Scope of Natural Plant Extract to Deactivate COVID-19

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Research Article

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Abstract

The outbreak of coronavirus disease 2019 (COVID-19) has emerged as a severe threat for public health and economy throughout the world. The structure of corona virus is composed of RNA based proteins that contains amino (-NH2) and carboxyl (-COOH) groups. It includes nucleocapsid protein (N-protein), spike protein (S-protein), envelope and hemagglutinin-esterase dimer (HE). These proteins affect adversely on human gastrointestinal system, heart, kidney, liver, and central nervous system leading to several organ damages. This investigation reveals that the extracted components of natural plants, especially hydroxyl (-OH) groups react chemically to deactivate the active components of the virus by esterification process. As a case study, using one of the natural resources, as for example, licorice (*Glycyrrhiza glabra*) which has the components of glycyrrhizin, glycyrrhetic acid, liquiritin and isoliquiritin that can be used to neutralize the activeness of COVID-19 and it can be used as an antiviral drug. The extracted licorice is further processed with PVA solution to form antiviral nano-membrane for potential application as wound dressing materials, musk, gloves and against skin infection by electrospinning. The morphology of the membrane is characterized using scanning electron microscope (SEM). The research suggests that the other plants having deactivate components against virus can be applicable to resolve the human health crisis of the globe.

1. Introduction

Yet now, researchers have been observed three different pathogenic human coronaviruses (CoVs), such as Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV), and a 2019 novel coronavirus (COVID-19) [1-3]. Among them, recently, the people of the world faces the biggest challenges of COVID-19 in relation to health issue. This COVID-19 has transmitting exponentially throughout the globe. Coronaviruses are relatively large in size occupying single-stranded positive-sense RNA genome enclosed in a membrane envelope.

In fact, this coronavirus is composed of four structural proteins, including spike (S), envelope (E), asamembrane (M), and nucleocapsid (N) proteins [2, 4, and 5]. Spike (S) protein is very crucial in connection of viral attachment, fusion and entry and this protein can be utilized to develop antibodies, entry inhibitors and vaccines [1, 6-12]. Human pathogenic coronaviruses (severe acute respiratory syndrome coronavirus [SARS-CoV] and SARS-CoV-2) bind to their target cells through angiotensin-converting enzyme 2 (ACE2), which is recognized by epithelial cells of the lung, intestine, kidney, and blood vessels [13]. Angiotensin-converting enzyme 2 (ACE2) is a membrane bound amino peptidase that effects on cardiovascular and immune systems [14]. Some literature [15, 16] mentioned that this virus effects in the lower respiratory system which is responsible for viral pneumonia. In addition to that, it may also deteriorate the conditions of the gastrointestinal system, heart, kidney, liver, and central nervous system leading to several organ damages. Following the adverse impact of a pandemic, researchers have been trying to realize nature of this new virus and the pathophysiology of this virus to seeking the

probable treatment process and searching out the reasonable therapeutic agents and vaccines. There are several attempts have been taken to find out the recovery system even though, there are no SARS-CoV-2-specific antiviral agents [17].

In this study, research has been carried out to find out the possible mechanisms to save the lives of people and produce the vaccines and drugs for future prevention.

The practice of using medicinal plants to treat human skin including healing of wounds and burn injuries, antifungal, antiviral, antibacterial applications against skin infections has long history and is going on for primary healthcare even in the modern age [18-20].

Usages of metal nanoparticles like as silver, zing in the existing wound dressing materials is not environment friendly due to its toxicity effect [18]. The altrtnative environment friendly similar materials such as licorice, neem, turmeric, honey, nigella etc. natural herbs have significant effect due to their inherent biomedicinal properties. As a result, the development of electrospun nanofibrous membrane by electrospinning technique from natural polymers is considered as novel approach in biomedical applications such as wound dressing, tissue engineering and drug delivery, leads to contemporary research field.

Synergistic effects of licorice on nanofibrous membrane have not yet been studied thoroughly. Thus, the newness of the present study is to fabricate nanofibrous membrane with licorice extract to explore its antibacterial and antiviral medicinal properties also.

2. Methodology

2.1 Virus Deactivation Strategy

The structure of RNA based COVID 19 virus is depicted in Figure 1. This virus is composed of different distinct proteins. The chemical and molecular structure of these proteins are different in nature. The human body is badly affected by this virus. The common symptoms of this virus are mentioned in Figure 2. The process of this research is to understand the chemical structure of these proteins for analyzing their effects. These most of the proteins of COVID 19 are formed by the amine ($-NH_2$) and carboxyl (-COOH) functional groups. Some chemical reaction oriented strategies are developed to neutralize the effect of amine ($-NH_2$) and carboxyl (-COOH) functional groups to protect the adverse effect of corona virus on human body. The leaves of hydroxyl group and bio-ingredients are processed for reacting with the amino acid compound to neutralize the chemical and molecular bonding of functional groups of S, N and M proteins.

