

Curative Effects of Traditional Chinese Medicine Quercetin in the Treatment of COVID-19 through Inhibiting Viral S-proteins of SARS-CoV-2 from Binding to its ACE-2 Receptor

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Abstract

SARS-CoV-2 is the virus that causes COVID-19, an infectious respiratory disease that broke out internationally at the beginning of 2020. SARS-CoV-2 enters its host cell as Spike (S) proteins bind to its ACE-2 receptor. Quercetin, a type of Traditional Chinese Medicine (TCM), has been shown by previous literature to have inhibitory effects in this pathway. However, quercetin has low bioavailability, and there is little literature on whether quercetin can lead to inhibitory effects in in-vivo conditions. This study will investigate whether SARS-CoV-2 will be inhibited from binding to its ACE-2 receptor in Syrian hamster cell lines if (1) quercetin is applied alone, (2) bromelain, a dietary supplement that can also increase quercetin's bioavailability, is applied alone, and (3) quercetin and bromelain are both applied. This study will use RT-qPCR and surface plasmon resonance (SPR) to measure the inhibitory effects of any of the treatments in this pathway and UV spectrophotometry to measure the solubility of the treatments in saline solution.

Keywords: SARS-CoV-2, S proteins, ACE-2 receptor, COVID-19, quercetin, traditional Chinese medicine

I. Introduction

The SARS-CoV-2 virus, a virus responsible for COVID-19, broke out towards the end of 2019 and has caused a total of “more than 6.7 million” deaths [1]. The S protein is “the main antigen component in all structural proteins of SARS-CoV-2”, playing a key role in the receptor recognition and the fusion of the cell membranes of the virus and the host cell [2]. In fact, S proteins are abundant on the surface of SARS-CoV-2, and eventually enters the host cell as it binds to the host cell receptor angiotensin-converting enzyme 2 (ACE-2). Once the virus is inside the cell, it begins releasing its viral RNA and synthesizing its viral proteins. This pathway is crucial to the entry of the virus into the host cell. Thus, if this pathway can be blocked by inhibiting the S proteins from binding to its ACE-2 receptor, the infection from SARS-CoV-2 may be prevented.

Quercetin, a plant pigment often used in Traditional Chinese Medicine (TCM), has been suggested by research using platforms like the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, to have the capability of binding to the ACE-2 receptor. This could suggest that quercetin may be able to inhibit S proteins from binding to its ACE-2 receptor and thus prevent the infection. Furthermore, quercetin has been suggested to be able to bind to the RBD domain of the S protein, highlighting how it may have the capability of neutralizing the virus in addition to blocking its receptor.

Despite the optimistic suggestions that have been made about quercetin's effects in preventing SARS-CoV-2 infection by binding to its receptor ACE-2, quercetin has extremely low bioavailability. In fact, research has estimated that “only 20% of the administered dose reaches the blood,” demonstrating how quercetin might not be able to perform its curative role in COVID-19 as a result [3]. Possible solutions that can increase quercetin's bioavailability include applying quercetin in combination with bromelain, a dietary supplement that is usually found in the fruit and stem of pineapples.

This paper aims to investigate whether there will be curative effects in the treatment of COVID-19 through inhibiting S proteins from binding to their ACE-2 receptors when quercetin is applied alone, when bromelain is applied alone, and when both quercetin and bromelain are applied.

This paper hypothesizes that quercetin and bromelain, if applied alone, will not be able to have curative effects, especially in *in-vivo* circumstances. However, quercetin, if applied in combination with bromelain, will be able to have significant curative effects due to increased bioavailability of quercetin.

II Methods

A. Cell lines

This experiment will use 25 Syrian hamsters cell lines as host cells, which are “small mammals that have been

used as models for infection with respiratory viruses,” including SARS-CoV-2 [4]. The cell lines will be kept at 37 °C, which is the usual optimal temperature for their growth. This study will apply random assignment by labeling each cell line with a unique number from 1-25, and using a random number generator to generate 5 unique numbers from 1-25, and assigning cell lines with the corresponding numbers to the negative control group. The study will continue selecting 5 unique numbers without replacement, and assign the corresponding cell lines to the positive control group. The same process will be used to assign the remaining cell lines randomly into the three experimental groups.

B. Negative and positive controls

The negative control group will consist of 5 cell lines treated with saline solution, and the positive control group will consist of 5 cell lines treated with Paxlovid, a Western antiviral drug for the treatment of COVID-19.

