Bromelain: A Potential Therapeutic Solution For COVID-19

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ABSTRACT

According To World Health Organization (WHO), the COVID-19 pandemic caused by the novel coronavirus has been a huge challenge to the medical personnel, healthcare systems and governments, infecting about 213 million people worldwide, causing 4.4 million deaths (WHO). There is also a dearth of drugs, vaccines and oxygen support. The present-day plethora of drugs tends to be exorbitant and excruciating for the patients affected with COVID-19. Amidst the distressing symptoms of COVID-19, these drugs also carry a multitude of side effects, making the patient's life an abyss of despair. Bromelain, a particularly novel and interesting concoction of enzymes, has lately been proved to have extensive therapeutic applications for COVID-19. This study gives an overview of the effects of COVID-19, the therapeutic targets for COVID drugs, the role of ACE2 receptor in COVID-19 infection, how blocking of ACE2 receptor can potentially inhibit COVID-19, the common medications presently used and finally, the potential of bromelain to be a cure for the existing pandemic. The unique properties of bromelain make it a potential drug capable of taking on the high virulence of Coronaviruses through cleavage of the S domain or binding to the ACE2 receptor. The smooth administration and negligible side effects allow us to view this as a game-changer in COVID-19 therapeutics.

KEYWORDS: COVID-19, ACE2 receptor, bromelain, SARS-CoV2

Impacts of the Coronavirus Disease 2019

In the past deadly diseases like the plague, Black Death, yellow fever progressed into a pandemic, causing massive fatalities across various parts of the world. But the novel coronavirus SARS-CoV 2 brought the entire world to a standstill, by rapidly spreading across all the continents of the world, turning Coronavirus disease 2019 (COVID-19) into a global pandemic. Coronavirus disease 2019 (COVID-19) is a highly infectious disease primarily affecting the respiratory tract and causing mild symptoms like fever, dry cough, breathlessness, loss of taste and smell. In the case of severe infection, acute respiratory distress syndrome (ARDS) and pneumonia are prevalent. If the infection progresses further, there is a rapid deterioration of the patient's health, leading to death. In some instances of infection, unusual symptoms like pink eye (conjunctivitis), nausea, vomiting, diarrhoea, rash and acral edema were observed [1]. Individuals affected with non-communicable diseases such as cardiovascular diseases, diabetes, cancer and respiratory ailments are highly susceptible to covid infection. These vulnerable individuals are more likely to require critical care due to their immune-compromised state. A study by Fedrica states that the inflammatory reaction associated with covid infection might reactivate dormant cancer cells (DCC) that

survived primary treatment. This reactivation of DCC may be due to the uncontrolled production of pro-inflammatory cytokines. The release of neutrophils due to covid associated inflammatory reaction specifically activates pre-metastatic cancer cells in the lungs[2]. Another study by Jungang represented the lytic reactivation of Kaposi's sarcoma-associated herpesvirus (KSHV) by anti-viral drugs like Azithromycin and Nafamostat mesylate, which are currently used to treat covid infection[3]

Properties of the Covid Spike protein

SARS-CoV-2 is an enveloped single, positive-stranded RNA virus that has structural proteins such as the spike protein (S), an envelope protein (E) and membrane protein (M) encoded by the genes S, E and M. It also contains non-structural proteins (nsps) such as papain-like protease and RNA dependent RNA polymerase [4].The S protein is a Type 1 transmembrane protein that produces the spike-like projections required for the entry of the virus into the host. The region of the S protein that extends into the extracellular space is called the ectodomain. This ectodomain of S protein has two subunits, S1 and S2. The S1 subunit contains the receptor-binding domain (RBD) that interacts with the compatible host cell receptor to enable the entry of the virus into the host's system. The anatomical site at which the virus enters the host's system is called the portal of entry. In the case of covid, the general portals of entry are the eyes, nose and mouth, but in certain complications like endotoxemia (presence of endotoxin within the blood) and thrombosis (clotting of blood), the intestine was identified as the portal of entry[5]. The entry of the virus into the host's system is a significant determinant of viral virulence and pathogenesis [6]. The receptor-binding domain (RBD) is a critical target for antiviral drugs to counterattack covid infection[7]. A study by Yuvan represented a 73-76% similarity between the RBD sequence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV 2) causing COVID-19 and severe acute respiratory syndrome coronavirus (SARS- CoV) causing SARS [8]. The S2 subunit helps in anchoring the spike protein S to the host's cell membrane. This subunit is also known as the membrane-fusion subunit. During the initial entry of the SARS-CoV2 virus, the S protein remains to inactivate. The activation of the S protein is caused due to the cleavage of the S1 and S2 subunits by the host cell proteases. Thus, the activation of S protein-mediated by proteolytic cleavage initiates primary infection.

Role of Angiotensin-Converting Enzyme 2(ACE2) in COVID-19

Angiotensin-Converting Enzyme 2 (ACE2) is a critical enzyme of the Renin-Angiotensin-Aldosterone System (RAAS). This hormone system plays a significant role in regulating blood pressure, sodium (Na) and water levels. We have constructed the Renin-Angiotensin-Aldosterone System (RAAS) pathway (fig3) using the modelling tool - cell designer[9]. Pathway 1 of RAAS biochemical pathway helps in sodium (Na)/water excretion, vasodilation and reducing inflammation [10]. These regulatory functions are brought about by the hydrolytic reaction catalyzed by Angiotensin-Converting Enzyme 2 (ACE2). ACE2 production counterbalances the activity of ACE, making it a highly efficacious drug for cardiovascular diseases. Pathway 2 of the RAAS biochemical pathway helps in sodium (Na)/water reabsorption, vasoconstriction and increased inflammation. These regulatory functions are brought about by the conversion of Angiotensin 1 (Ang1) to Angiotensin 2 (Ang2) catalyzed by Angiotensin-Converting Enzyme (ACE). The binding of SARS-CoV 2 to the host's Angiotensin-Converting Enzyme 2 (ACE2) receptor mediates the entry of the virus into the host's system. The Type 2 transmembrane serine protease (TMPRSS2) and other lysosomal proteases activate the trimeric S protein by proteolytic

cleavage. This initiates the replicative cycle of the virus resulting in the progression of the disease. The primary drug target site for covid is the ACE 2 receptor located on the short arm of the X chromosome. In a study conducted by Yuchong, males (46, XY) have comparatively higher mortality than females (46, XX) in COVID cases. Females have a mechanism to inactive the X-chromosome, and this phenomenon is named lyonization or X-inactivation. This dosage compensation gives them a genetic advantage over males as the latter lacks the mechanism. This results in higher fatalities in men when compared to women [11].