Fig. 1: Diagram of coronavirus virion structure showing spikes that form a "crown" like the solar corona, hence the name [21]

Fig. 2 Common symptoms and severe disease due to COVID-19 [22]

2.2 Preparation of PVA-Licorice Solution for Nanofibrous Membrane

The licorice roots were collected from the local area of Gazipur city, Dhaka, Bangladesh. Prior to extraction, licorice was checked to ensure that materials are free from dirt and impurities. Then, a small piece of licorice was oven-dried at 45°C and it was kept under water at ratio of 1:10 (w/v%) for 24hours. The crude extract of licorice was then filtered through nylon mesh three times.

On the other hand, 10gm polyvinyl alcohol (PVA) with molecular weight (MW) of 1, 15,000, DP of 1700–1800, viscosity: 26–32 cps, 99% hydrolyzed granules was sourced from Loba Chemical (India). PVA was mixed with 90mL deionized water to obtain 10wt % (w/v) solution. This mixture was stirred and heated to 80°C to get a clear and transparent solution.

Finally, the prepared PVA solution was blended with licorice solution at 50:50 ratio. A magnetic stirrer was used to make uniform and homogenous solution for 1hour. The prepared solution is then transferred via a syringe (30mL) to the pump of electrospinning machine.

Electrospinning machine (model TL-01, TONG LI TECH) shown in Fig. 3 was used to produce the nanofibrous membrane using PVA and licorice extract under optimized processing parameters.

Fig. 3 Electro-spinning machine for preparation of membrane

2.3 Morphological Analysis

The morphological analysis of nanofibrous membrane was observed by scanning electron microscopy (SEM) (SU 1510, Hitachi, Japan) at a magnification of × 1.5K and × 2.0K with a voltage of 10 kV to achieve actual images.

3. Results And Discussion

3.1 Mechanism of Deactivation COVID-19

Coronavirus disease 2019 (COVID-19) is a respiratory illness which is spread very rapidly from person to person. COVID-19 is a RNA based infected virus which is formed by nucleocapsid protein (N-protein), spike protein (S-protein), envelope and hemagglutinin-esterase dimer (HE). The nucleocapsid protein (N-protein) is a structural protein that binds to the coronavirus RNA genome, thus creating a shell (or capsid) around the enclosed nucleic acid. The spike protein performs two primary tasks that aid in host infection: 1) mediates the attachment between the virus and host cell surface receptors, and 2) facilitates viral entry into the host cell by assisting in the fusion of the viral and host cell membranes.

The CoV envelope (E) protein is a small, integral membrane protein involved in several aspects of the virus' life cycle, such as assembly, budding, envelope formation, and pathogenesis [23- 25]. The active components of these proteins are amino (-NH2) carboxyl (-COOH) groups [26]. The natural plant extract can be used to deactivate the active components of this virus. As a case study, licorice was selected as a sample to neutralize the activity of virus proteins. Similar agreements are found in literature [27-30] that state "licorice root extract is effect against HIV, RSB, herpes viruses and severe acute respiratory syndrome-related corona virus which causes a serious type of pneumonia". The contents of licorice are glycyrrhizin, glycyrrhetic acid, liquiritin and isoliquiritin [31] that are able to control the spreading of virus activity. Chemical structure of licorice components are illustrated in Fig. 5.

Fig. 5 Chemical structure of licorice components (a) glycyrrhizin, (b) isoliquiritin, (c) glabridin (d) glycyrrhetic acid and (e) liquiritin

Formation of licorice root extract in Fig. 6 can be effective to diminish the virus growth and having possibility to be used as antiviral drug.

Fig. 6 Formation of licorice extract solution.

All contents of licorice have hydroxyl groups that can attack the active group of virus. The mechanism is presented in Fig. 7.

Fig. 7 Mechanism of deactivation of COVID-19 using active component of plant extract

3.2 Characterization of Licorice Nanofibrous Membrane

The extraction solution of licorice was mixed with PVA solution at 50:50 ratio to form membrane by electrospinning machine. Voltage, pressure, heater power and collector distance and ambient condition were the parameters of electrospinning process. The optimum electrospinning conditions were reached by trial and error method at -12.3kV, +23 kV, 0.45 kW with a flow rate of 1.5 mL per hour, constant collector distance of 15 cm under ambient condition of 65% relative humidity and 27^0C respectively. The scanning electron microscope (SEM) image of the developed samples are shown in Fig. 8 which reflects the formation of smooth nanofiber having an average diameter of 245 nm.

Fig. 8: Morphology of electrospun PVA-licorice (a) natural image of membrane (b) SEM image at 10.0 kV, x1.00 k of front site view (c) SEM image at 10.0 kV, x1.00 k of back site view

4. Summary And Perspectives

RNA oriented COVID-19 virus is structured by some proteins which infect the internal parts of the human body adversely. As the amino and carboxyl groups are functional components of this virus, it causes infection human cells of respiratory. To deactivate the activity of this virus, hydroxyl group has significant role. As the artificial chemical ingredients have minor to severe side effects for human organs, this study proposed the extracted plants, as for example licorice, which can be used as a bio-chemical ingredients to diminish the effect of virus including reduced risk factor. The nano membrane produced from licorice having the antiviral characteristics and it can be used as wound dressing material. Some other supplementary bio-components can be added to improve the functional properties of the natural plant membrane. In future, how these hydroxyl groups related natural plants can be used to improve the immunity of human with proper drug delivery systems will be studied. The suggested some others nature plants are garlic, sambucus, astragalus, ginger, ginseng that can exhibit significant effect against COVID-19.

