Could pineapples be a new weapon against COVID-19?

By Dr. Tomislav Meštrović, MD, Ph.D.

Reviewed by Aimee Molineux

Sep 17 2020

This news article was a review of a preliminary scientific report that had not undergone peer-review at the time of publication. Since its initial publication, the scientific report has now been peer reviewed and accepted for publication in a Scientific Journal. Links to the preliminary and peer-reviewed reports are available in the Sources section at the bottom of this article.

Results of a recent research endeavor from the United States indicate that bromelain or bromelain rich pineapple stem may be utilized as an antiviral agent against coronavirus disease (COVID-19), but also for potential future coronavirus outbreaks. This exciting paper is currently available on the *bioRxiv** preprint server.

Study: Bromelain Inhibits SARS-CoV-2 Infection in VeroE6 Cells. Image Credit: 9comeback / Shutterstock

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is already well-known for its rapid human-to-human transmission, responsible for the relentless pandemic spread of dangerous COVID-19.

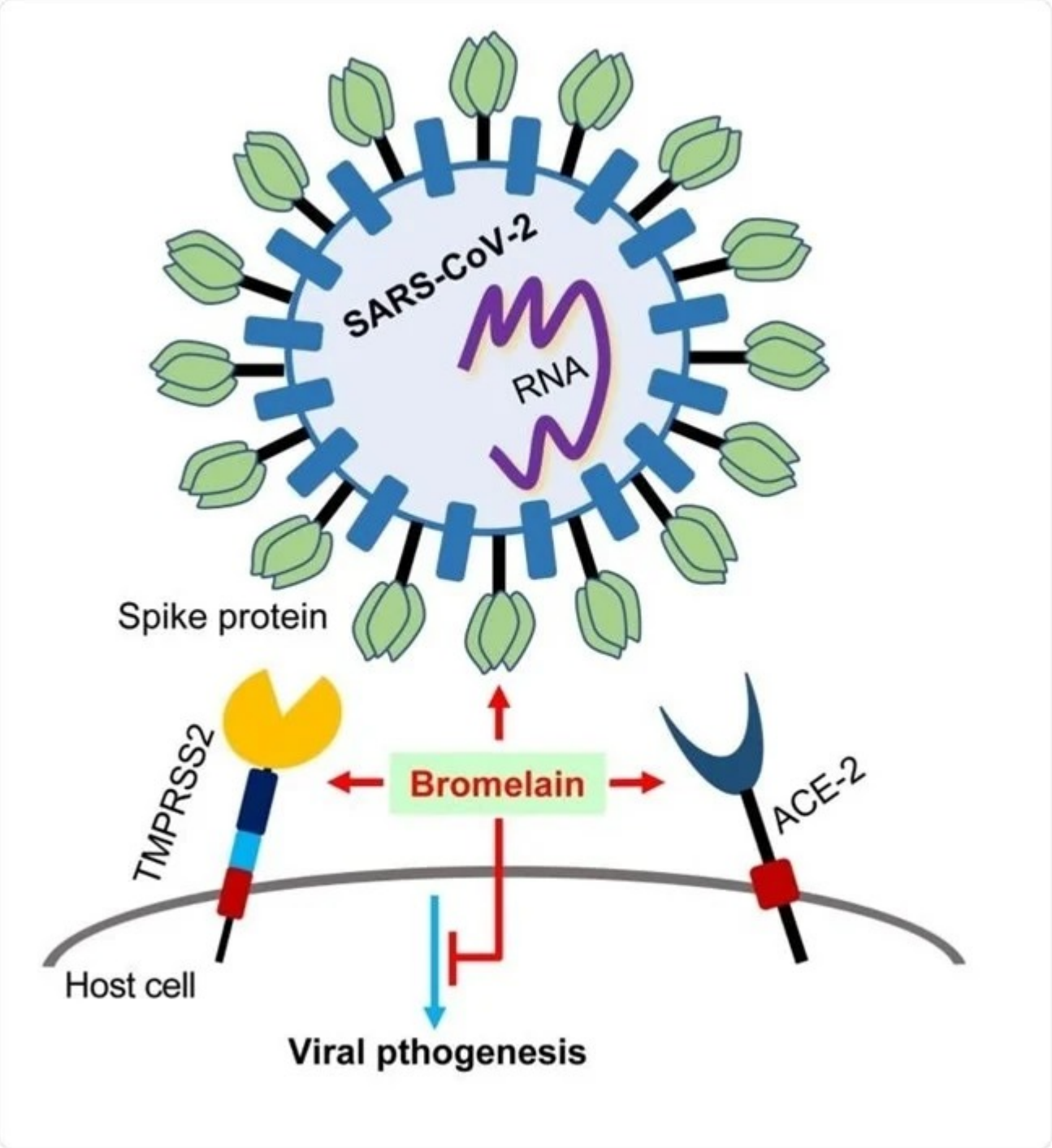
But every day, we learn a bit more about SARS-CoV-2 immunology. The initial interaction between Transmembrane Serine Protease 2 (TMPRSS2), primed spike glycoprotein (S-protein), and host cell receptor angiotensin-converting enzyme 2 (ACE-2) is a pre-requisite for cell entry and COVID-19 pathogenesis.

