Research Article

Mohamed Mohamady Ghobashy*, Dalal Mohamed Alshangiti, Sheikha A. Alkhursani, Samera Ali Al-Gahtany, Abeer S. Meganid, Mohamed Madani, and Ahmad S. Kodous

Aspects of the physiochemical properties of SARS-CoV-2 to prevent S-protein receptor binding using Arabic gum

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Abstract: With the number of people infected with the new coronavirus exceeding millions of confirmed infections, the world is turning to scientists and researchers, everyone is waiting – impatiently – for the results of the research that is being carried out in full swing to find an effective treatment for the virus. The recent development of the virus has witnessed at least 17 mutations that may affect its external shape, especially on the S-protein receptor-binding domain (RBD), which helps it attach to human cells' receptor angiotensin-converting enzyme-2 (ACE2) to make RBD–ACE2 interaction and entry to host cell. This interaction becomes stronger in the new strains of the coronavirus due to a mutation that occurs in the S-proteins that attach to human cells. For researchers and scientists to be able to confront this pandemic that has spread in the world like wildfire, they must be armed with accurate understanding and clear knowledge about coronavirus. This study focuses on polysaccharides, specifically negatively polysaccharides, that can interfere with the positive charge of the surface

of the SARS-CoV-2 and ACE2, thus inhibiting the virus's infectivity and destroying it. In addition, polysaccharides will boost the immune function of the vaccine, thereby fostering nonspecific immunity of the body and specific immunity of the body, cellular immunity, mucosal immunity, humoral immunity, and decreased pro-inflammatory expression. This research aims to reduce the attachment power and modify the pulling apart of the RBD and the angiotensin-converting enzyme 2 (ACE2) by polysaccharide molecules such as Arabic gum (AG) and carrageenan. The adapted fluorometric assay is used to investigate the probability of Arabic gum and ACE2 interactions. The obtained results confirmed that the interaction could take place between Arabic gum and ACE2. Several literature studies promote the use of the urchin egg as antiviral, especially for SARS-CoV-2, because it has sulfated fucan polysaccharide molecules that prevent interaction of SARS-CoV-2 with a host cell. But, to the best of our knowledge, we found that the effect of urchin egg as antiviral, especially for SARS-CoV-2 is very difficult due to the presence of immunoglobulin G (IgG) in the human cells containing sugars that terminate with N-glycolylneuraminic (Neu5Ac) as found in the sperm of sea urchin. So, most probably an interaction has occurred between Neu5Ac in IgG of human cells and sulfated fucan polysaccharide molecules of urchin egg.

Keywords: Arabic gum, RBD–ACE2 interaction, coronavirus, S-proteins

* **Corresponding author: Mohamed Mohamady Ghobashy**, Radiation Research of Polymer Chemistry Department, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority, P.O. Box 8029, Nasr City, Cairo, Egypt, e-mail: Mohamed.ghobashy@eaea.org.eg, Mohamed_ghobashy@yahoo.com, tel.: +20-222727413, fax: +20-222749298

Dalal Mohamed Alshangiti, Sheikha A. Alkhursani,

Abeer S. Meganid, Mohamed Madani: Faculty of Science and Humanities – Jubail, Imam Abdulrahman Bin Faisal University, Jubail, Saudi Arabia

Samera Ali Al-Gahtany: Faculty of Science, University of Jeddah, Jeddah, Saudi Arabia

Ahmad S. Kodous: Radiation Biology Department, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority, Cairo, Egypt

ORCID: Mohamed Mohamady Ghobashy 0000-0003-0968-1423

1 Introduction

The British government announced the discovery of a new variant of the emerging coronavirus in some areas of the country and indicated that investigations are continuing regarding this “surge” in the virus. The new mutation of the virus was identified after an increase in cases of this mutated type, in London and Kent County, in the southeast of the country. The new type of virus was

named “VUI-202012/01” [1]. The recent development of the virus has at least 17 mutations that may affect its external shape, especially on the S-protein, which helps it attach to human cells. It is possible that this mutation, in this particular part, gives the SARS-CoV-2 a greater ability to infect and spread more easily between people. The latest variant of the virus carries a set of mutations, including the N501Y mutation [2]. The N501Y mutation specifically occurred on the part that promoted the binding of virus protrusions receptor-binding domain (RBD) to the ACE2 receptors carried by human cells [3–5]. Not the first, another image of previous outcrops, preceded by another 614D mutation in the RBD [6–8], did not increase the risk of infection [7]. For nearly a year, the world remained hostage to the health conditions and safety measures imposed by the COVID-19 pandemic on the planet. Governments imposed a complete closure for about a year or more in some countries, and they partially returned sectors to work, not at their full capacity, in addition to imposing comprehensive closures in days in certain areas or specific areas. In most of these times, human activity, movement, trade, economy, culture, and freedom of movement had ceased. At a time when the government decided to provide aid packages to the most affected sectors, other sectors like this one related to the marine environment were overlooked, considering that it is a nonvital sector, although its damage is greater than that of many sectors. The waste treatment sectors were suspended, and this affected the environment in the long run, while those who could work were satisfied with the minimum levels of it, which led to significant economic impacts on their workers. Today, we stand on the cusp of the end of the first year of the COVID-19 pandemic, while uncertainty overwhelms everything, especially since we do not see an imminent end to the pandemic, which calls for an assessment of the damage caused to the various sectors, and we must present a roadmap for how we should manage in these sectors in the event of a prolonged crisis.