Reference

1. Du, Lanying, Yuxian He, Yusen Zhou, Shuwen Liu, Bo-Jian Zheng, and Shibo Jiang. "The spike protein of SARS-CoV—a target for vaccine and therapeutic development." *Nature Reviews Microbiology* 7, no. 3 (2009): 226-236.
2. Wang, Ning, Jian Shang, Shibo Jiang, and Lanying Du. "Subunit vaccines against emerging pathogenic human coronaviruses." *Frontiers in Microbiology* 11 (2020): 298.
3. Enitan, Seyi Samson, Isaiah Nnana Ibeh, Adeolu Sunday Oluremi, Ayodeji OluSola Olayanju, and Grace Eleojo Itodo. "The 2019 Novel Coronavirus Outbreak: Current Crises, Controversies and Global Strategies to Prevent a Pandemic." *International Journal of Pathogen Research* (2020): 1-16
4. Du, Lanying, Wanbo Tai, Yusen Zhou, and Shibo Jiang. "Vaccines for the prevention against the threat of MERS-CoV." *Expert review of vaccines* 15, no. 9 (2016): 1123-1134.

5. Zhou, Yusen, Shibo Jiang, and Lanying Du. "Prospects for a MERS-CoV spike vaccine." *Expert review of vaccines* 17, no. 8 (2018): 677-686.
6. Du, Lanying, Yang Yang, Yusen Zhou, Lu Lu, Fang Li, and Shibo Jiang. "MERS-CoV spike protein: a key target for antivirals." *Expert opinion on therapeutic targets* 21, no. 2 (2017): 131-143.
7. Lu, Lu, Qi Liu, Yun Zhu, Kwok-Hung Chan, Lili Qin, Yuan Li, Qian Wang et al. "Structure-based discovery of Middle East respiratory syndrome coronavirus fusion inhibitor." *Nature communications* 5, no. 1 (2014): 1-12.
8. Du, Lanying, Wanbo Tai, Yang Yang, Guangyu Zhao, Qing Zhu, Shihui Sun, Chang Liu et al. "Introduction of neutralizing immunogenicity index to the rational design of MERS coronavirus subunit vaccines." *Nature communications* 7, no. 1 (2016): 1-9.
9. He, Yuxian, Jingjing Li, Susanne Heck, Sara Lustigman, and Shibo Jiang. "Antigenic and immunogenic characterization of recombinant baculovirus-expressed severe acute respiratory syndrome coronavirus spike protein: implication for vaccine design." *Journal of virology* 80, no. 12 (2006): 5757-5767.
10. Liu, Shuwen, Gengfu Xiao, Yibang Chen, Yuxian He, Jinkui Niu, Carlos R. Escalante, Huabao Xiong et al. "Interaction between heptad repeat 1 and 2 regions in spike protein of SARS-associated coronavirus: implications for virus fusogenic mechanism and identification of fusion inhibitors." *The Lancet* 363, no. 9413 (2004): 938-947.
11. Wang, Qihui, Gary Wong, Guangwen Lu, Jinghua Yan, and George F. Gao. "MERS-CoV spike protein: Targets for vaccines and therapeutics." *Antiviral research* 133 (2016): 165-177.
12. Li, Fang, Wenhui Li, Michael Farzan, and Stephen C. Harrison. "Structure of SARS coronavirus spike receptor-binding domain complexed with receptor." *Science* 309, no. 5742 (2005): 1864-1868.
13. Wan, Yushun, Jian Shang, Rachel Graham, Ralph S. Baric, and Fang Li. "Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus." *Journal of virology* 94, no. 7 (2020).
14. Turner, Anthony J., Julian A. Hiscox, and Nigel M. Hooper. "ACE2: from vasopeptidase to SARS virus receptor." *Trends in pharmacological sciences* 25, no. 6 (2004): 291-294.
15. Su, Shuo, Gary Wong, Weifeng Shi, Jun Liu, Alexander CK Lai, Jiyong Zhou, Wenjun Liu, Yuhai Bi, and George F. Gao. "Epidemiology, genetic recombination, and pathogenesis of coronaviruses." *Trends in microbiology* 24, no. 6 (2016): 490-502.
16. Zhu, Na, Dingyu Zhang, Wenling Wang, Xingwang Li, Bo Yang, Jingdong Song, Xiang Zhao et al. "A novel coronavirus from patients with pneumonia in China, 2019." *New England Journal of Medicine* (2020).
17. Hatada, Ryo, Koji Okuwaki, Yuji Mochizuki, Kaori Fukuzawa, Yuto Komeiji, Yoshio Okiyama, and Shigenori Tanaka. "Fragment molecular orbital based interaction analyses on COVID-19 main protease-inhibitor N3 complex (PDB ID: 6LU7)." (2020).
18. Shahid, Md Abdus, Ayub Ali, Md Nur Uddin, Sumon Miah, Syed Maminul Islam, Md Mohebbullah, and Mohammad Salman Ibna Jamal. "Antibacterial wound dressing electrospun nanofibrous material

from polyvinyl alcohol, honey and Curcumin longa extract." *Journal of Industrial Textiles* (2020): 1528083720904379.