At the moment, infected patients are treated with different antiviral, anti-inflammatory, and antimalarial agents. Nevertheless, the response rate is relatively modest, and there is a need to confirm both the safety and efficacy profile of those drugs against COVID-19.

But repurposing existing drugs or develop new (either virus-based or host-based) antivirals against SARS-CoV-2 is still a way forward. The pertinent question is, could we maybe use bromelain – a dietary supplement isolated from pineapple stem used to treat patients with pain, inflammation, and

thrombosis – for COVID-19 patients as well?

This hypothesis was tackled by researchers from the University of Nebraska Medical Center and the FDA's Center for Biologics Evaluation and Research (CBER) in Silver Spring in the United States, with rather exciting findings.



Bromelain inhibits / cleaves the expression of ACE-2 and TMPRSS2, it cleaves / degrades SARS-CoV-2 spike protein and inhibits S-Ectodomain binding and SARS-CoV-2 infection.

Cloning and expressing recombinant proteins

Since ACE-2 and TMPRSS2 are full of cysteine residues that establish disulfide bonds to support the protein structure, this research group primarily appraised the effect of bromelain (which is a cysteine protease) on ACE-2 and TMPRSS2 expression.

The full-length genome sequences of 45 SARS-CoV-2 isolates were analyzed in-depth. At the same time, the African green monkey kidney epithelial cells (Vero E6) was chosen as the primary cell line utilized in this study.

The researchers cloned and subsequently expressed SARS-CoV-2 S-protein ectodomain that contains insect cell secretion signal. Next, they have determined the interaction between the purified S-Ectodomain and human recombinant ACE-2 using surface plasmon resonance technology (i.e., real-time detection of biomolecular interactions).

Finally, they have expressed SARS-CoV-2 S-Ectodomain tagged with a green fluorescent protein in Tni insect cells from *Trichoplusia ni* (i.e., cabbage looper). Surface resonance plasmon and Luminex assay were used to reveal the purified S-Ectodomain binding to human ACE-2, as well as immunoreactivity with COVID-19 positive samples.

Multiple ways of halting SARS-CoV-2

> *"We demonstrate that bromelain (isolated from pineapple stem and used as a dietary supplement) treatment diminishes the expression of ACE-2 and TMPRSS2 in VeroE6 cells and dramatically lowers the expression of S-Ectodomain", say study authors.*

More specifically, it was shown that bromelain decreases the expression of both ACE-2 and TMPRSS2 in a dose-dependent manner in Vero E6 cells. Moreover, bromelain's cysteine proteolytic activity was notably higher in ACE-2 when compared to TMPRSS2.

Even more important was the finding that the bromelain treatment was able to halt the interaction between S-Ectodomain and Vero E6 cells, significantly

diminishing the SARS-CoV-2 infection in this cell line.

Furthermore, this study indicates that the SARS-CoV-2 spike glycoprotein has both highly sialylated N- and O-linked glycans, and bromelain managed to cleave it. Consequently, a loss of negatively charged sialic acid groups in the N- and O-linked glycans may cause a decreased mobility shift of S-Ectodomain.