C. Treatment groups and levels

Quercetin alone will be applied at 50mg, 500mg, and 5,000mg doses in treatment 1 to the Syrian hamster cell lines. Bromelain alone will be applied at 50mg, 500mg, and 5,000mg doses in treatment 2. 50mg, 500mg, and 5,000 doses of each will be applied together in treatment 3. Inhibitory effects of each treatment at each level will be evaluated after one course of treatment, or 4 weeks, over a total of five courses of treatment.

D. RT-qPCR

RT-qPCR, the “most sensitive method for mRNA quantification”, will be used to investigate the amount of SARS-CoV-2, or the quantitative viral load, present in the Syrian hamster host cells every 48 hours [5].

E. SPR

SPR, a method of detecting COVID-19 infection based on antibodies, will also be used in addition to RT-qPCR. Since antibody responses to SARS-CoV-2 S proteins are “highly sensitive and specific”, the use of SPR will increase the accuracy of COVID-19 detection [6]. SPR will be performed on all cell lines every 48 hours in addition to RT-qPCR.

F. UV spectrophotometry

Aside from measuring the viral load, UV spectrophotometry will be used to study the solubility of quercetin and bromelain within a saline solution. By measuring the amount of light absorbed by a saline solution containing quercetin and a saline solution containing bromelain in comparison to a reference sample — a saline solution by itself, UV spectrophotometry can indicate the solubility

of quercetin and bromelain and shed light on their bioavailability.

G. Statistical analysis

This study will use a Student’s T test to analyze the quantitative data collected from UV spectrophotometry, RT-qPCR, and SPR, with a statistically significant result occurring if p-value < 0.05. All of the methods described above represent one trial of the study, and five trials of the study will be performed in total.

III Results

Since three methods are used in this study, namely RT-qPCR, SPR, and UV spectrophotometry, possible results from each method will be summarized in an individual results table. Each results table dedicated to one method will be presented first, followed by a description of the result from the method. Results tables and descriptions for the remaining two methods will be presented in the same manner.

Table 1 Solubility of quercetin and bromelain measured by UV spectrophotometry

	Result #1	Result #2	Result #3	Result #4
Quercetin	+	+	-	-
Bromelain	+	-	+	-

Note: a “+” means that the substance is soluble in a saline solution as measured by UV spectrophotometry, while a “-” means that the substance is insoluble in a saline solution.

A. Possible result 1: both quercetin and bromelain are soluble.

Through the use of UV spectrophotometry, which measures the amount of light absorbed by the sample as compared to a reference saline solution, both quercetin and bromelain have been indicated to be soluble to a significant extent in a saline solution.

B. Possible result 2: quercetin is soluble while bromelain is insoluble.

Through the use of UV spectrophotometry, quercetin has been indicated to have significant solubility in a saline solution, while bromelain has an insignificant solubility in a saline solution.

C. Possible result 3: bromelain is soluble while quercetin is insoluble.

Through the use of UV spectrophotometry, bromelain has been indicated to have significant solubility in a saline solution, while quercetin has an insignificant solubility in a saline solution.

D. Possible result 4: both quercetin and bromelain are insoluble.

Through the use of UV spectrophotometry, neither quercetin nor bromelain have demonstrated significant solubility within a saline solution.

Table 2 SARS-CoV-2 viral load measured by RT-qPCR

	Result #1	Result #2	Result #3	Result #4	Result #5	Result #6	Result #7	Result #8
Querce-tin	+	+	+	-	+	-	-	-
Brome-lain	+	+	-	+	-	+	-	-
Querce-tin and Brome-lain	+	-	+	+	-	-	+	-

Note: a “+” means that the viral load detected using RT-qPCR is less than that in the NC, while a “-” means that the viral load detected is more than or equal to that in the NC.

A. Possible result 1: applying quercetin alone, bromelain alone, or quercetin and bromelain together will all significantly reduce the viral load.

As measured by RT-qPCR, all 3 treatments will have a significant inhibitory effect in the S protein and ACE-2 receptor pathway, as indicated by a measured amount of viral load in the cell lines that is less than that measured in the negative control group (NC).

B. Possible result 2: applying quercetin alone or bromelain alone will significantly reduce the viral load, while applying quercetin and bromelain together will not significantly reduce the viral load.

As measured by RT-qPCR, quercetin or bromelain alone will have significant inhibitory effects in the S protein and ACE-2 receptor pathway, as indicated by a measured amount of viral load that is less than that of the NC. However, quercetin and bromelain, when applied together, will not have significant inhibitory effects as indicated by a measured amount of viral load that is equal to or larger than that of the NC.

C. Possible result 3: applying quercetin alone or applying quercetin and bromelain together will significantly reduce the viral load, while applying bromelain alone will not significantly reduce the viral load.