From this standpoint, this research provides a physical and chemical conception of the coronavirus to understand more about its nature and what helps in saving the lives of people and reducing the size of the damage caused by the pandemic, its devastating effects, and also the ability of sectors to adapt, and whether some of them were able to cope with the current situation and continue to provide its products and exploring some of the successful business models developed by the sectors to neutralize the effects of the pandemic, or at least reduce its effects.

The possibility of chemical treatment will also be discussed, according to what will be explained later.

What we learn from the emerging coronavirus crisis, similar to what we have experienced in previous epidemics, is important to be organized. While there are various scenarios, all of them presume that the coronavirus can propagate in waves, which means that it must be cyclical in the process of tackling it. Countries not yet affected should begin to prepare while beginning to develop response plans. This would facilitate the process of adjustment once the crisis occurred and reduce its negative effects to a minimum. Accordingly, understanding the nature of the virus chemically is our main goal to preserve human revolution.

In this field, the role of scientists is prominent to find solutions from different points of view and build on them. We will build on our scientific experiences in the field of chemistry and physics to increase the understanding of the virus, as it will be dealt with as a chemical substance. Health officials and health policymakers can use this information in research as an opportunity to create new models for sterilization, medicine, and vaccines. The structure of SARS-CoV-2 is like SARS-CoV-1, which is composed of a protein membrane with a diameter of 50–200 nm, and inside there is RNA [9]. The nanosize gives viruses a large surface area like several nanomaterial compounds [10–18]. The virus consists of four types of structural proteins that contribute to the formation of the body structure of the virus, including the protein (S), which is known as (the itch protein), which forms the spinal protuberances on the surface of the virus and gives it the characteristic coronary shape [19]. The results of the study showed that the mutations that occurred on the emerging coronavirus made it more fierce and dangerous, and although both viruses bind to the same receptor on human cells – the receptor for angiotensin-converting enzyme-2 (ACE2) – the emerging coronavirus can bind to it more tightly. Thanks to the mutations that occurred to the genetic material and resulted in changes in the virus's structure as a result of changing some amino acids in the receptor-binding domain, which is the part that binds to the ACE2 receptor, known as RBD, which led to an increase in its affinity for and suitability to these receptors [20]. It was found that virus proteins stick to the cells of the human airway so that the virus can enter [21]. The elasticity of proteins holds great importance for the success of the virus and its attachment to the surface of the respiratory tract cells. This study aims to study the sites that reduce the binding strength and change the pulling apart of the RBD and the ACE2 by polysaccharide molecules. The electrostatic binding of viruses and polysaccharide molecules was studied *in vitro* by Kumar et al. [22]. The authors used influenza, fibrinogen, and adenoviruses

as a model of viruses that immobilized on the substrate brushes from polysaccharides having groups of α -mannose, β -glucose, or β -galactose besides ionizable groups like amino-methyl to create positively charged sites on the surface of substrates. The level of viruses attached on the substrate was determined by measuring the change of the carbohydrate layer resistance. The polysaccharide molecules and amino-methyl groups as positive sites were co-immobilized in different ratios. The results indicated that low amounts of viral particles were adsorbed on the substrate. This is due to the electrostatic repulsion between positively charged sites that effectively blocked the virus from interacting with the substrate coated by polysaccharides. A previous study recognized that the electrostatic interaction or repulsion between viruses and polysaccharide molecules can be achieved. From this aspect, we chose polyanionic carbohydrates to bind with ACE2 to protect them from coronavirus.