Bromelain as a broad-spectrum antiviral

> *"For the first time, our results demonstrate that bromelain can inhibit SARS-CoV-2 infection by targeting all three host ACE-2 and TMPRSS2, and SARS-CoV-2 S-proteins", summarize study authors in this bioRxiv paper.*

Previous studies have demonstrated that bromelain can be utilized to treat patients with inflammation and pain and that the compound is well absorbed and with prolonged biological activity. All of these advantages can be exploited when treating patients with COVID-19.

In conclusion, either bromelain or bromelain rich pineapple stem represents a viable option as an antiviral for treating not only COVID-19 but also potential future outbreaks of other coronaviruses.

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Journal references:

- **Preliminary scientific report.** Sagar, S. et al. (2020). Bromelain Inhibits SARS-CoV-2 Infection in VeroE6 Cells. *bioRxiv*. https://doi.org/10.1101/2020.09.16.297366.
- **Peer reviewed and published scientific report.** Sagar, Satish, Ashok

Kumar Rathinavel, William E. Lutz, Lucas R. Struble, Surender Khurana, Andy T. Schnaubelt, Nitish Kumar Mishra, et al. 2021. "Bromelain Inhibits SARS-CoV-2 Infection via Targeting ACE-2, TMPRSS2, and Spike Protein." *Clinical and Translational Medicine* 11 (2). https://doi.org/10.1002/ctm2.281, https://onlinelibrary.wiley.com/doi/10.1002/ctm2.281

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Dr. Tomislav Meštrović is a medical doctor (MD) with a Ph.D. in biomedical and health sciences, specialist in the field of clinical microbiology, and an Assistant Professor at Croatia's youngest university - University North. In addition to his interest in clinical, research and lecturing activities, his immense passion for medical writing and scientific communication goes back to his student days. He enjoys contributing back to the community. In his spare time, Tomislav is a movie buff and an avid traveler.

LIFESTYLE

Covid 19 coronavirus: Pineapples could be key to treating virus

news.com.au
By Lauren McMah

19 Aug, 2020 02:44 AM 2 mins to read

Judith Collins challenges the government's claim that border employees refused to take Covid-19 tests.

Australian researchers are testing a breakthrough treatment for Covid-19 derived from the humble pineapple.

Cancer specialist Professor David Morris, from St George Hospital in Sydney, and his team have repurposed a drug he had already developed to treat cancer patients.

The drug, BromAc – which is made with an enzyme found in pineapples -was found to dissolve the spike proteins that Covid-19 uses to infect human cells.

It has been repurposed into a nasal spray that researchers hope will stop the virus' spread from the nose and throat to the lungs. A trial on patients at the Royal Melbourne Hospital could start next month.

As for the pineapple link, one of the ingredients in BromAc is an enzyme called Bromelain, which is derived from pineapple stems, and is already widely used in medicine – including to treat burns patients.

BromAc has already been trialled in 36 cancer patients where it extended their lives and has been found to be safe for human use, the Herald Sun reported.

"We've taken a drug in development for more than a decade and asked whether it can be adapted for treating people infected with Covid-19," Prof David Morris said in a statement.

"Our lab results show the new drug renders the Covid-19 spike ineffective, stopping it from infecting other cells.

"We hope the results will show the treatment can confine Covid to the nose and throat and prevent lung infection, and stop infected patients from passing on the live virus."

The drug won't be a vaccine, but it could be used as for prevention or treatment, the researchers said.

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LETTER TO EDITOR | Open Access

Bromelain inhibits SARS-CoV-2 infection via targeting ACE-2, TMPRSS2, and spike protein

Satish Sagar, Ashok Kumar Rathinavel, William E. Lutz, Lucas R. Struble, Surender Khurana, Andy T. Schnaubelt, Nitish Kumar Mishra, Chittibabu Guda, Nicholas Y. Palermo ... See all authors