As measured by RT-qPCR, quercetin alone or quercetin and bromelain together will have significant inhibitory effects in the S protein and ACE-2 receptor pathway, as indicated by a measured amount of viral load that is less than that of the NC. Bromelain alone will not have significant inhibitory effects as indicated by a measured amount of viral load that is equal to or larger than that of the NC.

D. Possible result 4: applying bromelain alone or applying quercetin and bromelain together will significantly reduce the viral load, while applying quercetin alone will not significantly reduce the viral load.

As measured by RT-qPCR, bromelain alone or quercetin and bromelain together will have significant inhibitory effects in the S protein and ACE-2 receptor pathway, as indicated by a measured amount of viral load that is less than that of the NC. Quercetin alone will not have significant inhibitory effects as indicated by a measured amount of viral load that is equal to or larger than that of the NC.

E. Possible result 5: only quercetin alone will significantly reduce the viral load.

As measured by RT-qPCR, only quercetin alone will have significant inhibitory effects in the S protein and ACE-2 receptor pathway, as indicated by a measured amount of viral load that is less than that of the NC. Bromelain alone or quercetin and bromelain together will not have significant inhibitory effects as indicated by a measured amount of viral load that is equal to or larger than that of the NC.

F. Possible result 6: only bromelain alone will significantly reduce the viral load.

As measured by RT-qPCR, only bromelain alone will have significant inhibitory effects in the S protein and ACE-2 receptor pathway, as indicated by a measured amount of viral load that is less than that of the NC. Quercetin alone or quercetin and bromelain together will not have significant inhibitory effects as indicated by a measured amount of viral load that is equal to or larger than that of the NC.

G. Possible result 7: only quercetin and bromelain together will significantly reduce the viral load.

As measured by RT-qPCR, only quercetin and bromelain together will have significant inhibitory effects in the S protein and ACE-2 receptor pathway, as indicated by a measured amount of viral load that is less than that of the NC. Quercetin alone or bromelain alone will not have significant inhibitory effects as indicated by a measured amount of viral load that is equal to or larger than that of

the NC.

H. Possible result 8: quercetin alone, bromelain alone, or quercetin and bromelain together will all reduce the viral load by only an insignificant amount.

As measured by RT-qPCR, quercetin alone, bromelain alone, or quercetin and bromelain together will all have insignificant inhibitory effects on the S proteins and ACE-2 receptor pathway as indicated by a measured amount of viral load that is equal to or larger than that of the NC.

Table 3 Blockage of S protein’s interaction with its ACE-2 receptor as measured by SPR

	Possible result #1	Possible result #2	Possible result #3	Possible result #4	Possible result #5	Possible result #6	Possible result #7	Possible result #8
Querce-tin	+	+	+	-	+	-	-	-
Brome-lain	+	+	-	+	-	+	-	-
Quercet-in and Brome-lain	+	-	+	+	-	-	+	-

Note: a “+” means that S proteins were blocked from interacting with its ACE-2 receptor more than in the NC, while a “-” means that S proteins were blocked from interacting with its ACE-2 receptor less than or equal to in the NC.

A. Possible result 1: quercetin alone, bromelain alone, or quercetin and bromelain together will all significantly block the S-proteins from interacting with its ACE-2 receptor.

As measured by SPR, quercetin alone, bromelain alone, or quercetin and bromelain together will all have significant inhibitory effects on the S proteins and ACE-2 receptor pathway as indicated by a lower interaction between the S proteins and its ACE-2 receptor than that observed in the NC.

B. Possible result 2: applying quercetin alone or bromelain alone will significantly block the S-proteins from interacting with its ACE-2 receptor, while applying quercetin and bromelain together will not have significant effects.

As measured by SPR, quercetin or bromelain alone will have significant inhibitory effects in the S protein and ACE-2 receptor pathway, as indicated by a a lower interaction between the S proteins and its ACE-2 receptor than that observed in the NC. However, quercetin and bromelain, when applied together, will not have significant inhibitory effects as indicated by a measured amount of interaction between S protein and its ACE-2 receptor that is equal to or larger than that of the NC.

C. Possible result 3: quercetin alone or quercetin and bromelain together will significantly block the S-proteins from interacting with its ACE-2 receptor, while bromelain alone will not have significant effects.

As measured by SPR, quercetin alone or quercetin and bromelain together will have significant inhibitory effects in the S protein and ACE-2 receptor pathway, as indicated by a a lower interaction between the S proteins and its ACE-2 receptor than that observed in the NC. Bromelain alone will not have significant inhibitory effects as indicated by a measured amount of interaction between S protein and its ACE-2 receptor that is equal to or larger than that of the NC.

