing if Ashwagandha Withanolides Wi-A and Wi-N would have anti-COVID19 activity and discovered its potential as COVID-19 warrior as follows

ASHWAGANDHA MAY INHIBIT THE ENTRY OF SARS-COV-2 INTO THE HOST CELLS BY BLOCKING AND DIMINISHING HOST CELL PROTEIN TMPRSS-2

As described above, TMPRSS2 protein acts as a gate for entry of virus to host cells. It was confirmed in athat SARS-CoV-2 requires TMPRSS2 for successful entry to cells [5]. The main catalytic residues of TMPRSS2 involved in its proteolytic activity are His296, Asp345 and Ser441. It has been shown to be dispensable in mice knockout studies that demonstrated no significant effect on development, survival or normal organ structure or functions, suggesting its functional redundancy with other transmembrane serine proteases. In these premises, TMPRSS2 has emerged as an attractive therapeutic target against COVID-19. Camostat mesylate, TMPRSS2 inhibitor, was shown to indeed block entry of SARS-CoV-2 virus into the cells. It, similar to the other non-specific serine

protease inhibitors, targets a highly conserved catalytic domain comprising of His296-Asp345-Ser441 triad [13]. Although the exact molecular mechanism of action of camostat remains elusive, to be effective against different serine proteases, it must target this active site harboring the conserved catalytic triad residues. Based on the proved anti-viral activity of Camostat mesylate, we decided to use it as a reference molecule. We first modelled its interactions with the catalytic residues of TMPRSS2 and then compared them with our Ashwagandha Withanolides; Wi-A and Wi-N. Complex of TMPRSS2 with Wi-A and Wi-N showed docking scores that are compatible with its potential to block its catalytic site of TMPRSS2. Detailed analyses showed that the overall conformation of TMPRSS2-Wi-A, and TMPRSS2-Wi-N were comparable to that of TMPRSS2-Camostat mesylate, and were stable (Figure 1). Wi-N made significantly stronger contact with amino acid residue Ser441 that is directly involved in catalytic activity of TMPRSS2.

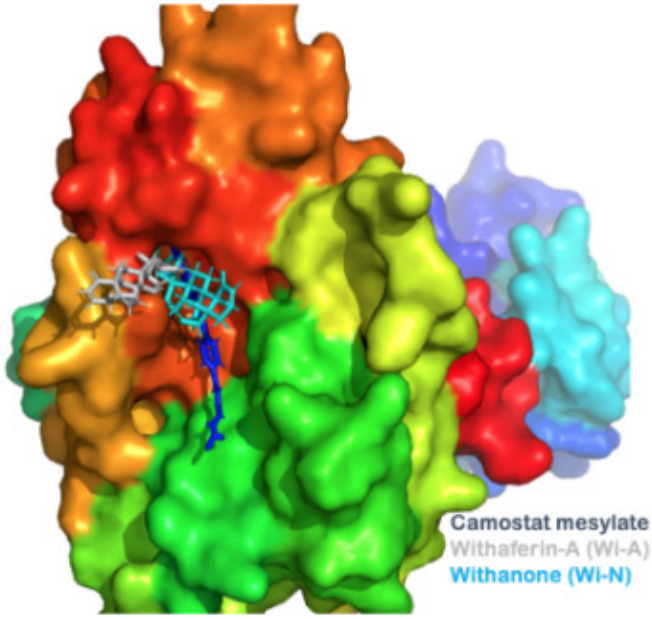

Figure 1: Surface view of TMPRSS2 showing binding of Wi-A, Wi-N and Camostat at its catalytic site.

Downregulation of cell surface proteins that are involved in entry of virus to host cells is another approach towards preventing transmission of coronavirus. Peptide-conjugated Phosphorodiamidate Morpholino Oligomers (PPMO), single-stranded-DNA-like antisense agents that targeted TMPRSS2 were shown to cause significant suppression of viral titers [14]. We examined if Wi-N, in addition to the predicted inhibition of TMPRSS2, have capability to diminish the expression of TMPRSS2 and discovered that human breast carcinoma (MCF7) treated with non-toxic doses of Wi-N had 40-50% reduction in TMPRSS2 transcript suggesting that Wi-N may have dual impact on TMPRSS2, i.e. inhibition of its catalytic activity as predicted by molecular modelling data and transcriptional downregulation of the protein. Both of these may collectively block the cleavage of viral protein and entry of the virus to host cells, effectively.