2 Materials and methods

2.1 Screening assay of ACE2 inhibition

An ACE2 Inhibitor Screening Assay Kit with a Fluorogenic Substrate (Catalog #79923) was provided by BPS Bioscience (San Diego, CA), which was modified to evaluate the exopeptidase activity of ACE2 in the presence and absence of inhibitors. Following the manufacturer's instructions, the fluorescence assay was carried out in a black flat-bottom 96-well plate with a final reaction volume of 50 μ L. The Arabic gum (AG; provided by Merck-Schuchardt) and DX600 compounds were prepared in various concentrations of 100, 10, 1, and 0.1 μ g·mL⁻¹. Triplicates of each experiment were carried out. Each plate contained a positive control of enzyme treated with vehicle alone (10% DMSO), and a blank control with no enzyme. Each reaction consisted of 20 μ L of recombinant human ACE2 protein (0.5 ng· μ L⁻¹) in ACE2 buffer, 5 μ L of Arabic gum (different concentrations to each well-designated test inhibitor), or 5 μ L of standard ACE2 inhibitor (DX600 various concentrations) to each well-designated comparison inhibitor. About 5 μ L of 10% DMSO in water (inhibitor buffer) was added to the wells designated "positive control" and "blank." About 25 μ L of ACE2 fluorogenic substrate was also added. The total volume of the reaction was 50 μ L. The reaction mixtures were kept at room temperature for 2.5 h, sheltered from light. After that, the fluorescence intensity of the samples was determined using a Beckman Coulter DTX880 multimode plate reader (excitation = 555 nm; emission = 585 nm). The IC₅₀ value was calculated using GraphPad prism program 8.4.3.

3 Results and discussion

3.1 The most prominent information about the virus

A virus is a genetic material usually found inside an organic particle that invades living cells, and it works to produce new generations of virus particles by using the metabolism of the host body [23]. It is worth noting that these viruses use different methods when doing this. Some viruses introduce their genetic material into the host's DNA. When the host cell reproduces, it produces some new viruses at the same time. And some other viruses can cause the host cell to explode during its expansion in numbers, and this process or state is known as the lytic cycle of reproduction, and it must be noted that the sizes of viruses vary from each other. The nature of viruses has baffled many researchers and scientists, especially whether they are considered living organisms or not. Finally, they are considered inactive particles moving through the environment. Once they become part of the living cell, viruses acquire the living characteristics of these host cells, in addition to their biochemistry metaphor for this living cell to carry out the process of reproduction within it. As for the composition of viruses, they consist of a nucleus of the genetic material and this genetic material may be DNA or it may be RNA; these viruses are usually surrounded by a protective layer called the capsule (capsid), which consists of protein. In some cases, this cover may be surrounded by another spiky envelope, and it must be noted that the virus can attach to the host cells and enter inside them as well. Viruses can be considered living organisms because of their DNA and their ability to reproduce and regenerate. At the same time, they cannot be considered as living because of their inability to read the information contained in these nucleic acids that they own and deal with independently, that is, they cannot reproduce unless they are present inside the body of a host. As for the DNA, whether DNA or RNA is found inside the virus, it could be an independent one, or it could be a duplicate, in addition, that it is what makes up the genome, or the sum of the genetic information for this virus. Viral genomes are usually characterized by being small in size in general, as they are encoded only for essential proteins such as capsule proteins, enzymes, and proteins needed for proliferation within the host cell. Viruses are infectious agents that are characterized by a set of living and nonliving characteristics, and it is possible for these viruses to infect animals, plants, and even microorganisms as well. Among the

most important and prominent of these characteristics are the following: they multiply at good and wonderful rates, but this is only done once inside the host cell, and they can mutate and change. As for the nonliving characteristics, they are the following: they are considered acellular, as they do not contain the cytoplasm or even any of the other cellular organelles. Most viruses contain DNA or RNA, but not both. Viruses cannot metabolize alone, and they need the host cell to reproduce, and their reproduction is usually through the metabolism of this host cell. That is, these viruses, in general, do not grow or divide, and instead, new virus components are synthesized, assembled, and formed inside the host cells infected with this virus. Coronaviruses are positive overall RNA viruses enveloped in the cytoplasm that replicate [24]. The coronavirus-cell entry method involves fusing the envelope with the cellular membrane controlled by viral S-proteins to carry the nucleocapsid into the human host (Figure 1) [24].

3.2 Spike proteins (S-protein)

S-protein is present on the surface of viruses that allows them to bind and enter the host cells to cause a bad infection. S-Protein is a trimeric class I TM glycoprotein found in all types of HCoV and other viruses like AIDS, influenza, paramyxo, and Ebola. Like other coronaviruses, the S-protein of SARS-CoV-2 mediates receptor recognition, cell penetration, and fusion causing infection [25]. Glycoprotein (combined protein with carbohydrates) is present

on the lipid bilayer surface of cell membranes. It is hydrophilic and allows them to bind with other molecules, where they act in cell-cell recognition. Glycoprotein is a protein that includes chains of oligosaccharides covalently attached to amino acid groups. This reaction is known as the glycosylation process. The glycosylation process is a highly important mechanism of secondary amino protein within cells. It plays a critical role in determining the structure, function, and stability of the protein. The glycoprotein is found on the surface of the viruses. Then, the viral envelope fuses with the cell membrane of the host and capsid of the viral genome to infect the host cell (Figure 2). Based on their structure and the mechanism of production, there are three kinds of glycoproteins: N-linked glycoproteins, O-linked glycoproteins, and nonenzymatic glycosylated glycoproteins.