Medications used to treat COVID-19

At present, the most commonly used antiviral to treat covid infection is Remdesivir that is administered intravenously. Other medications such as Favilavir(antiviral), Chloroquine, Hydroxychloroquine(antimalarial), Lopinavir, darunavir(viral protease inhibitors) are also used [12]. However, the efficacy, response rate, effect across different populations, safety, side effects and long term impact of these medications must be established through further clinical trials. The rapid mutation of SARS-CoV 2 has resulted in numerous strains of the virus that differ in structure, function and pathogenicity. The currently identified mutant strains of SARS-CoV2 are alpha, beta, gamma and delta variants. The current challenge for scientists worldwide is to develop a single efficacious drug that counterattacks all variants of the SARS-CoV2 with minimum side effects. Herbal remedies for COVID-19 includes *Glycyrrhiza uralensis, Armeniacae semen amarum, Ephedrae herba, Gypsum fibrosum, Scutellariae radix, Atractylodis rhizoma, Poria sclerotium, Citri reticulatae pericarpiu, Forsythiae fructus and Magnoliae officinalis cortex* [13]. Another potential natural medication that could be used against SARS-CoV2 is bromelain.

Bromelain - A promising natural remedy for COVID-19

A member of the family *Bromeliaceae*, *Ananas comosus* or commonly called pineapple, yields a sulfhydryl protease called bromelain, which is a combination of various thiol endopeptidases and glucosidase, cellulase, peroxidase, phosphatase and many protease inhibitors synthesized in the stem and fruit of pineapples [14]. Thailand, the Philippines, Costa Rica, Brazil, China, Indonesia, Hawaii, India, and Bangladesh are the leading pineapple-producing countries [15]. Based on the extraction site, bromelain is differentiated into Stem bromelain – EC 3.4.22.32 and Fruit bromelain – EC 3.4.22.33. According to the studies conducted by Rajendra, a higher concentration of bromelain was found in the pineapple stem than in the fruit. Since the stem is a non-edible waste by-product, it serves to be an efficient and inexpensive material for bromelain extraction[16]. Bromelain is generally recognized as safe (GRAS**)** by the U.S. Food and Drug Administration (FDA). It was established in various studies that bromelain has anti-inflammatory properties as they inhibit prostaglandins that assist in inflammation and have anti-inflammatory, anti-oedematous, analgesic, anti-thrombotic activities by influencing the arachidonic acid and kallikrein-kinin pathways leading to cerebrovascular and cardiovascular effects [17]–[20]. Anti-cancer applications were also seen as bromelain suspends cell proliferation through activation of apoptosis [21]–[23]. In a study conducted by Saptarini et al., bromelain was found to have immunomodulatory activity due to anti-oxidant abilities and protease activity. Subsequently, it activates NK cells, heightens tumour necrosis factor-a (TNF-ab), IL-b IL-1, IL-2, IL-6, IL-8, Interferon γ and granulocyte-macrophage stimulating factor and suppresses CD4+ T cells and CD25 expression [23]–[25]. It also is a non-invasive therapy for metabolic disorders like osteoarthritis [26]. Reduction of detectable connective tissue in meat, also called tenderization, can be done enzymatically using bromelain [27], [28]. Bromelain's abilities as a phytotherapeutic drug are unmatched as it has a plethora of applications in sinusitis,

bronchitis, surgical trauma, thrombophlebitis and high absorption of drugs [24], [29], [30]. Keeping in mind the patient's comfort, the increased economic nature and comfort given by oral administration of drugs are singularly the most effective administration and bromelain available through natural sources like pineapple increases its therapeutic potential [23], [30]–[32]. Acceleration of wound healing through proliferation and clearing up skin debris results in its applications in the cosmetic field [23], [33]. In addition to that, bromelain has benefits for the beverage and textile sectors.

Role of Bromelain in COVID-19

When host cells encounter penetration of foreign genetic material like the COVID-19 virus, the foreign proteins try to bind with the host receptors. The translation of viral RNA and the subsequent multiplication through the lytic or lysogenic cycle occurs using the host's resources. Then the viral proteins produced replicate until they are in a large quantity to launch an attack against the host's defence mechanism. Proteins coordinate many crucial stages in the development of the virus. The proteolytic activity of bromelain is one of the methods to curb the proliferation of the virus [34], [35].

ACE2 is the primary receptor for viral entry into the host. The zinc metalophosphatase domain of the ACE 2 binds to the viral spike protein(S) of SARS-CoV- 2. Both the ACE2 receptor and TMPRSS2 required for the entry of the virus and activation of the spike protein respectively are enriched with cysteine residues.ACE-2 is found to have six cysteine residues with three disulphide bonds for stabilization. TMPRSS2 is predicted to have 18 cysteine residues with nine disulphide bonds for stabilization. Bromelain, a cysteine protease, binds with the cysteine-rich ACE-2 and TMPRSS2, reducing their expression in a dosage-dependent manner. A study by Satish Sagar using Vero E6 cell lines demonstrated that bromelain‘s cysteine photolytic activity is higher in ACE-2 than TMPRSS2. SARS-CoV-2 spike protein has 30 cysteine residues with 15 disulphide bonds in the ectodomain for stabilization. The cysteine protease – bromelain interacted with the ectodomain of the spike protein and reduced the expression of the S-ectodomain in a dosage dependant manner [12]. In a study conducted by Panagiotis, administration of bromelain and curcumin(a natural phenol found in turmeric) together boosts the individual's immune response and helps prevent COVID-19 [36].

Fig 10 represents a study done by Sagar highlighting the therapeutic properties of bromelain. Immunoprobing was done to bromelain treated S-ectodomain in Tni insect cell. Through Simply Blue-staining and negative staining transmission, viral particles were analysed by adding vehicle control (left) and bromelain (right). In the end, bromelain (magenta and yellow) was found to be docked with S protein (green) [37].Fig 11 depicts the blind protein docking of chain A of SARS COV-2 spike protein with chain A of Bromelain using H-DOCK server[38].A docking score less than -60 is said to be a good score, so bromelain is found to have good affinity towards COVID-19 spike protein[39]–[44]