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Dear Editor,

The new coronavirus, SARS-CoV-2, transmits rapidly from human-to-human resulting in the ongoing pandemic. SARS-CoV-2 infects angiotensin-converting enzyme 2 (ACE-2) expressing lung, heart, kidney, intestine, gall bladder, and testicular tissues of patients, leading to organ failure and sometimes death.[1, 2] Currently, COVID-19 patients are treated with different agents, including favilavir, remdesivir, chloroquine, hydroxychloroquine, lopinavir, darunavir, and tocilizumab.[3, 4] However, the safety and efficacy of those drugs against COVID-19 still need further confirmation by randomized clinical trials. Hence, there is an emergent need to repurpose the existing drugs or develop new virus-based and host-based antivirals against SARS-CoV-2. Bromelain is a cysteine protease isolated from pineapple stem and is used as a dietary supplement for treating patients with pain, inflammation,[5] thrombosis,[6] and cancer.[7]

Recently, studies have shown that SARS-CoV-2 homotrimeric viral spike protein (S1) binds to the Transmembrane Serine Protease 2 (TMPRSS2) primed host cell's receptor ACE-2 for initial entry, followed by S2-mediated membrane fusion.[8] Of several normal and cancerous cells tested, VeroE6 and Calu-3 cells showed ACE-2 protein expression (Fig. 1A), as well as a basal level of TMPRSS2 protein (Fig. 1B). Since ACE-2[9] and TMPRSS2 (UniProtKB-O15393) contains cysteine residues with disulfide bonds to stabilize the protein structure, we investigated the effect of bromelain on ACE-2 and TMPRSS2 expression. Bromelain-induced a dose- and time-dependent reduction of ACE-2 and TMPRSS2 expression in VeroE6 cells (Fig. 1C and D) but do not alter ACE-2 expression in Calu-3 cells (Fig. 1E). However, bromelain reduces the expression of TMPRSS2 in Calu-3 (Fig. 1E) and ACE-2 negative normal bronchial epithelial (BEAS-2B) and lung adenocarcinoma (A549) cells (Fig. 1F and G). Cysteine protease inhibitor (E-64) treatment further confirmed that bromelain's cysteine protease activity could cleave/reduce the expression of ACE-2 and TMPRSS2 (Fig. 1H). Surface plasmon resonance (SPR) analysis revealed that purified SARS-CoV-2 S-ectodomain binds with ACE-2 in a concentration-dependent manner and has a comparable binding affinity as control RBD (Fig. 1I). The calculated molecular weight of the purified S-ectodomain-GFP protein is ~165 kDa; however, we observed a higher molecular weight of S-ectodomain (~215 kDa), which

may be due to heavy N- and O-linked glycosylation (Fig. 1I intent). A serological assay showed a significantly increased median fluorescent intensity (MFI) of purified S-ectodomain with COVID-19 positive patients' samples (Fig. 1J). These two results indicated that purified S-ectodomain is a properly folded and functionally active protein.