D. Possible result 4: bromelain alone or quercetin and bromelain together will significantly block the S-proteins from interacting with its ACE-2 receptor, while quercetin alone will not have significant effects.

As measured by SPR, bromelain alone or quercetin and bromelain together will have significant inhibitory effects in the S protein and ACE-2 receptor pathway, as indicated by a a lower interaction between the S proteins and its ACE-2 receptor than that observed in the NC. Quercetin alone will not have significant inhibitory effects as

indicated by a measured amount of interaction between S protein and its ACE-2 receptor that is equal to or larger than that of the NC.

E. Possible result 5: only quercetin alone will significantly block the S-proteins from interacting with its ACE-2 receptor.

As measured by SPR, only quercetin alone will have significant inhibitory effects in the S protein and ACE-2 receptor pathway, as indicated by a lower interaction between the S proteins and its ACE-2 receptor than that observed in the NC. Bromelain alone or quercetin and bromelain together will not have significant inhibitory effects as indicated by a measured amount of interaction between S protein and its ACE-2 receptor that is equal to or larger than that of the NC.

F. Possible result 6: only bromelain alone will significantly block the S-proteins from interacting with its ACE-2 receptor.

As measured by SPR, only bromelain alone will have significant inhibitory effects in the S protein and ACE-2 receptor pathway, as indicated by a lower interaction between the S proteins and its ACE-2 receptor than that observed in the NC. Quercetin alone or quercetin and bromelain together will not have significant inhibitory effects as indicated by a measured amount of interaction between S protein and its ACE-2 receptor that is equal to or larger than that of the NC.

G. Possible result 7: only quercetin and bromelain together will significantly block the S-proteins from interacting with its ACE-2 receptor.

As measured by SPR, only quercetin and bromelain together will have significant inhibitory effects in the S protein and ACE-2 receptor pathway, as indicated by a lower interaction between the S proteins and its ACE-2 receptor than that observed in the NC. Bromelain alone or quercetin alone will not have significant inhibitory effects as indicated by a measured amount of interaction between S protein and its ACE-2 receptor that is equal to or larger than that of the NC.

H. Possible result 8: quercetin alone, bromelain alone, or quercetin and bromelain together will all lead to only insignificant reductions in the interaction between S protein and its ACE-2 receptor.

As measured by SPR, quercetin alone, bromelain alone, or quercetin and bromelain together will have insignificant inhibitory effects in the S protein and ACE-2 receptor pathway, as indicated by a larger or equal amount of

interaction between the S proteins and its ACE-2 receptor than that observed in the NC.

IV Discussion

Overall, there are 20 possible results: 4 from using UV spectrophotometry, 8 from using RT-qPCR, and 8 from using SPR. In order to avoid repetitive analysis of identical results, results from RT-qPCR and SPR will be viewed and analyzed in combination as an indication of whether the treatment had significant inhibitory effects on the S proteins and ACE-2 receptor pathway. For example, if 4 out of the 5 trials of the experiment show that quercetin alone does not have significant inhibitory effects as measured by both RT-qPCR and SPR, the paper will view the combination of results as a sign of how quercetin does not have significant inhibitory effects in this study.

Firstly, Possible results 1 from UV spectrophotometry, where both quercetin and bromelain are soluble in saline solution, partially supports my hypothesis. The fact that bromelain is soluble is consistent with previous research, especially the finding that bromelain is capable of increasing quercetin's bioavailability. On the other hand, quercetin is unlikely to have a high solubility in saline solution as suggested by its low bioavailability, demonstrating why this result would only partially support my hypothesis. The result could have happened because of systematic errors with the use of UV spectrophotometry, such as accidentally leaving a finger print on the cuvette containing the solution with quercetin, which would lead to an overestimation of the sample's absorption of light and consequently an overestimation of the concentration of quercetin that has been dissolved in the sample. To re-conduct this experiment, it is crucial to make sure that all of the cuvettes are entirely clean, and that the UV spectrophotometry is used correctly.

Possible results 2 from UV spectrophotometry fully contradicts my hypothesis, as quercetin is soluble while bromelain is not. An amount of bromelain lower than intended might have been accidentally added, while a systematic error mentioned in possible results 1 from UV spectrophotometry might have occurred and caused an overestimation of the solubility of quercetin. To avoid making the same mistake in future trials, dosages must be carefully measured, and UV spectrophotometry equipment must be carefully checked and made clean.

Possible results 3 from UV spectrophotometry fully supports my hypothesis, as quercetin is insoluble while bromelain is soluble. This is consistent with previous studies on the low bioavailability of quercetin and the higher solubility of bromelain, which can also help it increase the bioavailability of quercetin when applied

together.