Also, several receptors on the cell surface are glycoproteins. Glycoproteins frequently function like receptors of other glycoproteins. It causes a chain reaction within the cell when a specific molecule binds to its receptor. The desired result will be produced by this chain response. Protein glycosylation involves the attachment of a glucose moiety to a protein molecule. For protein molecules involved in cell membrane development, it is a typical post-translational modification. It includes the molecules glucose, mannose, and N-acetylglucosamine. More than 50% of them are considered to be potentially glycosylated in the particular case of human proteins, most of them at several glycosylation sites.

The RBDs of the N-terminal domain (NTD) and C-terminal domain (CTD) are found on the surface of the coronaviruses (S-protein). Polysaccharides, specifically

Figure 1: Structure and information of SARS-CoV-2.

Figure 2: (a) The coronavirus has a large number of spike glycoproteins (S-protein). S-protein exists in trimeric form, having an RBD domain unit in each subunit (S1 and S2) that binds to the (ACE2) receptor on the human cell membrane. The RBD-ACE2 interaction occurs in a part of the cell membrane of the heart, kidneys, lungs, and intestinal epithelium. As mentioned above, the virus spike protein/receptor pairing is a key to infecting a new host cell by RBD-AEC2 interaction. The angiotensin-converting enzyme 2 (ACE2) is a mono-carboxypeptidase that hydrolyses angiotensin II. This means ACE2 is negatively charged [31,32]. So, if we used polyanionic polysaccharides, they could prevent RBD-AEC2 interaction. Many polyanionic polysaccharide efforts neutralize antibodies of RBD to prevent viral entry and fusion with host cells. (b) This model shows the surface SARS-CoV-2 virus has spike proteins that infect the human host cells; the polysaccharide molecules (especially anionic) shield the folded peptide inside. The green site is charged hydrophilic regions, and the blue site is hydrophobic regions. The two regions help polysaccharides to attach to the protein of virus's human cells that would be effective to prevent RBD-ACE2 interaction.

negatively charged polysaccharides, can interfere with the positive charges of the surface of the virus, thus inhibiting the virus's infectivity or directly destroying it. Polysaccharides will boost the immune function of the

vaccine, thereby fostering nonspecific immunity of the body and specific immunity of the body including cellular immunity, mucosal immunity (MI), humoral immunity (HI), and decreased pro-inflammatory expression [26].

3.3 The new strain of coronavirus

The world has entered strongly in a second wave, more ferocious from the spread of the new mutation of coronavirus that occurred in the proteins that attach to human cells, with infections reaching record levels in Europe and other parts of the world, at a time when the efforts of research institutions and pharmaceutical companies have not yet succeeded to adopt a vaccine that stops this rapid spread, despite the arrival of a number of them. Many have reached advanced levels of clinical trials. In a new attempt to explain this mystery, an international team led by Daly et al. discovered a second key to coronavirus connection with cells, making the virus more contagious and widespread in the body; the protein is neuropilin-1 (NRP-1) [27]. Neuropilin-1 is present in the cells lining the nasal cavity, which makes it suitable for the virus to establish a refuge inside our bodies and multiply to form a viral family, before spreading by moving to a new host. The study revealed that neuropilin-1 is considered an extra piece of protein that allows the coronavirus to adhere to host cells for a sufficient period before they enter. As shown in Figure 2, it is known that the first key to coronavirus connection with human cells is the ACE2 receptor, which was published in several studies, most notably a previous study that had compared the emerging coronavirus and its closest relative of the coronavirus family, which is a virus, and concluded that genetic mutations that occurred on coronavirus made it more virulent and dangerous than SARS, and although both viruses bind to the ACE2 receptor, coronavirus can bind more tightly, and this helped it to spread more than SARS. The charge of neuropilin-1 is negative [28–30] and allows b1 domain binding of SARS-CoV-2.

3.4 SARS-CoV-2 blood clotting syndrome

Research indicates that clotting occurs when the SARS-CoV-2 virus attacks the endothelial cells that line blood vessels [33–35]. the virus binds to the ACE2 receptors located in the endothelial cell membrane, and as soon as it binds to the receptors, the blood vessels release the proteins that cause blood clots [33,36]. Studies also indicate that SARS-CoV-2 causes the body's immune system to stimulate an overactive inflammatory response, and this inflammation may cause blood clotting, and patients who need hospital care due to infection have other risk factors for blood clots. A new study found that one of the possible causes of blood clots in the context of COVID-19 is the presence of a certain level of a