19. Ali, Ayub, and Md Abdus Shahid. "Polyvinyl Alcohol (PVA)–Azadirachta indica (Neem) Nanofibrous Mat for Biomedical Application: Formation and Characterization." *Journal of Polymers and the Environment* 27, no. 12 (2019): 2933-2942.
20. Ali, Ayub, Md Abdus Shahid, Md Delwar Hossain, and Md Nurul Islam. "Antibacterial bi-layered polyvinyl alcohol (PVA)-chitosan blend nanofibrous mat loaded with Azadirachta indica (neem) extract." *International journal of biological macromolecules* 138 (2019): 13-20.
21. https://commons.wikimedia.org/wiki/File:3D_medical_animation_corona_virus.jpg
22. https://en.wikipedia.org/wiki/Coronavirus_disease_2019
23. de Haan, Cornelis AM, Lili Kuo, Paul S. Masters, Harry Vennema, and Peter JM Rottier. "Coronavirus particle assembly: primary structure requirements of the membrane protein." *Journal of virology* 72, no. 8 (1998): 6838-6850.
24. Nieva, José Luis, Vanesa Madan, and Luis Carrasco. "Viroporins: structure and biological functions." *Nature Reviews Microbiology* 10, no. 8 (2012): 563-574.
25. Schoeman, Dewald, and Burtram C. Fielding. "Coronavirus envelope protein: current knowledge." *Virology journal* 16, no. 1 (2019): 69.
26. Seebach, Dieter, Albert K. Beck, and Daniel J. Bierbaum. "The world of β-and γ-peptides comprised of homologated proteinogenic amino acids and other components." *Chemistry & biodiversity* 1, no. 8 (2004): 1111-1239.
27. Wang, Liqiang, Rui Yang, Bochuan Yuan, Ying Liu, and Chunsheng Liu. "The antiviral and antimicrobial activities of licorice, a widely-used Chinese herb." *Acta Pharmaceutica Sinica B* 5, no. 4 (2015): 310-315.
28. Fukuchi, Kunihiko, Noriyuki Okudaira, Kazunori Adachi, Reina Odai-Ide, Shigeru Watanabe, Hirokazu Ohno, Masaji Yamamoto et al. "Antiviral and antitumor activity of licorice root extracts." *in vivo* 30, no. 6 (2016): 777-785.
29. Yeh, Chia Feng, Kuo Chih Wang, Lien Chai Chiang, Den En Shieh, Ming Hong Yen, and Jung San Chang. "Water extract of licorice had anti-viral activity against human respiratory syncytial virus in human respiratory tract cell lines." *Journal of ethnopharmacology* 148, no. 2 (2013): 466-473.
30. Cinatl, J., B. Morgenstern, G. Bauer, P. Chandra, H. Rabenau, and H. W. Doerr. "Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus." *The Lancet* 361, no. 9374 (2003): 2045-2046.
31. Sharma, V., A. Katiyar, and R. Agrawal. "Glycyrrhiza glabra: chemistry and pharmacological activity." Sweeteners: Pharmacology, biotechnology, and applications (2017): 1-14