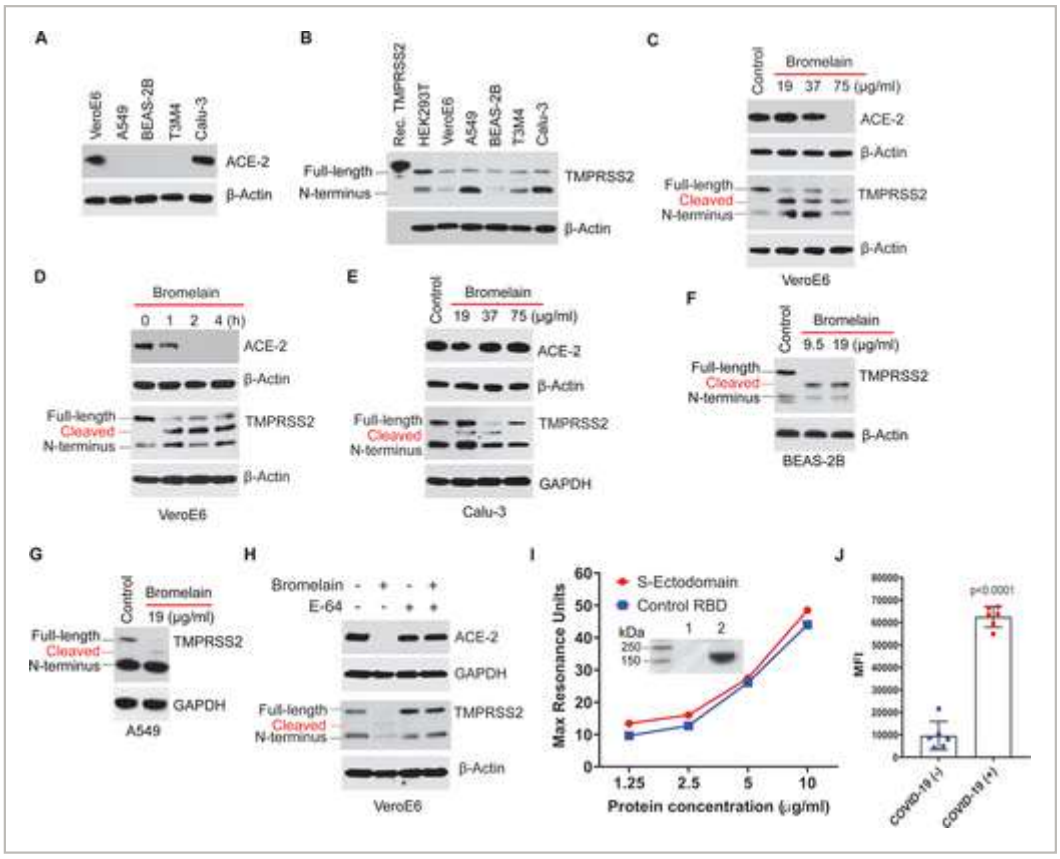

FIGURE 1

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Bromelain inhibits ACE-2 and TMPRSS2 expression. (A and B) Immunoprobing of ACE-2 and TMPRSS2 expression in various normal and cancerous cells. (C and D) Immunoblotting of ACE-2 and TMPRSS2 in VeroE6 cells treated with varying dose (19, 37, and 75 μg/ml for 48 h) and time (75 μg/ml for 1–4 h), respectively. (E) Immunoblotting of ACE-2 and TMPRSS2 in Calu-3 cells treated with varying dose (19, 37, and 75 μg/ml for 48 h). (F and G) Immunoblotting of TMPRSS2 in BEAS-2B and A549 cells treated with varying dose (9.5 and 19 μg/ml for 48 h). (H) ACE-2 and TMPRSS2 expression in bromelain (75 μg/ml) plus E-64 (4 μM) treated VeroE6 cells. β-Actin and GAPDH served as a loading control. (I) SPR Max Resonance Units (RSU) at equilibrium as a function of the concentration of control RBD and S-ectodomain using immobilized ACE-2. S-ectodomain-eGFP purified from Tni insect cells and showed ~215 kDa molecular weight (intent; 1, mock control; 2, S-ectodomain-eGFP). (J) Luminex assay median fluorescent intensity (MFI) of S-ectodomain with COVID-19 positive (n = 6) and negative (n = 6) patients' samples.

The S-ectodomain has 30 cysteine amino acids with 15 stabilizing disulfide bonds (UniProtKB: P0DTC2) (Fig. 2A). The RBD domain alone has nine cysteine residues, eight of which form four disulfide linkages. Bromelain-induced a dose- and time-dependent cleavage of S-ectodomain in Tni insect cell supernatant (Fig. 2B and C) and purified S-ectodomain

(Fig. 2D). Heat inactivation and cysteine proteinase inhibitor (E-64) treatment inhibited bromelain mediated digestion of S-ectodomain (Fig. 2E and F). Further, SARS-CoV-2 with bromelain treatment showed the loss of Spike protein on the viral surface (Fig. 2G). Our docking studies between homotrimeric (RBD containing chain A) S protein and stem bromelain revealed that bromelain cleaves the S-protein equally likely at the 131–166 (50.4%) and 617–649 (49.6%) disulfide bonds (Fig. 2H). Though the catalytic site is not directly engaged, protein–protein docking places the enzyme in close proximity to these bonds. These results demonstrate that bromelain's cysteine protease activity is responsible for the cleavage of host cells' ACE-2 and SARS-CoV-2 S-protein.