protein in patients' blood called von Willebrand (VW) [37,38]. This protein is synthesized in endothelial cells and platelets, and its main function is to form a framework for adhesion. So far, how the level of this protein in the blood is regulated has not been fully studied; however, it is known that it is stored in vascular endothelial cells, and as soon as some damage occurs, such as that caused by the SARS-CoV-2 virus, blood clotting occurs. Which protein plays an important factor in its occurrence, and which is a complex process during which the blood forms clots is not clear. It is worth noting that the level and activity of this protein in the blood in humans vary significantly between healthy people, for example, it is found in men at higher rates than women, and in adults more than children, as well as among the elderly and young people. Research has linked this protein to the blood groups, as its levels are lower among people who have O blood type; in contrast, it is higher among those who have A blood type [39]. Low levels of VW protein are associated with patients called VW, which is a permanent disorder that causes bleeding in which the blood does not clot normally, and people with this disease have low levels of VW factor or the protein does not work as well as it should [40,41]. From the chemistry aspects, blood clot is formed due to an increase in the level of CO₂ (hypercapnia) and slow delivery of oxygen to the tissues. This situation may be due to the interaction of the positive charges of SARS-CoV-2 with the negative charges of O₂, thereby preventing the ventilation-perfusion process. This hypothesis may need further study.

3.5 Aspects to prevent S-protein receptor binding

The S-proteins present on the virus surface play a key role in coronavirus infection. S-proteins are a trimeric glycoprotein of class I TM necessary for viral fusion to host cells, and it exists in all types of coronaviruses. The S-protein contains two domains: an NTD S1 domain that is responsible for receptor binding and a C-terminal S2 domain responsible for fusion (Figure 3). The S1 domain containing RBD primarily functions for virus binding to the receptor of the host cell and is the important unit that binds to the receptor of human (ACE2), while the HR domain, including HR1 domain and HR2 domain, is closely linked to viral fusion [42]. As described above, the S-protein on the surface of SARS-CoV-2 is connected to the host cell of humans by recognizing the human receptor ACE2 [43]. ACE2 is present mainly in the intestine, lung,

Figure 3: The N-terminal and CTDs of SARS-CoV-2 S-proteins exhibit positive charges, while the human cells (ACE2) and NPR-1 have negative charges that could establish RBD–ACE2 binding.

kidneys, and heart [44]. In this section, we assessed how to reverse the RBD–ACE2 binding and inactivate SARS-CoV-2 using anionic polysaccharides. The S protein of SARS-CoV-2 is believed to be positively charged [31] more than SARS-CoV and may lead to interactions with other negative molecules through specific and nonspecific interactions. Predominantly, in human cells, ACE2 is negatively charged and may provide a salt bridge and a possible binding site between the RBD in the S-protein of SARS-CoV-2 (having positive charge), which was recently specified and identified as a salt bridge formation between Asp30 in (ACE2) the human cell with a positively charged Lys417 in the S-protein [45–47]. Hence, an increase in the amount of positively charged amino acids on the surface of SAR-CoV-2 could greatly increase the binding strength of SAR-CoV-2 as it passes ACE2 and enters the human host cells. This situation occurred for the new mutation of SAR-CoV-2, called D614G mutation.

3.6 Estimation of coronaviruses movement in water

The movement of coronaviruses as charged particles in water was estimated in water vapor, airborne, or through sneezing from an infected person. As mentioned above, coronaviruses are positive charged particles so we chose the sea urchin egg as a model to explain how charged particles can move in water easily. The sea urchin egg is

surrounded by a gelatinous extracellular matrix called the jelly (Figure 4). This jelly layer can cause egg mobility and sperm agglutination. According to their chemical compositions, the net charge of urchin egg and sperm are negative and positive, respectively. The sperm head consists of sialic acid polysaccharides such as N-acetylneuraminic acid (Neu5Ac) [48,49]. On the other hand, the surface of the jelly layer in the urchin egg consists of 20% short peptides and 80% sulfated fucan glycoproteins [50–52]. The negative charge of sulfated fucan polysaccharide molecules from the egg jelly regulates cell-cell adhesion with positively charged sperm. This situation is very close to the attachment of SARS-CoV-2 with the host of human cells as explained later.

Several literature studies promote the use of urchin egg as antiviral, especially for SARS-CoV-2 because it has sulfated fucan polysaccharide molecules that prevent interaction of SARS-CoV-2 with the host cell [53–56]. But, to the best of our knowledge, we found that the effect of urchin egg as antiviral, especially for SARS-CoV-2, is very difficult and impossible. Due to immunoglobulin G (IgG) being an antibody-protein found in humans, This antibody indicates that patient may have had COVID-19 in the recent past and have developed antibodies that may protect him from future infection. IgG glycosylation contains sugars that terminate with N-glycolylneuraminic (Neu5Ac) like found in sperm of sea urchin [57–59]. So, most probably, an interaction occurred between Neu5Ac in

Figure 4: The interaction of egg and sperm of sea urchin taking place via the sperm protein.