ASHWAGANDHA MAY INHIBIT SARS-COV-2 MAIN PROTEASE (MPRO) THAT IS ESSENTIAL FOR ITS SURVIVAL

Based on the above results, we next tested Wi-N for its capability to interact with SARS-CoV-2 main protease (Mpro) [15] that is crucial for its replication and assembly inside the host cell. SARS-CoV-2 Mpro and N3 co-crystalized complex was used as reference. N3 inhibitor showed interaction within the substrate binding pocket of the SARS-CoV-2 main protease; the two main residues forming polar interaction with the inhibitor were Thr190 and Glu166. Wi-A, Wi-N were able to dock comparable to that of N3 inhibitor (Figure 2). Detailed analysis showed that whereas Wi-A did not show good affinity for Mpro, Wi-N interacted with a residues important for the catalytic activity of Mpro suggesting that it has potential to inhibit SARS-CoV-2 Mpro.

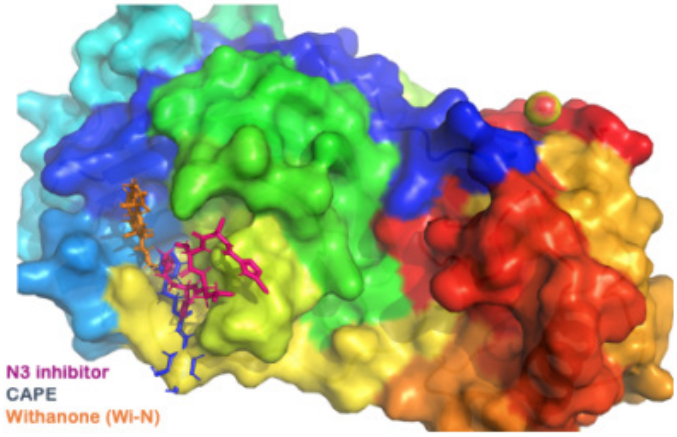

Figure 2: Surface view of viral Mpro protein showing N3 inhibitor and Wi-N embedded at the same region.

Based on these predictions (Wi-N, but not its closely related withanolide,Wi-A is capable of interacting with and inhibiting viral Mpro), it was extremely important that we define the source of Wi-N. We examined extracts of Ashwagandha root, stem and leaves for their Wi-A and Wi-N content and discovered that Wi-N is enriched in Ashwagandha stem. Together, it is strongly predicted that Wi-N possesses preventive and therapeutic potential for COVID-19. Ashwagandha stem and/or its Wi-N rich extract may provide an easy and economic resource for management of the COVID-19 pandemic. Interestingly, two clinical trials for Ayurvedic formulation have also been initiated in India by Ministry of Aayush, Government of India in collaboration with Council of Scientific & Industrial Research (CSIR). These formulations include Ashwagandha and may provide the clinical proof and efficacy to the bioinformatics and laboratory results discussed here (Figure 3).

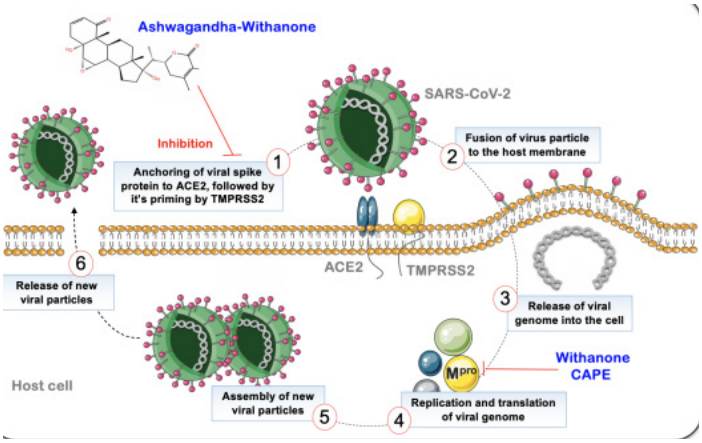

Figure 3: Schematic representation of host and viral proteins being targeted by natural compounds to be used against SARS-CoV-2 for the prevention and treatment of COVID -19.

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