CONCLUSION

To overcome the global pandemic caused by COVID-19 has been the prime goal for scientists across the globe. The side effects and genetic modifications caused by medications and vaccines used to treat covid are yet to be identified. Furthermore, the genetic mutations caused by SARS-CoV 2 virus in the infected population is yet to be established through clinical trials. Thus, combating COVID-19 with an easily available, natural, non-invasive and efficacious mode of medication would guarantee safety against longer-term impacts. Having the potential to block the initial entry of the virus into the host's system, bromelain could be

the most efficacious natural mode of covid treatment. The majority of the covid medication are administered as oral tablets or intravenous injections. But bromelain could be obtained by including pineapple in our day-to-day diet or as a concentrated supplement.People with diabetes are advised to keep away from pineapple as much as possible as it has a medium glycemic index (GI) But in case they consume pineapple, they are advised to pair it with foods that have a lower glycemic index (GI). According to the U.S. Food and Drug Administration (FDA) regulations, bromelain may be used alone or combined with other natural medications. Hence, the multi-organ involvement in covid could be treated efficiently by using a combination treatment of bromelain with other medicines. Bromelain, like all promising therapeutics, has extended bioavailability and ease of administration. Historically, Asian food diets have been filled with vitamins, minerals and flavonoids. That could also be the reason for the minimal impact of COVID-19 in Asian countries. Pineapple, amongst other fruits, has a special ingredient that makes it a promising cure. Therefore, bromelain is a promising natural medication that could help end the global pandemic with negligible long-term implications.

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Competing interests

The authors declare no competing financial interests.

TABLES

The target for antiviral drugs	Description
Viral attachment and entry	Entry of the virions into the host cell by adsorption
Penetration	Non-enveloped viruses are engulfed by a phagocytosis process called viropexis. But enveloped viruses fuse with the plasma membrane of the host cell.
Uncoating	Stripping the outer layers and capsid of the virus for release of nucleic acid
Early protein synthesis	Production of the capsid protein and other regulatory proteins to shut down the standard cellular mechanism.
Nucleic acid synthesis	Production of viral nucleic acid to direct the production of the viral components.
Maturation and Viral release	Assembly and release of viral progeny host cell lysis

Table.1 Targets for the development of antiviral drugs[45]

Description	**Alpha**	**Beta**	**Gamma**	**Delta**

First identified	United Kingdom	South Africa	Japan/Brazil	India
Spread	Spreads much faster than other variants	May spread faster than other variants	Spreads faster than other variants	Spreads much faster than other variants
Severe illness and death	May potentially cause more people to get sicker and to die	Not deadlier than other variants	Not deadlier than other variants	May cause more severe cases than the other variants
Vaccine	Currently, authorized vaccines work efficiently against this variant.	Currently, authorized vaccines do work efficiently against this variant	Currently, authorized vaccines do work efficiently against this variant.	Currently, authorized vaccines work inefficiently against this variant.
Treatments	Treatments are effective against this variant	Lower efficiency of certain monoclonal antibody treatment observed against this variant	Lower efficiency of certain monoclonal antibody treatment observed against this variant	Lower efficiency of certain monoclonal antibody treatment observed against this variant

Table.2 Variants of the coronavirus (source: Centers for Disease Control and Prevention)

Description	Stem bromelain	Fruit bromelain

Primary component	Cysteine endopeptidase	Aspartic endopeptidase
Site of extraction	Pineapple stem	Pineapple fruit juice
Optimum temperature	50-60 ºC	37-70 ºC
Optimum pH	6-7	3-8
Enzymatic activity	Higher	Lower
Proteolytic activity	Lower	Higher
Specificity for peptide bond	Lower	Higher

Table.3 Difference between stem bromelain and fruit bromelain [46], [47]

HDock Docking Score	Interaction Statistics		
	Hydrogen bonds	Electrostatic bonds	Hydrophobic bonds
-293.60	7	1	7

Table.4 Docking score and interaction statistics[50–54,56]

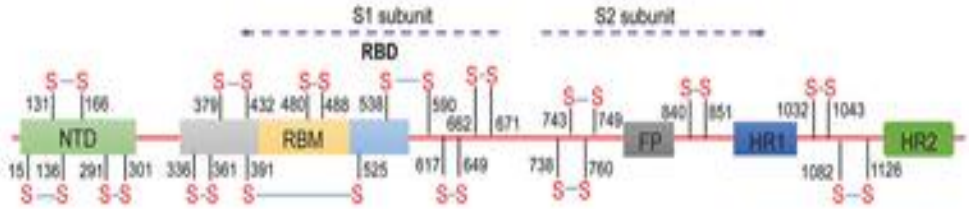

Fig.1 Schematic representation of in SARS-CoV-2 S-ectodomain [48]

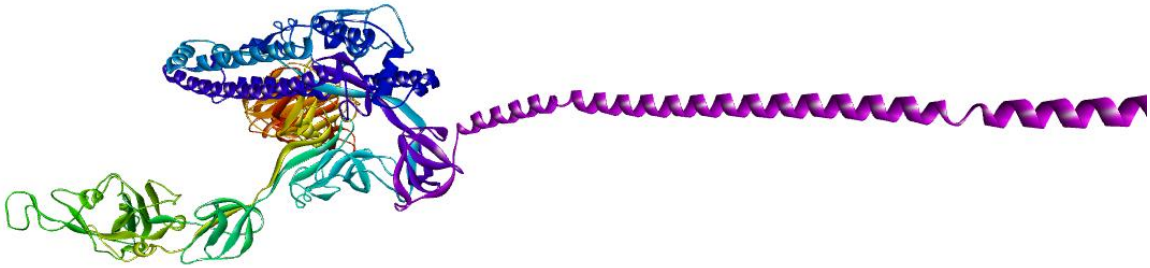

Fig 2. A schematic representation of SARS-CoV-2 spike protein [49]

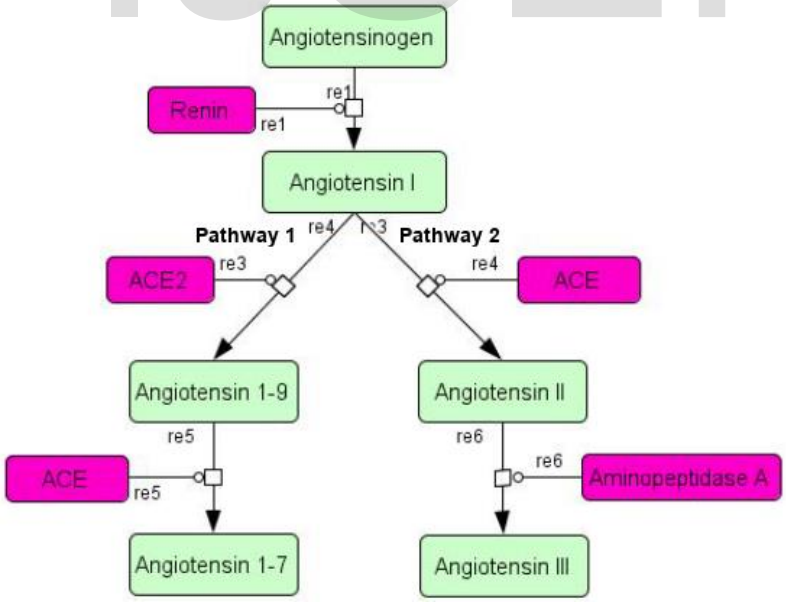

Fig.3 Renin-Angiotensin-Aldosterone System (RAAS) biochemical pathway[50]