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Figures

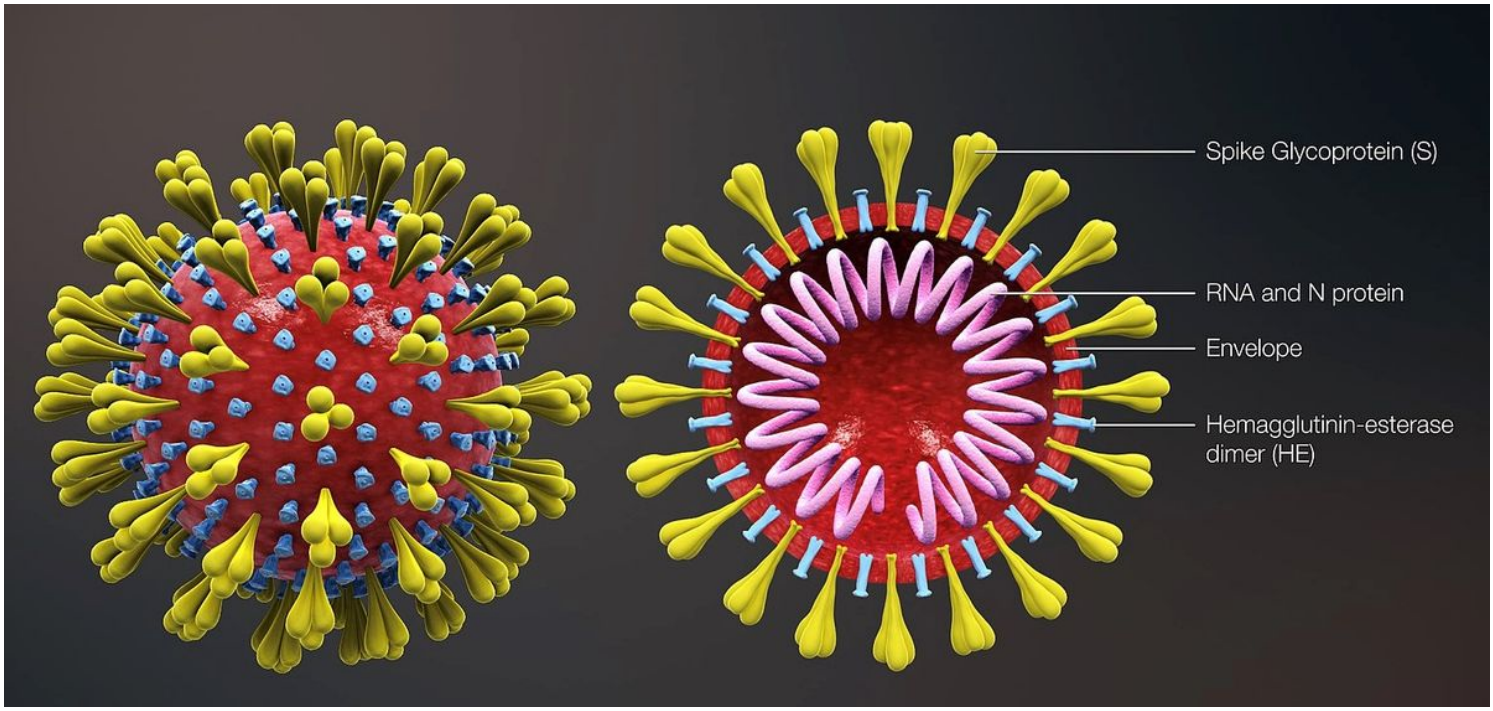

Figure 1

Diagram of coronavirus virion structure showing spikes that form a "crown" like the solar corona, hence the name [21]

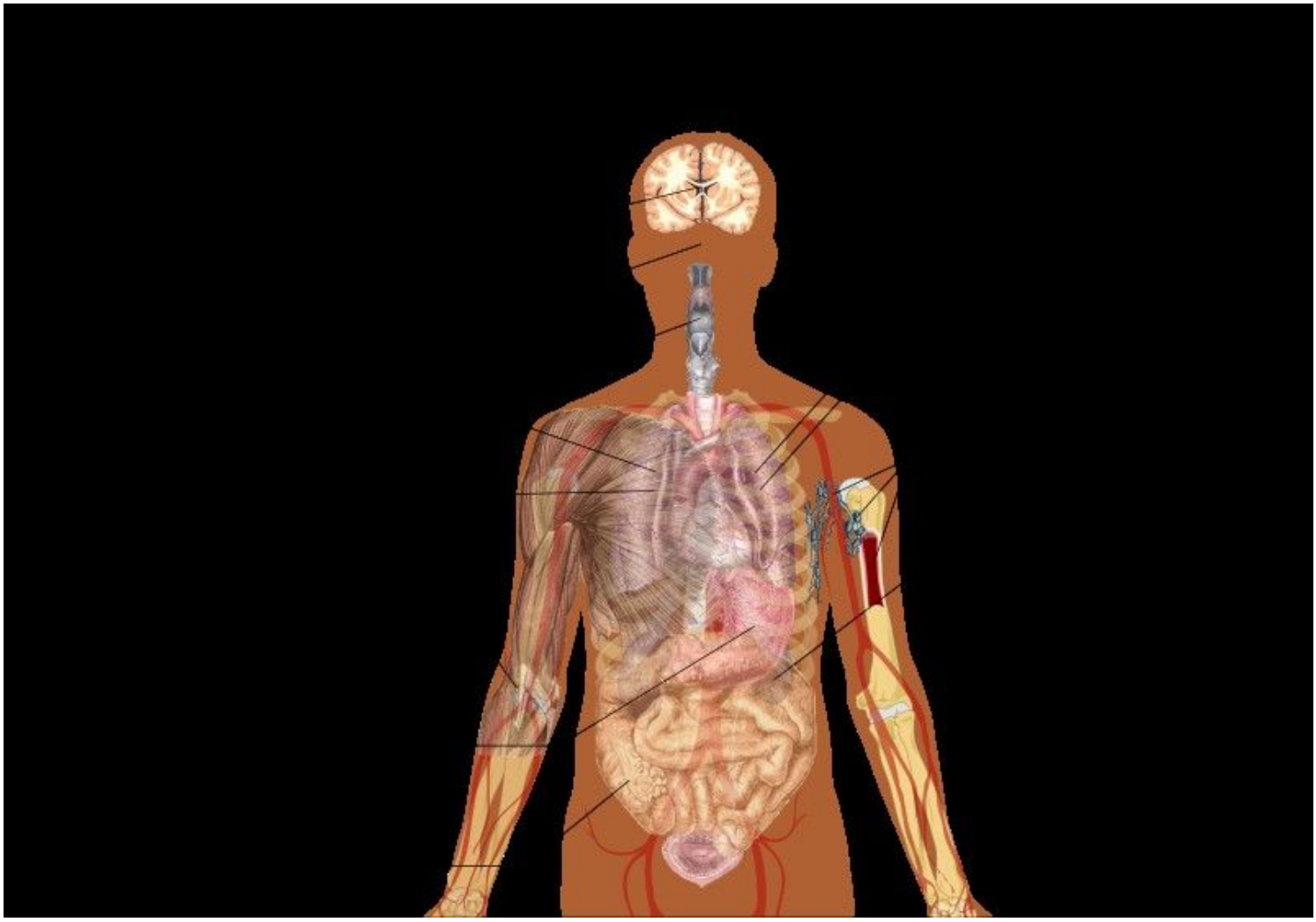

Figure 2

Common symptoms and severe disease due to COVID-19 [22]