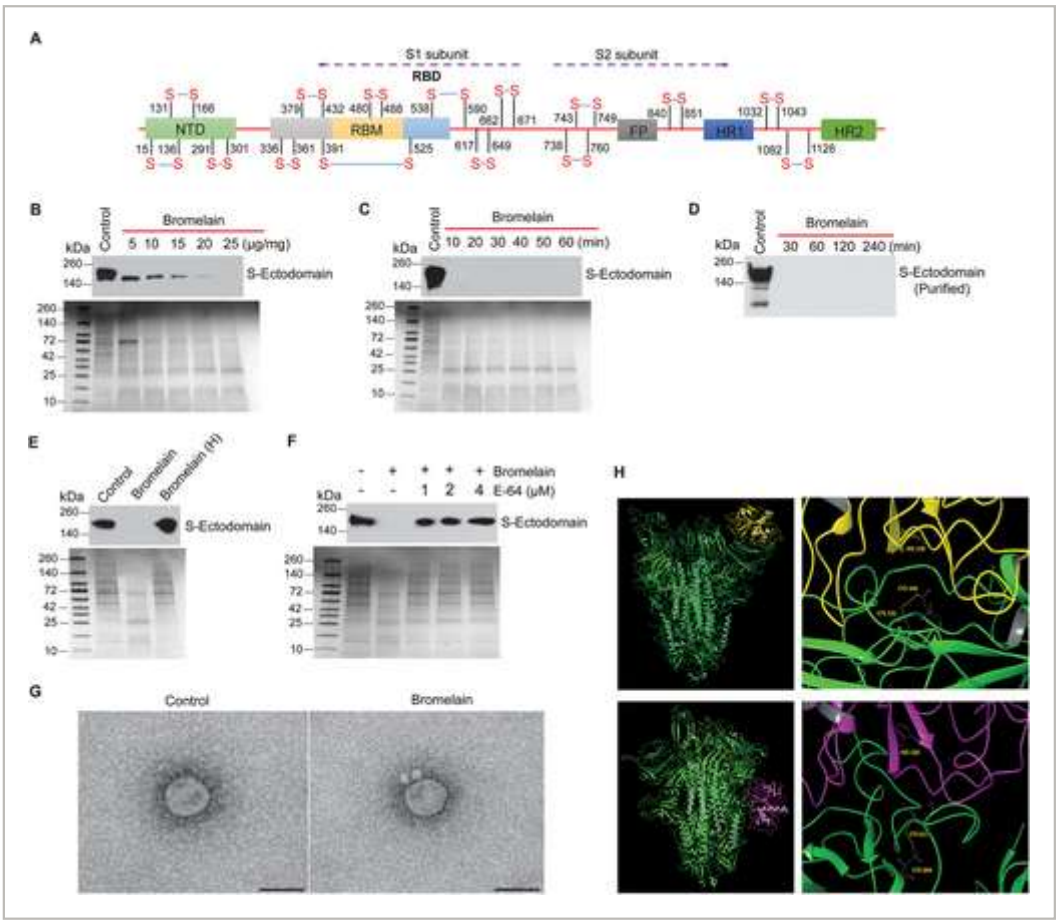

FIGURE 2

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Bromelain cleaves SARS-CoV-2 S-ectodomain. (A) Schematic representation of predicted cysteine amino acid position and disulfide bridges in SARS-CoV-2 S-ectodomain. (B and C) Immunoblotting of S-ectodomain in bromelain-treated Tni insect cell supernatant varying dose (5, 10, 15, 20, and 25 µg/mg of total protein) and time (25 µg/mg of total protein for 10–60 min), respectively. (D) Immunoprobing of bromelain-treated purified S-ectodomain (1:10 ratio) with 30, 60, 120, and 240 min. (E and F) Immunoprobing of S-ectodomain in heat-inactivated bromelain (80°C/8 min) and bromelain (25 µg/mg of total protein) plus E-64 (1, 2, and 4 µM) treated Tni supernatant, respectively. SimplyBlue-stained gel images of bromelain-treated Tni supernatant served as a loading control. (G) Negative-staining transmission-EM of bromelain-treated (250 µg /ml, at 37°C for 1.5 h) SARS-CoV-2. Viral particles treated with vehicle control (left) and bromelain (right). Scale bar = 100 nm. Experiments were performed thrice, and a representative image is presented. (H) Representative

conformation of bromelain (yellow and magenta) docked with the S protein (green) near the 131–166 (upper-left; closer view upper right) and 617–649 (lower-left; closer view lower right) disulfide bridge