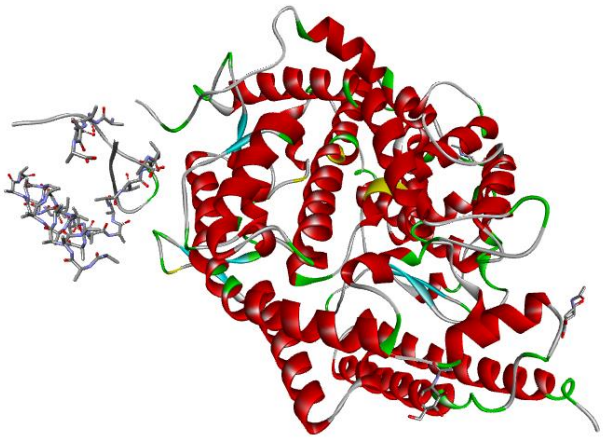

Fig. 4 A schematic representation of Angiotensin-Converting Enzyme 2 [51] [52]

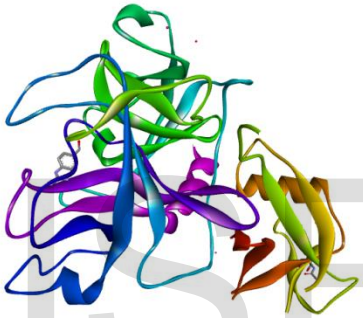

Fig.5 A schematic representation of Type 2 transmembrane serine protease (TMPRSS2) [53]

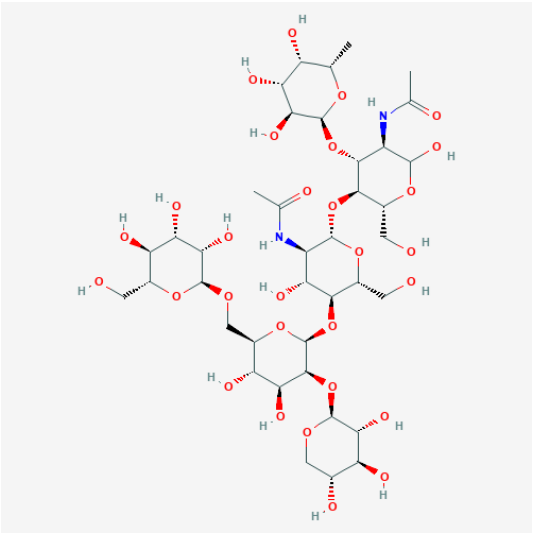

Fig. 6 Structure of bromelain (N-[(2S,3R,4R,5S,6R)-2-[(2R,3S,4R,5R)-5-acetamido-6-hydroxy-2-(hydroxymethyl)-4-[(2S,3S,4R,5S,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxyoxan-3-yl]oxy-5-[(2S,3S,4S,5S,6R)-4,5-dihydroxy-6-[[(2S,3S,4S,5S,6R)-3,4,5-

trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]-3-[(2S,3R,4S,5R)-3,4,5-trihydroxyoxan-2-yl]oxyoxan-2-yl]oxy-4-hydroxy-6-(hydroxymethyl)oxan-3-yl]acetamide) [54]

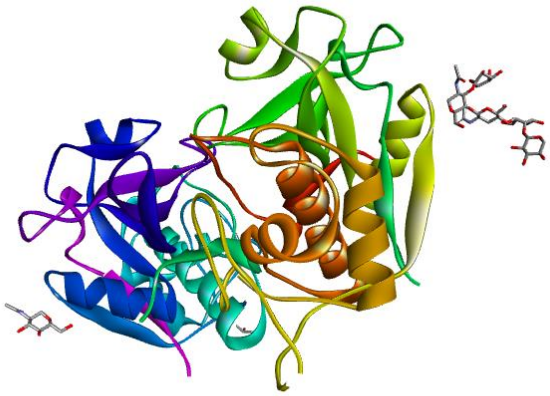

Fig.7 A schematic representation of bromelain protease [55] [56]

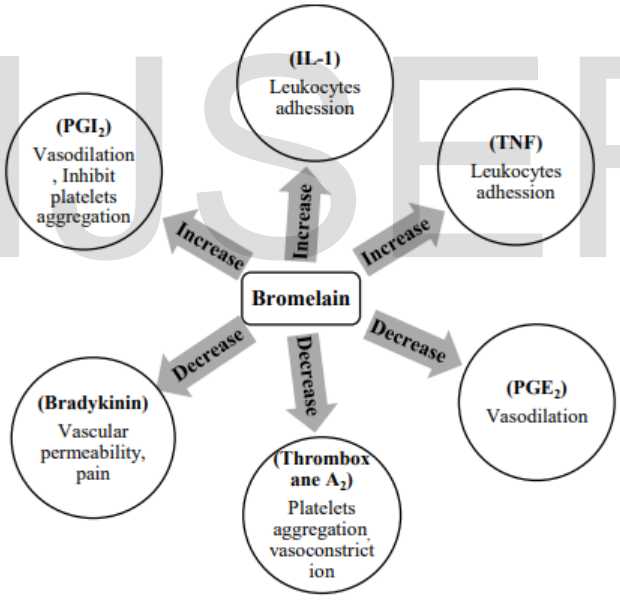

Fig.8 Anti-inflammatory activities of bromelain [57]

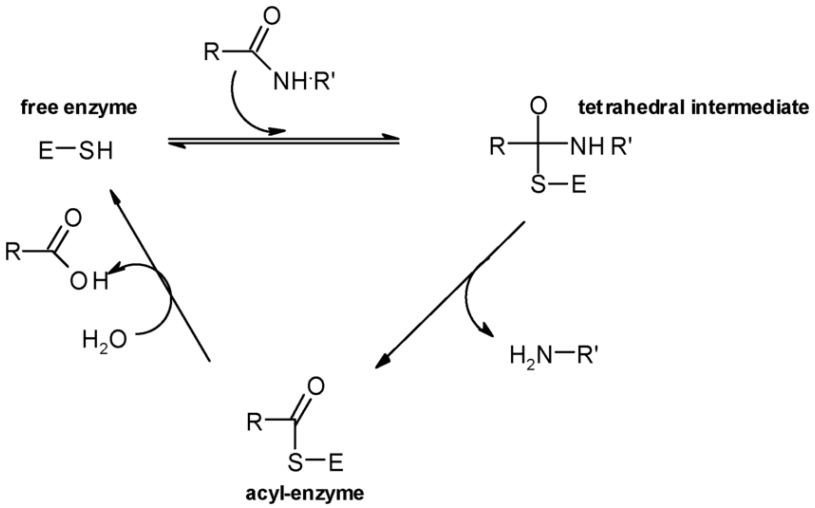

Fig.9 Mechanism of action of cysteine protease [58]