Figure 3

Electro-spinning machine for preparation of membrane

Figure 4

Legend not available in this version

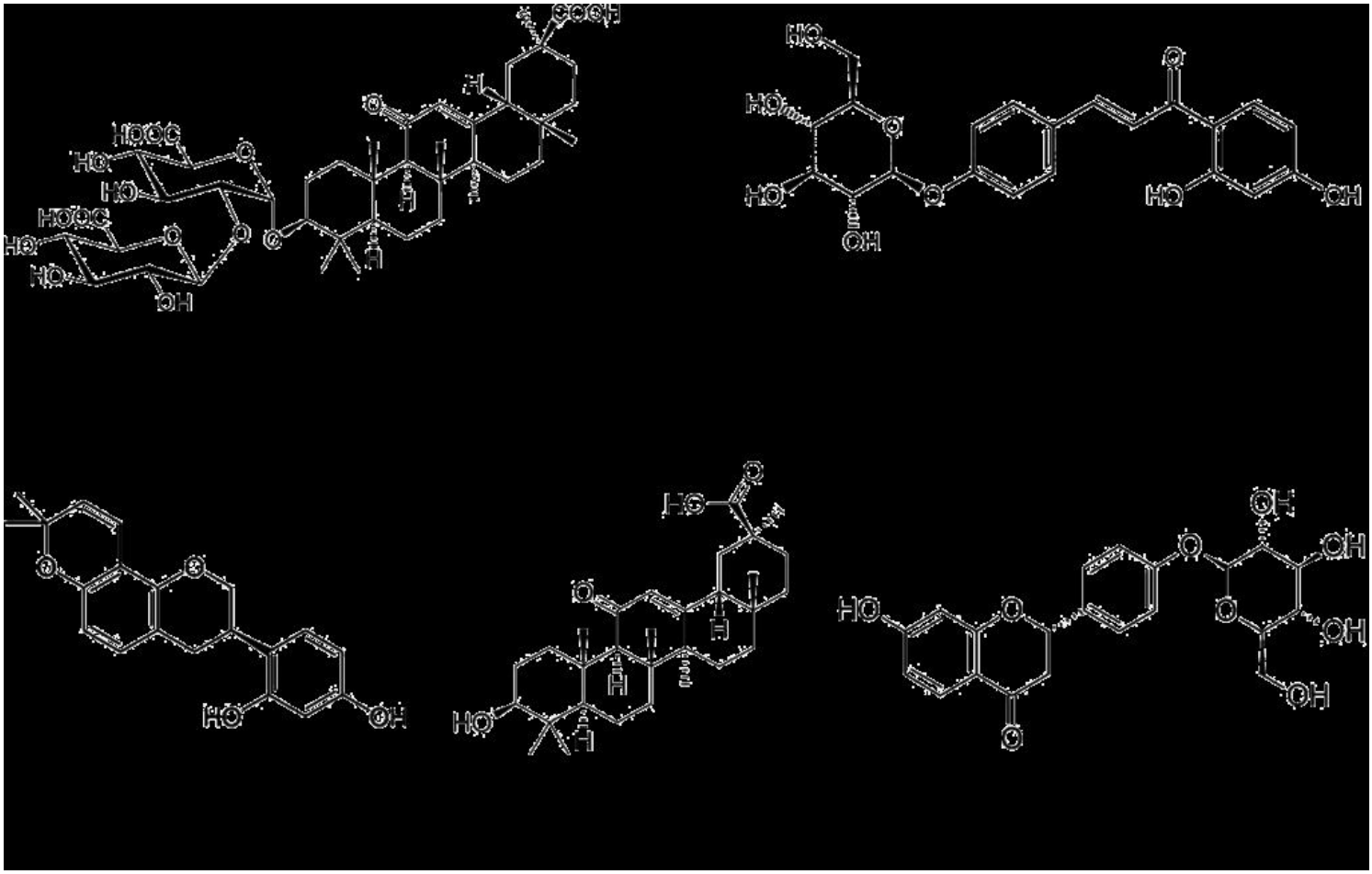

Figure 5

Chemical structure of licorice components (a) glycyrrhizin, (b) isoliquiritin, (c) glabridin (d) glycyrrhetic acid and (e) liquiritin