IgG of human cells and sulfated fucan polysaccharide molecules of urchin egg.

3.7 Reversing RBD–ACE2 binding by polysaccharide molecules

This study proposed the unbinding of the S-protein from the RBD with the receptor of human cells (ACE2). The stability of RBD–ACE2 binding is equal to $71 \text{ kcal}\cdot\text{mol}^{-1}$. Both spikes of SARS-CoV-2 and SARS-CoV show different binding strengths. Several other investigations have reported that SARS-CoV-2 is much more infective unlike SARS-CoV and SARS-CoV-2 displays much greater membrane fusion relative to SARS-CoV because its spike comes into contact more strongly with ACE2 [60]. The molecular dynamics (MD) calculations run on RBD–PD interactions of both SARS-CoV-2 and SARS-CoV viruses show that only 6 are retained in SARS-CoV out of 15 interactions of various forms [61]. Although SARS-CoV has various hydrophobic interactions, no salt bridges were found in the interaction with ACE2 [62,63]. Several different RBD–ACE2 interactions, which include 13 hydrophobic, 12 hydrogen bonds, a few salt bridges, and 6 electrostatic interactions and two electrostatic interactions, were found by the researchers. It became clear that stable RBD–ACE2 interactions were mainly due to the hydrophobic part of the RBD. This indicates a potent target for preventing infection with SARS-CoV-2 [64,65].

3.8 Information of Arabic gum polysaccharide

Arabic gum (acacia) is a polyanion edible biopolymer obtained naturally from exudates of *Acacia senegal* trees (Figure 5) and *Acacia seyal* that principally grows in Africa [66,67]. The source of the Arabic gum plant tree is *Vachellia nilotica* or *Acacia nilotica* from the Fabaceae family. *Vachellia nilotica* or *Acacia nilotica* is commonly known as a tree of Arabic gum, thorn mimosa, babul, Egyptian acacia, or *Senegalia senegal*. The Arabic gum trees are native to Africa, the Middle East, and India. Arabic gum gives a nonviscous liquid when dissolved in water or ethanol, which are fiber-rich. The significant purposes of Arabic gum (acacia) date back to the second century BC, when it was used as an ink and adhesive by the Egyptians. Arabic gum made its way to Europe over time, and it began to be called “gum Arabic” because it was shipped from Arab ports. Chemically, Arabic gum is a mixture of different macromolecules and mainly consists of carbohydrates and proteins. Figure 5 shows the proposed structure of Arabic gum as the wattle blossom model. Arabic gum acid is composed of six different carbohydrate molecules (such as galactose, arabinopyranose, rhamnose, arabinofuranose, 4-O-methyl glucuronic, and glucuronic acid) and four molecules of protein (such as hydroxyproline, serine, proline) with three fractions of AG – arabinogalactan 88%, AGP – arabinogalactan 10%, and GP – glycoprotein 1%, and they contain different

Figure 5: Illustration of the Arabic gum structure and how it can inactivate SARS-CoV-2.

contents of polypeptides (20, 50, and 30, respectively). The protein is localized on the AGP unit. In addition, Arabic gum contains more than 1,600 amino acids [68].

The properties and characteristics of Arabic gum have now been extensively investigated and developed to be used in various fields of industry, like textiles, lithography, ceramics, and pharmaceuticals. In the pharmaceutical industry, it is used as a thickener, a stabilizer, and an emulsifier agent in the pharmaceutical formulation (e.g., syrup and creams). Arabic gum is used as an active pharmaceutical ingredient for antimicrobial activity. Additionally, recent studies have highlighted that Arabic gum has antioxidant

properties, plays a role in lipid metabolism, and used in therapies for many degenerative diseases like kidney failure and cardiovascular disease, and shows good results. Therefore, ample evidence shows that Arabic gum may play a positive role in human health. Therefore, we aim to describe the general aspects of the effect of Arabic gum (positively charged surface) on the deactivation of the coronaviruses (negatively charged surface). This work was done to establish the efficiency of Arabic gum for coronaviruses treatment and their chemical properties. A number of physicochemical properties are listed below to improve their benefits to choose for coronaviruses treatment. Arabic

Table 1: Effects of Arabic gum ($0.1\text{--}100\ \mu\text{g}\cdot\text{mL}^{-1}$) on ACE2 enzymatic activity

Code	IC ₅₀	Concentration ($\mu\text{g}\cdot\text{mL}^{-1}$)	log	% inhibition	T2	T1	ΔT	RFU2	RFU1	ΔRFU	Slope	K. activity	EC
Arabic gum	4.688	100	2	86.63	30	0	30	13.37	0	13.37	3.3333	16	120
		10	1	52.98	30	0	30	47.02	0	47.02	3.3333	56.4	120
		1	0	30.61	30	0	30	69.39	0	69.39	3.3333	83.3	120
		0.1	-1	13.18	30	0	30	86.82	0	86.82	3.3333	104	120
EC				0	30	0	30	100	0	100	3.3333	120	120

gum that also includes polysaccharides beside glycoproteins has been shown to be immunopotential [69,70]. Research studies have shown that dendritic cells can be activated by Arabic gum [71]. Analysis of Arabic gum by XRD has shown that it is completely amorphous in nature; it is known that amorphous biomaterial is more effective than crystalline biomaterial and this is achieved for Arabic gum [12,72]. The major functional groups in Arabic gum have hydrophilic and hydrophobic characters [70,73–75].