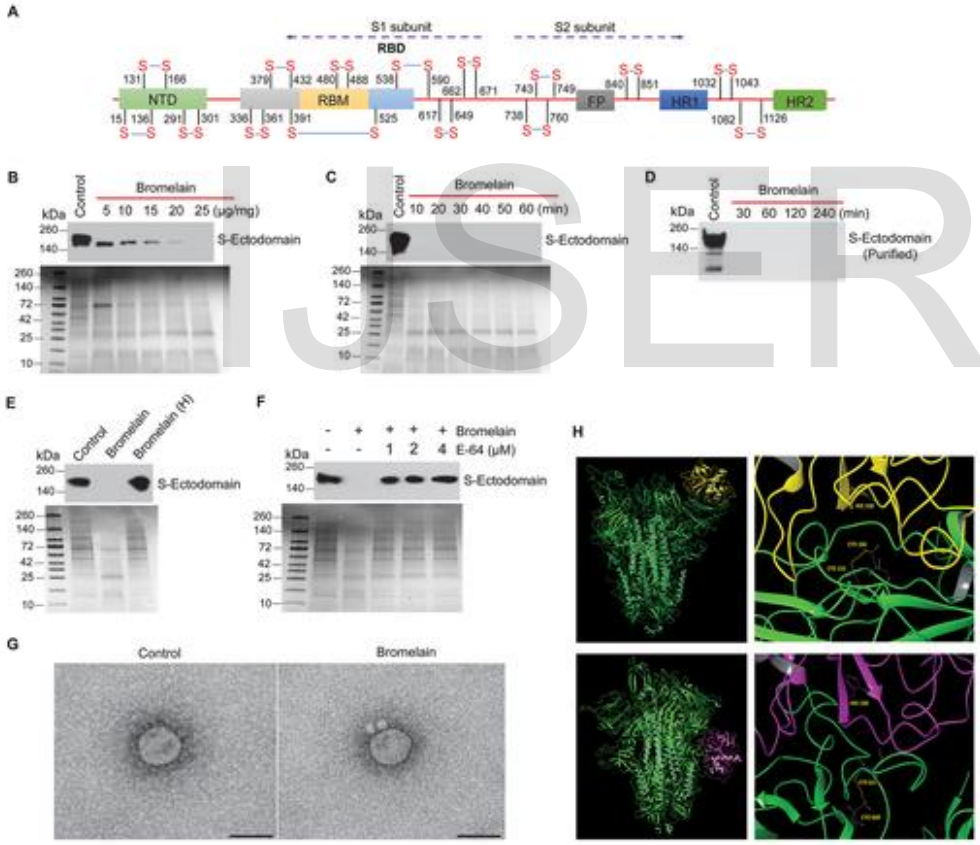

Fig. 10 Docking of bromelain with the S-ectodomain of SARS CoV2[37]

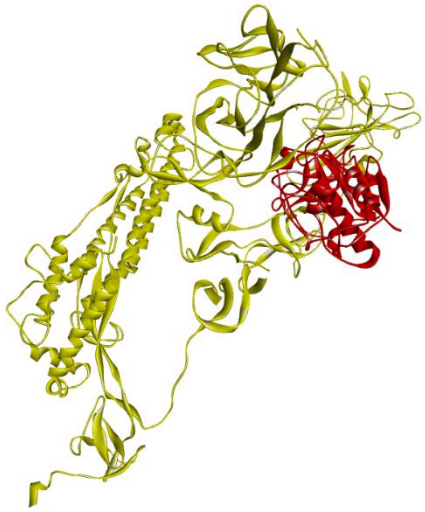

Fig. 11 Blind protein docking of A chain of SARS COV-2 spike protein with A chain of Bromelain [38]–[43]