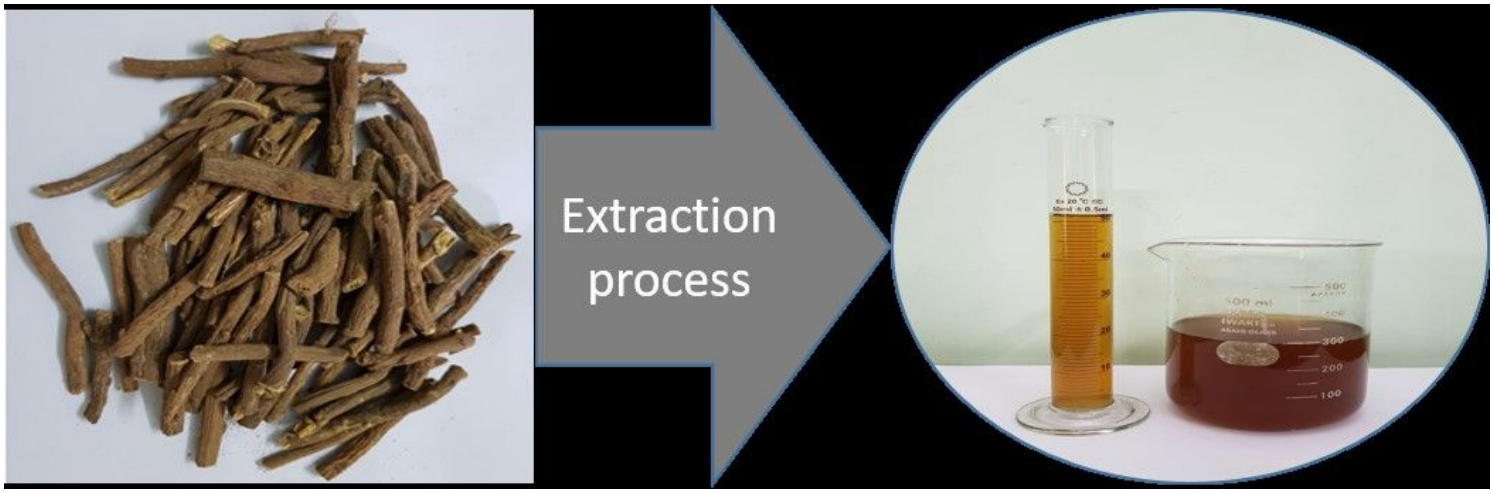

Figure 6

Formation of licorice extract solution

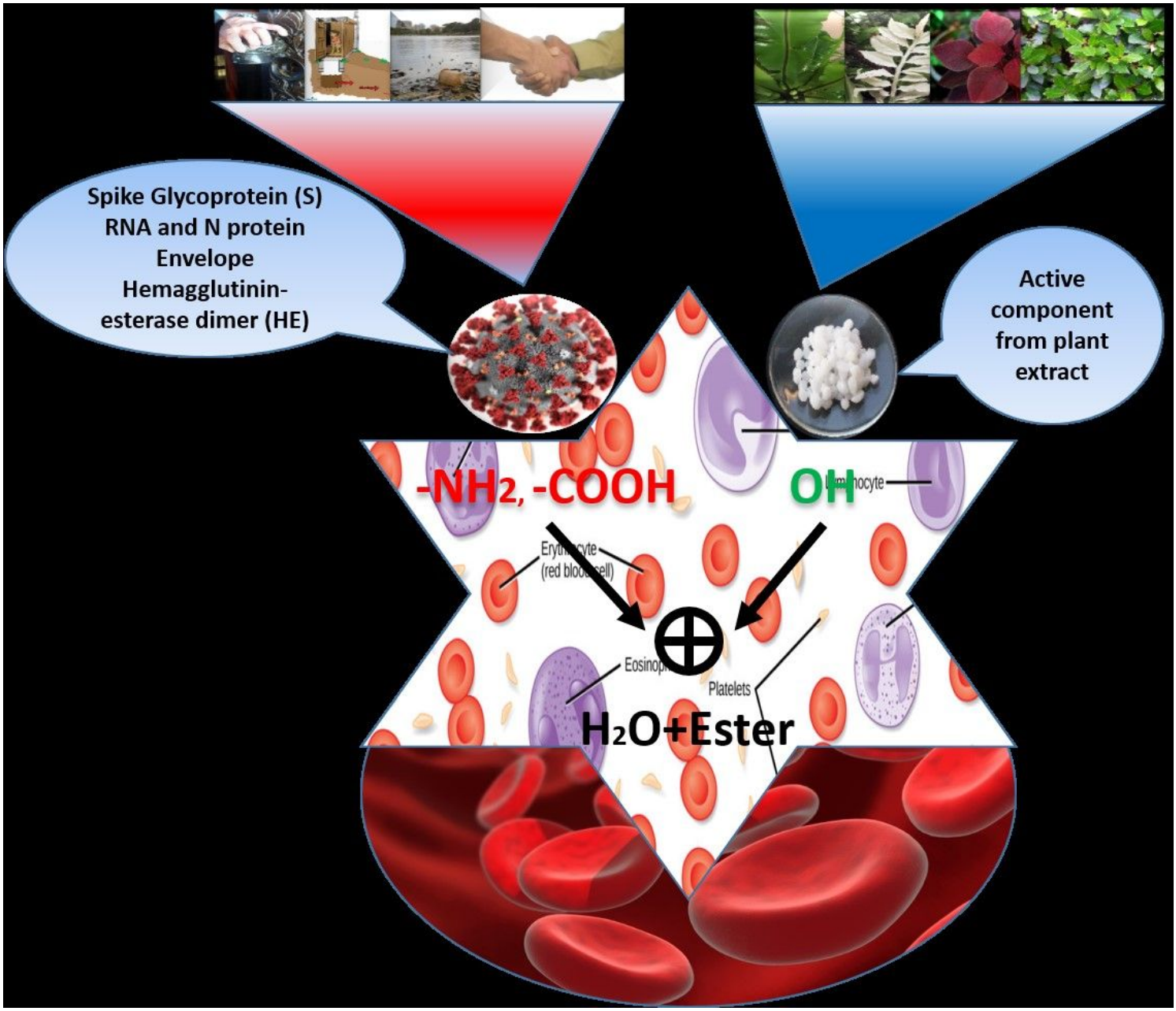

Figure 7

Mechanism of deactivation of COVID-19 using active component of plant extract