3.9 The adapted fluorometric assay to investigate the probability of Arabic gum and ACE2 interactions

Effects of Arabic gum and ACE2 exopeptidase activity. We determined the effect of Arabic gum on the exopeptidase activity of ACE2 using an adapted fluorometric assay. We determined the effect of Arabic gum in comparison to DX600 as a potent inhibitor on the exopeptidase activity of ACE2 using an adapted fluorometric assay (BPS Bioscience). We revealed that Arabic gum at different concentrations (0.1, 1, 10, and $100\ \mu\text{g}\cdot\text{mL}^{-1}$) inhibited ACE2 activity by 13.18%,

30.61%, 52.98%, and 86.63%, respectively, with IC₅₀ value of $4.688 \pm 0.24\ \mu\text{g}\cdot\text{mL}^{-1}$ (Table 1 and Figure 6). The results showed that the most potent inhibition concentration of Arabic gum was $100\ \mu\text{g}\cdot\text{mL}^{-1}$ (86.63%). These results proved for the first time that Arabic gum is a targeted inhibitor for ACE2. DX600 (0.1, 1, 10, and $100\ \mu\text{g}\cdot\text{mL}^{-1}$) inhibited ACE2 activity by 40.36%, 62.72%, 86.15%, and 94.14%, respectively, with an IC₅₀ value of $0.235 \pm 0.01\ \mu\text{g}\cdot\text{mL}^{-1}$ (Table 2 and Figure 6).

COVID-19's spike protein has been shown to facilitate the virus's entry into the cell. One of the most effective ways to inhibit COVID-19 from spreading the infection is to prevent the spike protein from binding to the host cell's ACE2 [9,10]. Many small molecule compounds that inhibit SARS-CoV-2 virus infection have been found, as have licensed medicines that are likely to be repurposed for COVID-19 therapy, although there are currently a few effective treatments demonstrated in WHO clinical studies [11]. According to our findings, Arabic gum inhibits ACE2 internalization and hence inhibits SARS-CoV-2 virus infection. The molecular mechanism behind Arabic gum's inhibition of ACE2 internalization remains unknown. Arabic gum showed effective suppression of ACE2 internalization owing to pseudovirus infection, while Arabic gum inhibited SARS-CoV-2 infection in a concentration-dependent manner but not through a competitive mechanism. Kato et al. [11] revealed that at high concentrations, some compounds bind to the S protein at the RBD domain's end, inhibiting the ACE2-S protein interaction. However, the weak binding and the inhibition potency impact on SARS-CoV-2 infection by them are not completely explained [11]. Arabic gum prevents SARS-CoV-2 virus-induced ACE2 internalization, and more studies are needed to pinpoint a molecular target. According to Anderson et al. [12], six identified RBD amino acids (Leu455, Phe486, Gln493, Ser494, Asn501, and Tyr505) were used to map the binding site of SAR-CoV-2 RBD. These amino acids have been demonstrated to be important for binding to ACE2 receptors and for defining the host range of SARS-CoV-like viruses [12]. According to Dwarka et al. [13], the anti-SARS-CoV-2 potential of

Figure 6: Arabic gum (AG) in comparison to DX600 noncompetitively inhibits ACE2. DX600 IC₅₀ = $0.235 \pm 0.01\ \mu\text{g}\cdot\text{mL}^{-1}$ and Arabic gum IC₅₀ = $4.688 \pm 0.24\ \mu\text{g}\cdot\text{mL}^{-1}$.