FIGURES

Fig.1

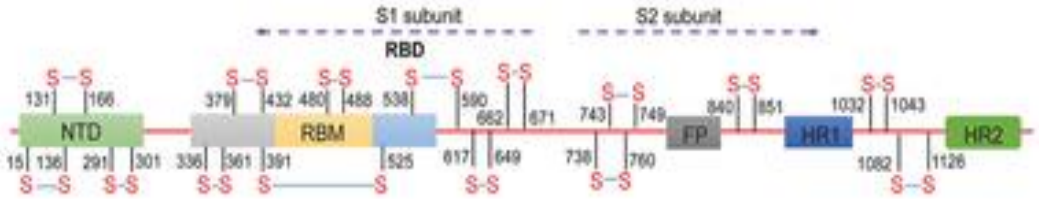

Fig.2

FIG.3

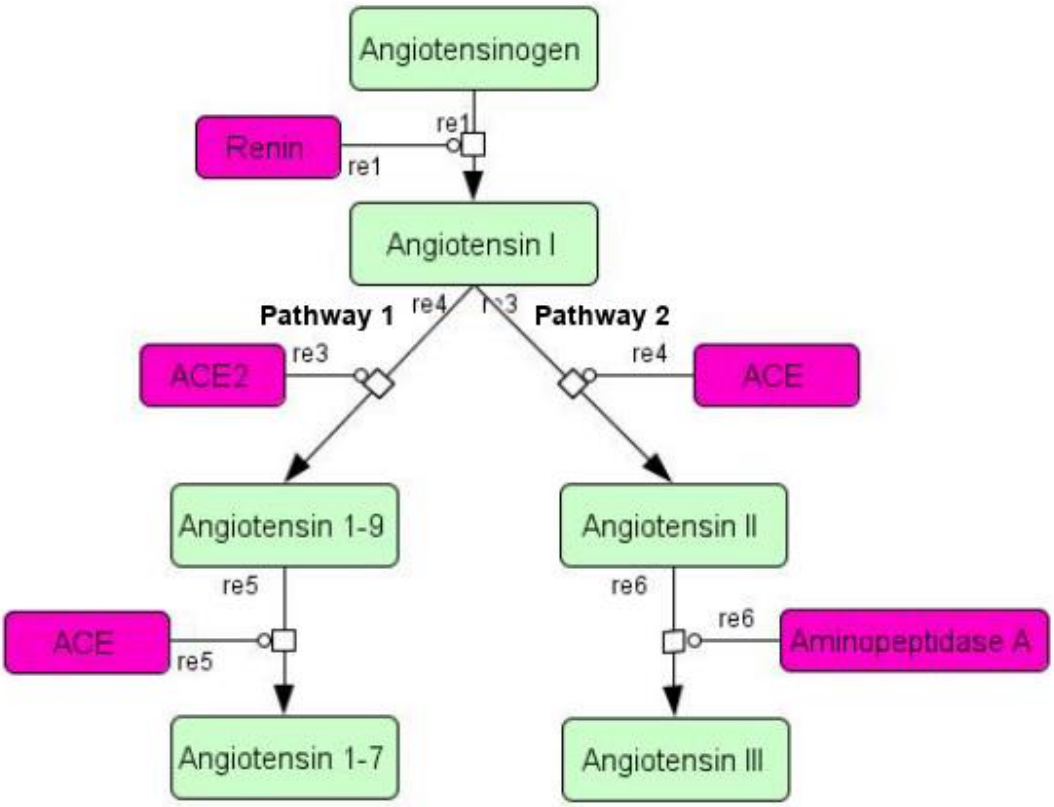
Angiotensinogen
re1
Renin
re1
Angiotensin I
Pathway 1
re4
re3
Pathway 2
re3
ACE2
re4
ACE
Angiotensin 1-9
Angiotensin II
re5
re6
re6
ACE
re5
Aminopeptidase A
Angiotensin 1-7
Angiotensin III