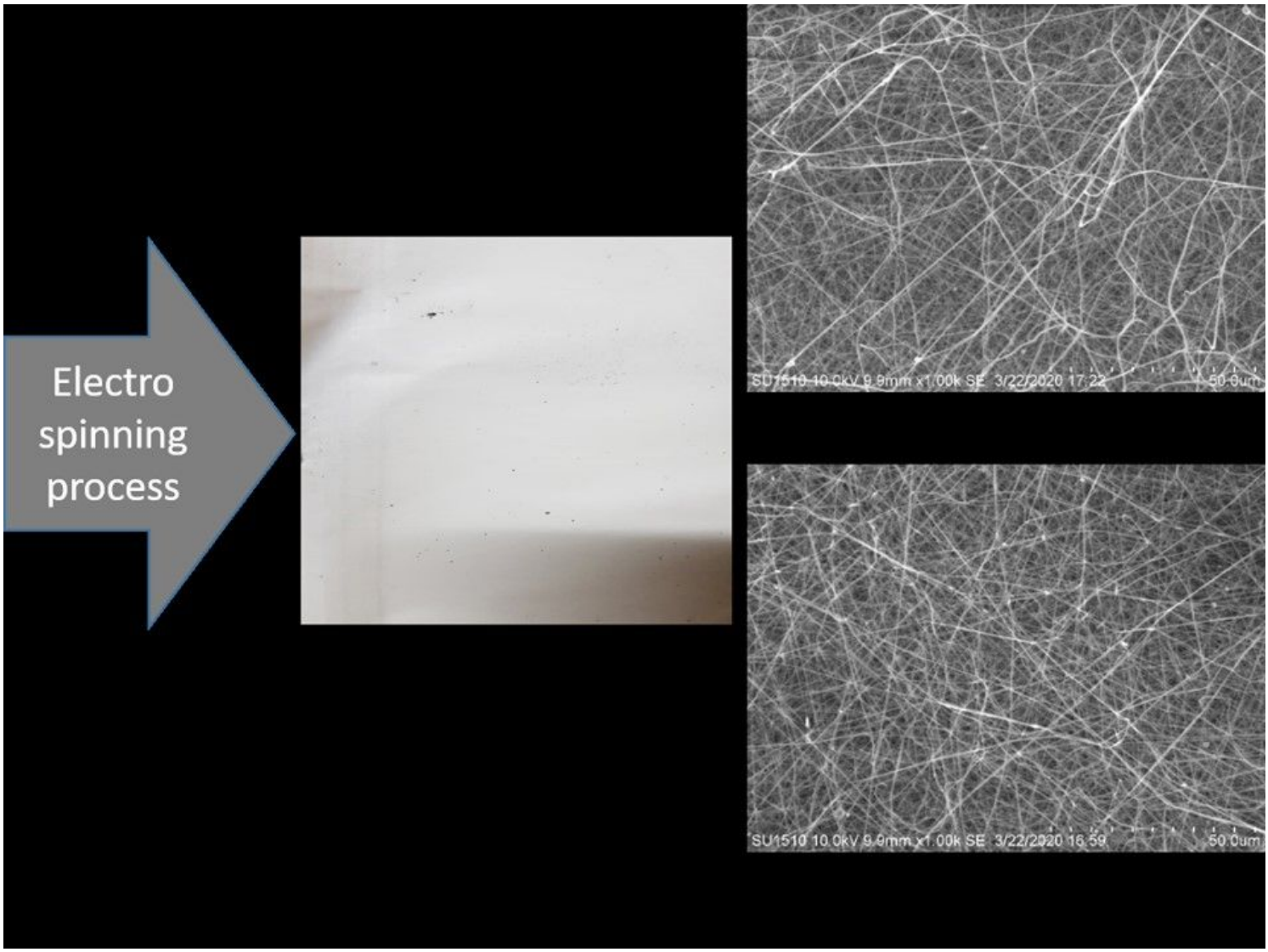

Figure 8

Morphology of electrospun PVA-licorice (a) natural image of membrane (b) SEM image at 10.0 kV, x1.00 k of front site view (c) SEM image at 10.0 kV, x1.00 k of back site view