Since bromelain digested ACE-2 and S-ectodomain, we investigated the effect of bromelain on the interactions of S-ectodomain and SARS-CoV-2 with VeroE6 cells. Bromelain significantly reduced the binding of S-protein to VeroE6 cells (Fig. 3A and B) and was further confirmed by cysteine protease inhibitor (E-64) treatment (Fig. 3C). Interestingly, bromelain pre-treatment significantly decreased SARS-CoV-2 viral binding in VeroE6 cells (P = .0021) (Fig. 3D). Most importantly, VeroE6 cells or SARS-CoV-2 or both with bromelain reduces the viral infection (Fig. 3E and F). Additionally, we found significantly reduced SARS-CoV-2 viral RNA copies in bromelain-treated VeroE6 (P = .0010) and Calu-3 (P = .0099) cells (Fig. 3G and H, respectively). Collectively, these results suggest that bromelain could inhibit SARS-CoV-2 binding and infection in VeroE6 and Calu-3 cells. Studies have demonstrated that SARS-CoV-2 S-protein has high homology among other coronaviruses (76% identity with SARS-CoV) with conserved cysteine amino acids (UniProtKB: P59594). This indicates that bromelain may be used as a broad antiviral agent against SARS-CoV-2 and other related family members.

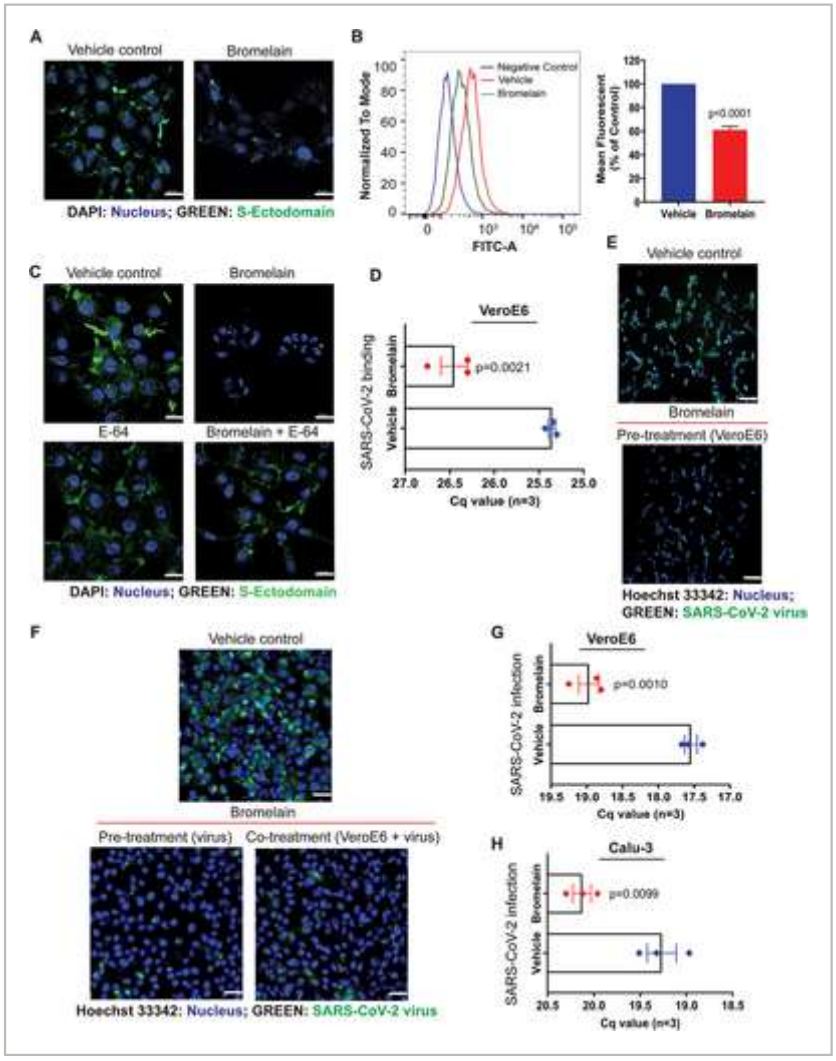

FIGURE 3

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Bromelain inhibits SARS-CoV-2 binding and infection. (A and B) Immunofluorescence and flow cytometry analysis of S-ectodomain in vehicle- (PBS) and bromelain (75 μg/ml/2 h)-treated VeroE6 cells (n = 3), respectively. The mean fluorescent intensity was measured by using FlowJo software. Experiments were performed twice, and one set of representative data is presented. Mean ± SD. (C) Immunofluorescence of S-ectodomain in-vehicle, bromelain (75 μg/ml) with and without E-64 (4 μM)-treated VeroE6 cells (n = 3) for 2 h. Nuclei were stained with DAPI. Scale bar = 20 μm. (D) qRT-PCR analysis of SARS-CoV-2 RNA in vehicle and bromelain pre-treated VeroE6 cells. The Cq values were graphically represented. Mean ± SD (n = 3). Immunofluorescence analysis of SARS-CoV-2 in bromelain pre-treated VeroE6 cells (75 μg/ml/2 h) (n = 6) (E), bromelain pre-treated SARS-CoV-2 (75 μg/ml/1 h) and bromelain co-treated SARS-CoV-2 plus VeroE6 cells (75 μg/ml/1 h) (n = 4) (F). Scale bar = 50 μm. Nuclei were stained with Hoechst 33342. (G and H) qRT-PCR analysis of SARS-CoV-2 RNA infected bromelain-treated VeroE6 and Calu-3 cell culture supernatants, respectively (n = 3). The Cq values were graphically represented. Mean ± SD (n = 3). $P < .05$ was considered statistically significant with an unpaired Student's t-test