FIG.4

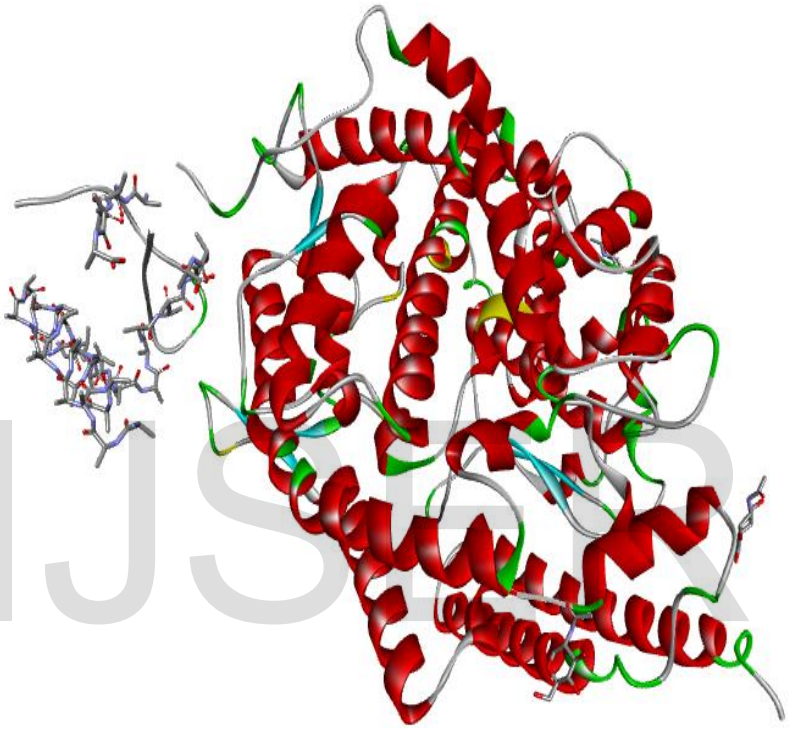

FIG 5

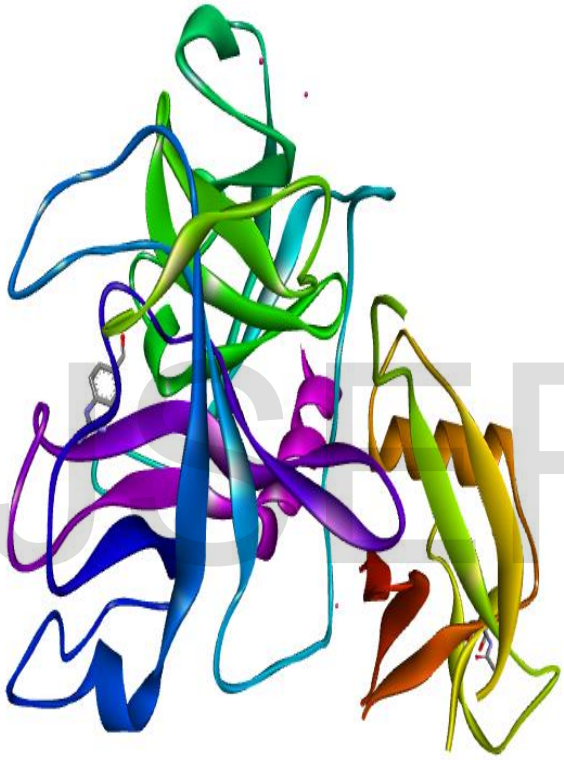

FIG-6

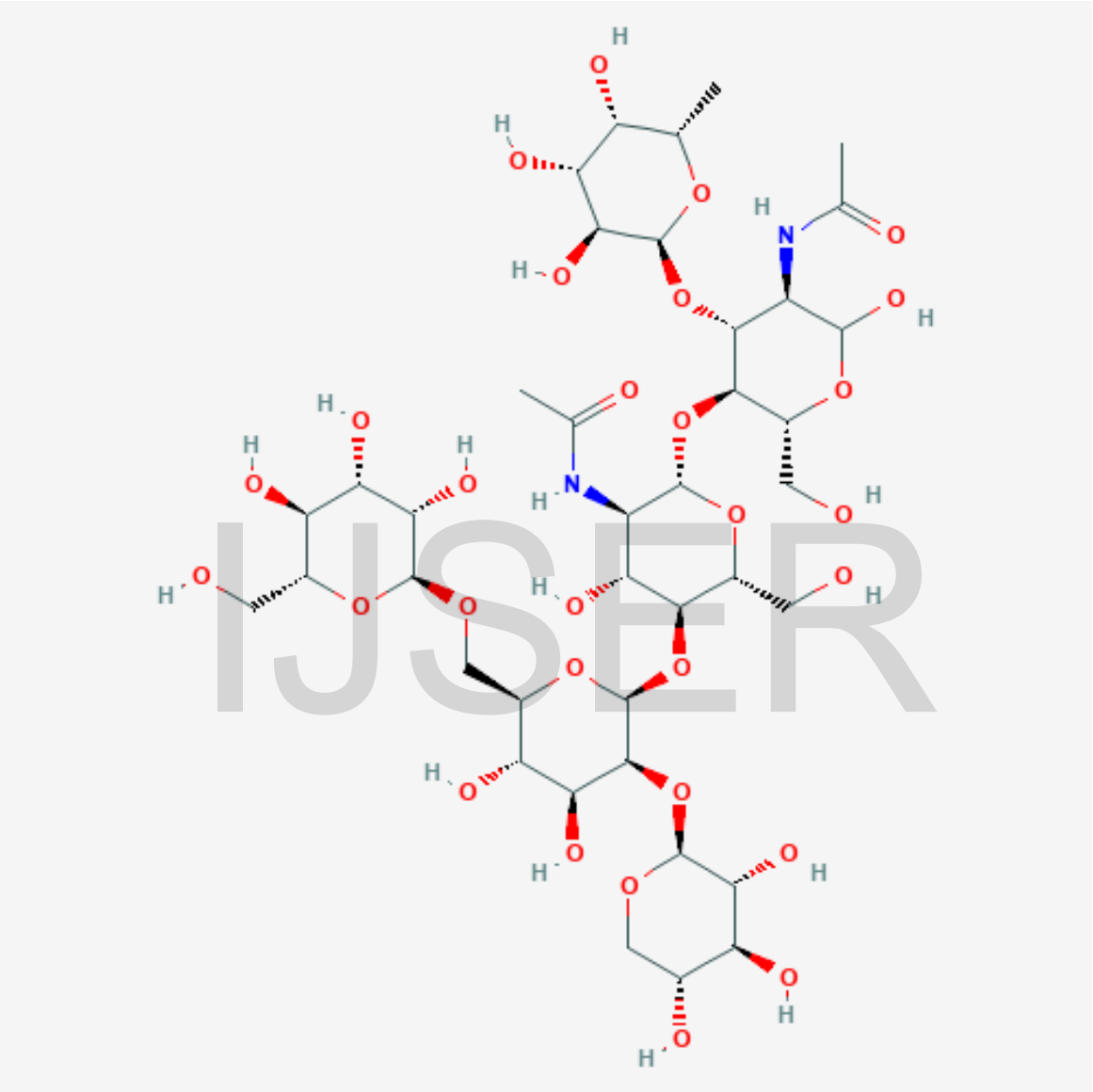

FIG-7

FIG-8

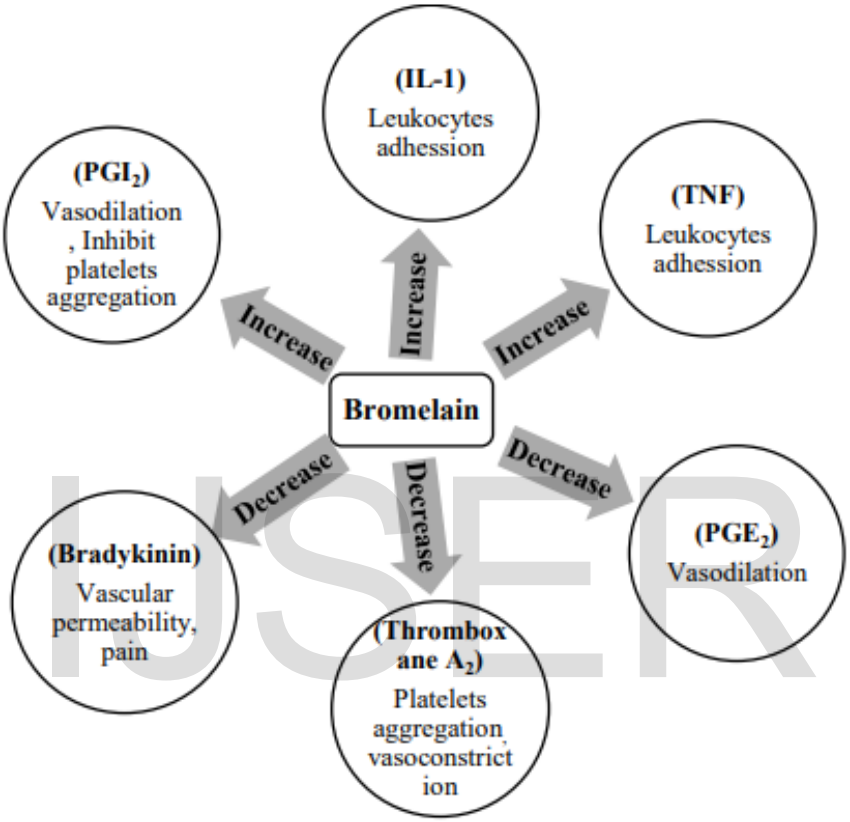
(IL-1)
Leukocytes adhession
(PGI2)
Vasodilation , Inhibit platelets aggregation
(TNF)
Leukocytes adhession
Increase
Increase
Increase
Bromelain
Decrease
Decrease
Decrease
(Bradykinin)
Vascular permeability, pain
(Thromboxane A2)
Platelets aggregation, vasoconstriction
(PGE2)
Vasodilation