Table 2: Effects of DX600 (0.1–100 $\mu\text{g}\cdot\text{mL}^{-1}$) on ACE2 enzymatic activity

Code	IC ₅₀	Concentration ($\mu\text{g}\cdot\text{mL}^{-1}$)	log conc	% inhibition	T2	T1	ΔT	RFU2	RFU1	ΔRFU	Slope	K. activity	EC
DX600	0.235	100	2	94.14	30	0	30	5.86	0	5.86	3.3333	7.03	120
		10	1	86.15	30	0	30	13.85	0	13.85	3.3333	16.6	120
		1	0	62.72	30	0	30	37.28	0	37.28	3.3333	44.7	120
		0.1	–1	40.36	30	0	30	59.64	0	59.64	3.3333	71.6	120
EC				0	30	0	30	100	0	100	3.3333	120	120

active chemicals identified in South Africa traditionally utilized herbs. The compounds were evaluated in silico for their ability to inhibit 3 CLpro, SARS-CoV-2 RBD, and SARS-CoV-2 RdRp. Molecular docking demonstrated that Arabic acid from *Acacia senegal* discovered in *Sutherlandia frutescens* binds to the active site of 3CLpro with favorable binding modes and strong interactions [13]. Dwark et al. [13] revealed that Arabic acid from *Acacia Senegal* bound strongly with SARS-CoV-2 3C-like main protease (3CLpro) binding site residues, HiArabic gum63 [14], Val308, Ser144, Leu141, Asn142, Gly143, and CyArabic gum45 [15] by hydrogen bonds. Ser144, CyArabic gum45, Leu141, and Asn142 all appear to be important for inhibitor binding such as Arabic acid [13]. It was reported that the common cold, flu, respiratory disorders, and viral infections can all be treated with Arabic acid [16] because it has the best docking scores for SARS-CoV-2 biological targets, which should be investigated further as a possible SARS-CoV-2 therapy [13]. In previous research, Towler et al. [17] reported that ACE2, a carboxypeptidase, is a type I integral membrane protein consisting of about 805 amino acids that belong to zinc metalloproteases with structural homology for a catalytic motif, containing one HEXXH + E zinc-binding consensus sequence and binding sites for inhibitors or specific substrates, respectively [17]. The essential residues of ACE2 for binding with zinc are His374, His378, and Glu402 [17]. Many studies revealed ACE2 as a human cell receptor for SARS-CoV-2 [18–20] as well as a protector of the lung against stark COVID-19 complications [21] and acute respiratory distress syndrome (ARDS) [22]. Olaleye et al. [23] showed clioquinol (CLQ) as an inhibitor of SARS-CoV-2 by metal and zinc chelation, resulting in the inhibition of the interaction of rhACE2 with SARS-CoV-2 spike (RBD) protein, all critical steps/processes in the pathogenesis of COVID-19 [23].

4 Conclusion

Polysaccharides, specifically negatively charged polysaccharides, can interact with the positive charges on the

surface of the virus, thus inhibiting the virus's infectivity or directly destroying it. Polysaccharides will boost the immune function of the vaccine, thereby fostering non-specific and specific immunity of the body including cellular immunity (CI), MI, HI, and decreased pro-inflammatory expression. It is indicated that the anionic polysaccharides such as Arabic gum and carrageenan can inhibit the activation of coronaviruses, especially SARS-CoV-2 by preventing the pathway of RBD–ACE2 interaction, which may help to suppress the danger of coronavirus. Arabic gum at different concentrations (0.1, 1, 10, and 100 $\mu\text{g}\cdot\text{mL}^{-1}$) inhibited ACE2 activity by 13.18%, 30.61%, 52.98%, and 86.63%, respectively, with an IC₅₀ value of $4.688 \pm 0.24 \mu\text{g}\cdot\text{mL}^{-1}$. The results showed that the most potent Arabic gum inhibition concentration was 100 $\mu\text{g}\cdot\text{mL}^{-1}$ (86.63%). These results proved for the first time that Arabic gum is a targeted inhibitor for ACE2. Understanding how anionic polysaccharides play an important role in preventing RBD–ACE2 interaction will give new ideas for inactive and SARS-CoV-2 and COVID-19 treatment. Polysaccharides, specifically negatively charged polysaccharides such as Arabic gum and carrageenan can interact with the positive charges of SARS-CoV-2, thus inhibiting the SARS-CoV-2 infectivity and directly destroying it. But, we prefer Arabic gum in COVID-19 treatment as it contains about 1,500 different types of amino acids that can improve the vaccine immune effect, thus promoting body nonspecific and body-specific immunity. In addition, Arabic gum is preferred rather than other polysaccharide molecules because it has hydrophilic and hydrophobic regions; besides, it is a composite of protein and carbohydrate like glycoproteins found on the surface of SARS-CoV-2. Several literature studies promote the use of urchin egg as antiviral, especially for SARS-CoV-2 because it has sulfated fucan polysaccharide molecules that prevent interaction of SARS-CoV-2 with the human host cells. But, to the best of our knowledge, we found that the effectiveness of urchin egg as antiviral, especially for SARS-CoV-2, is very difficult and impossible. Due to IgG being antibody proteins found in human had infected by COVID-19 in the recent past, IgG glycosylation contain sugars which terminate with N-glyco lylneuraminic (Neu5Ac) like founded in sperm of sea urchin. So, most probably an

interaction occurred between Neu5Ac in IgG of human cells and sulfated fucan polysaccharide molecules of urchin egg.

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