In conclusion, the currently used drugs against SARS-CoV-2 have potential side effects. Vaccine trials have started against COVID-19, but the host immune response against SARS-CoV-2 is not fully understood. It differs between individuals, and also re-infection of individuals with SARS-CoV-2. For the first time, our results demonstrate that bromelain can inhibit SARS-CoV-2 infection via targeting ACE-2, TMPRSS2, and SARS-CoV-2 S-protein. Also, thrombosis development is a significant risk factor of multiorgan failure and death in COVID-19 patients.[10] Since bromelain inhibits SARS-CoV-2 infection, and its profound fibrinolytic activity [6] suggests that bromelain or bromelain-rich pineapple could be used as an antiviral against SARS-CoV-2 and future outbreaks of other coronaviruses.

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CONFLICT OF INTEREST

Satish Sagar and Prakash Radhakrishnan have ownership interest (including patents) in a pending patent.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The institutional review board (IRB) of the University of Nebraska Medical Center approved clinical samples used in this study.

AUTHOR CONTRIBUTIONS

Prakash Radhakrishnan conceived the idea; Surender Khurana, St. Patrick M. Reid, Gloria E. O. Borgstahl, and Prakash Radhakrishnan designed research; Satish Sagar, Ashok Kumar Rathinavel, William E. Lutz, Lucas R. Struble, St. Patrick M. Reid, Tobias Hoffmann, and Mara J. Broadhurst performed research; Nitish Kumar Mishra, Chittibabu Guda, Nicholas Y. Palermo, and Kenneth W. Bayles contributed new reagents/analytic tools; Satish Sagar, Ashok Kumar Rathinavel, Andy T. Schnaubelt, Gloria E. O. Borgstahl, St. Patrick M. Reid, and Prakash Radhakrishnan analyzed data; Gloria E. O. Borgstahl, and Prakash Radhakrishnan wrote the paper.

DATA AVAILABILITY STATEMENT

Materials are available upon a reasonable request from the corresponding author.

Supporting Information

Filename	Description
ctm2281-sup-0001-SuppMat.docx 28.7 KB	Detailed materials and methods and key resources are included in the supplementary information.

Please note: The publisher is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing content) should be directed to the corresponding author for the article.

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