FIG9

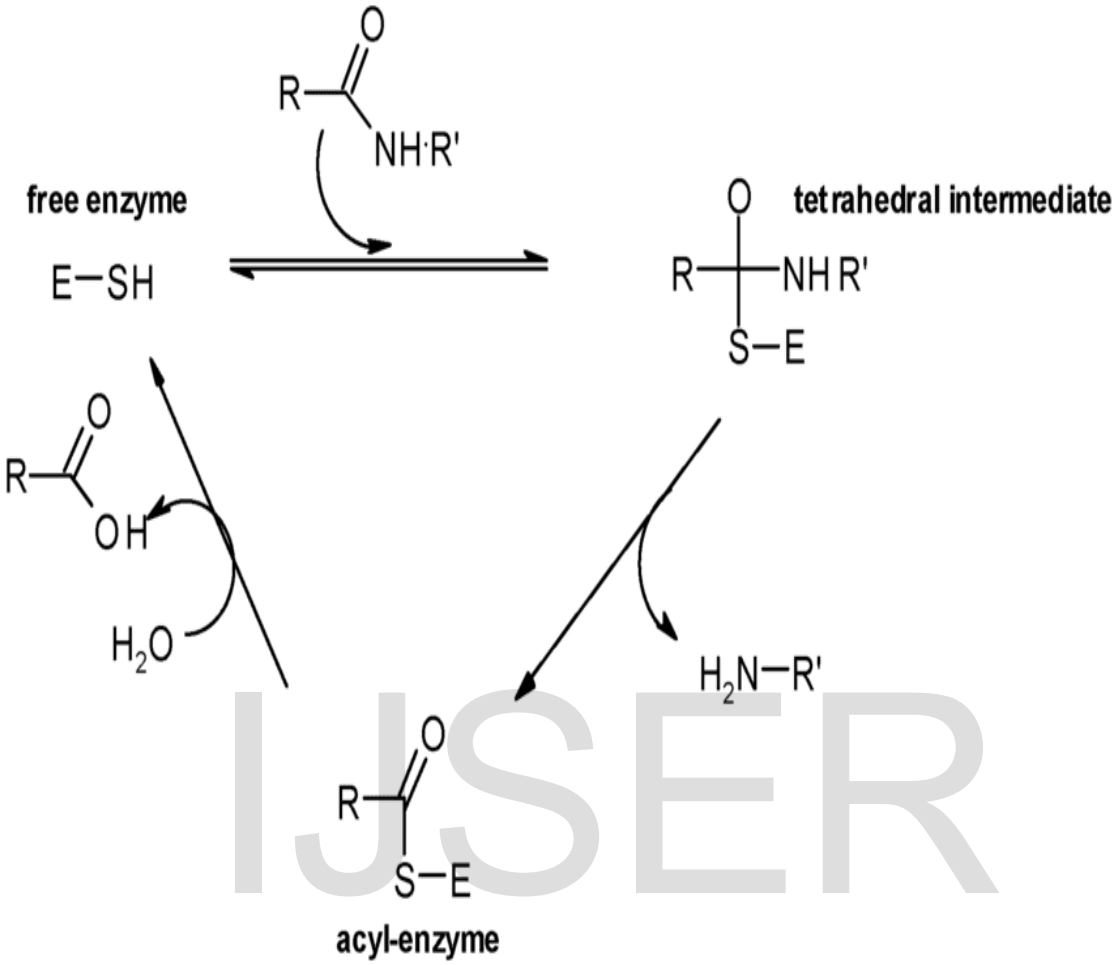
free enzyme
E—SH
tetrahedral intermediate
acyl-enzyme
H_2O
$H_2N—R'$

FIG 10

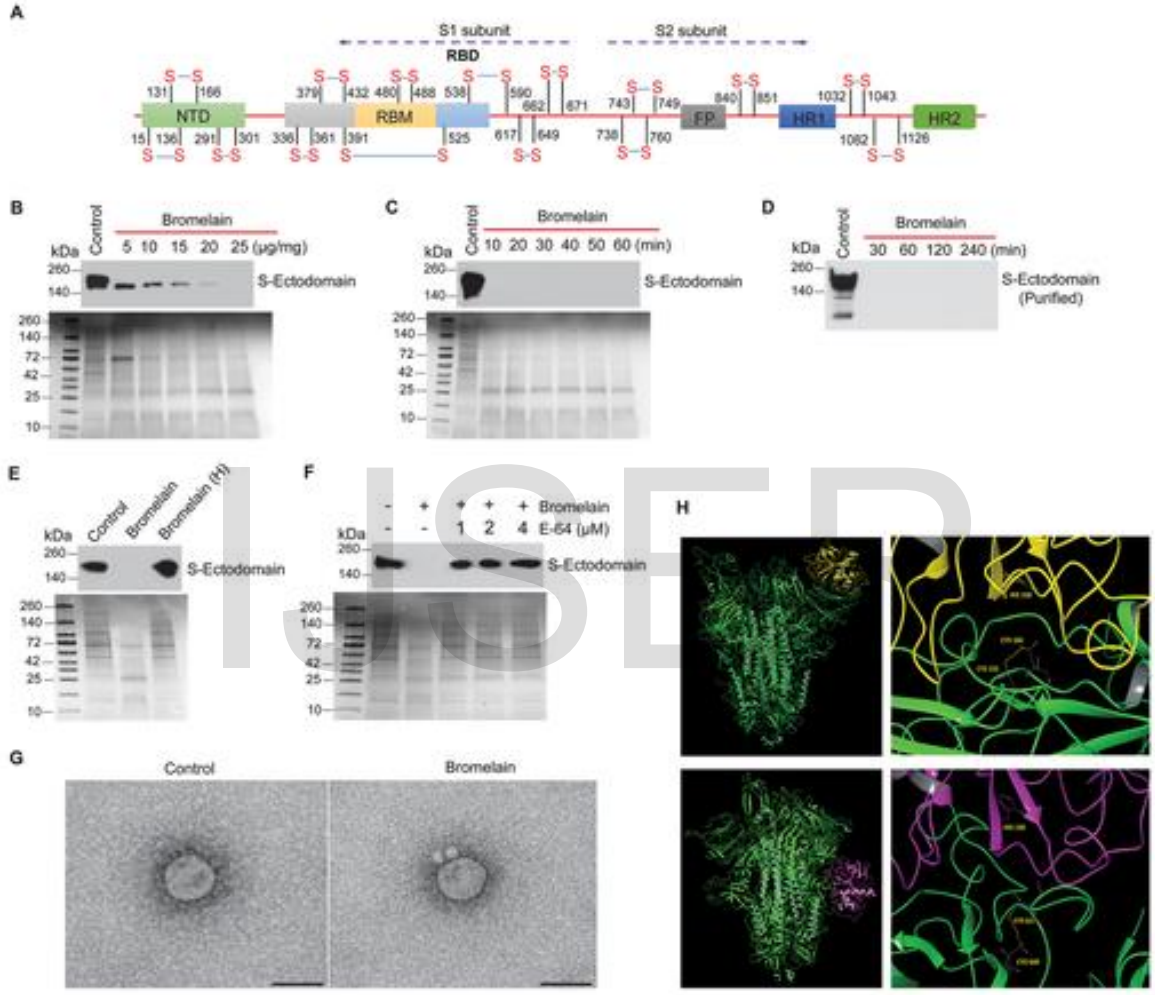
A
S1 subunit
S2 subunit
RBD
NTD
RBM
FP
HR1
HR2
B
Control
Bromelain
5 10 15 20 25 (μg/mg)
kDa
S-Ectodomain
C
Control
Bromelain
10 20 30 40 50 60 (min)
kDa
S-Ectodomain
D
Control
Bromelain
30 60 120 240 (min)
kDa
S-Ectodomain
(Purified)
E
Control
Bromelain
Bromelain (H)
kDa
S-Ectodomain
F
Bromelain
E-64 (μM)
1 2 4
kDa
S-Ectodomain
G
Control
Bromelain
H

FIG 11