Possible results 4 from UV spectrophotometry partially supports my hypothesis, as both quercetin and bromelain are measured to be insoluble. A lower than intended amount of bromelain might have been added to cause this result, and it is important to make sure that dosages are carefully measured and double-checked for future trials, and that the equipment used to measure dosages are carefully calibrated and accurate.

Possible results 1 from RT-qPCR and SPR — where quercetin alone, bromelain alone, and quercetin and bromelain together all have significant inhibitory effects in the S proteins and ACE-2 receptor pathway — partially supports my hypothesis. Due to quercetin's low bioavailability, quercetin alone should not be able to significantly prevent S proteins from binding to its ACE-2 receptors. Furthermore, though bromelain is a dietary supplement, its inhibitory effects in the S proteins and ACE-2 receptor pathway is not supported by a significant amount of previous literature. Lastly, quercetin and bromelain together is predicted to have significant inhibitory effects, since bromelain is effective in increasing the bioavailability of quercetin and can thus amplify the inhibitory effects quercetin has. Quercetin alone and bromelain alone might have been measured to have significant inhibitory results because (1) a higher dosage of each were accidentally added, (2) other materials that can increase quercetin's bioavailability — such as Vitamin C — may have been unintentionally added in the cultivation of the Syrian hamster cell lines in preparation for the study, or (3) quercetin was accidentally added into the cell lines instead of bromelain alone, which may lead to increased inhibitory effects. For future trials, careful records about materials added to the cell lines should be checked to make sure that only the treatment at the correct doses are applied.

Possible results 2 from RT-qPCR and SPR fully contradicts my hypothesis, as quercetin alone and bromelain alone have significant inhibitory effects, while quercetin and bromelain together do not. Aside from the 3 reasons mentioned in the discussion of result 1 from RT-qPCR and SPR that might have led to an overestimation of the inhibitory effects of quercetin and bromelain alone, inhibitory effects of quercetin and bromelain together might not have been measured because a much lower dosage of each substance was accidentally applied, or because bromelain can not increase quercetin's bioavailability to an extent large enough for quercetin's inhibitory effects to fully take place. Doses must be carefully measured and applied at different intended amounts, namely 50mg, 500mg, and 5,000mg each, to ensure that inhibitory effects can be measured across

small, medium, and large doses and to prevent false negatives.

Possible results 3, 4, 5, and 6 from RT-qPCR and SPR partially support my hypothesis. The results either show that quercetin alone has significant inhibitory effects, bromelain alone has significant inhibitory effects, quercetin and bromelain together have insignificant inhibitory effects, or combinations of the three. Reasons such as higher dosages, accidental addition of quercetin to bromelain alone, or accidental addition of substances like vitamin C to quercetin alone might have led inhibitory effects to be observed. Lower dosages of each might have led no inhibitory effects to be observed for quercetin and bromelain together. Random errors like incorrect dosages and additions of materials that will affect the study undesirably should be prevented for future studies, as mentioned more specifically in the discussion of possible results 1 and 2 from RT-qPCR and SPR.

Possible result 7 from RT-qPCR and SPR fully support my hypothesis, as quercetin and bromelain alone do not exhibit significant inhibitory effects, while bromelain and quercetin together do. Quercetin's low bioavailability would likely prevent it to sufficiently block S protein from interacting with its ACE-2 receptor despite its potential in doing so, as little of quercetin would actually be absorbed by the cell line. Furthermore, though bromelain — being a dietary supplement — can have strengthening effects on the immune system, its effects of disrupting the S protein and ACE-2 receptor pathway is not widely found in the current body of literature. Lastly, since bromelain is a dietary supplement and can likely increase the bioavailability of quercetin, it may allow the inhibitory effects of quercetin to take place to a significant extent. This would suggest that quercetin and bromelain together will successfully block S proteins from interacting with its ACE-2 receptors.

Lastly, possible result 8 from RT-qPCR and SPR partially supports my hypothesis, as no significant inhibitory effects of quercetin and bromelain together have been measured. This may be because both quercetin and bromelain were added in dosages that were too low for an effect to occur, and it is crucial to make sure that different dosages of quercetin and bromelain are added for five trials of the experiment and for future experiments so it will be clear whether inhibitory effects will occur at larger doses.

V Conclusion

Overall, this study has investigated whether quercetin alone, bromelain alone, or quercetin applied in combination with bromelain, will have significant curative effects in the treatment of COVID-19. The potentially controversial results can indicate whether it is worthy to further invest in the usage of quercetin to cure COVID-19,

and whether bromelain can increase the bioavailability of quercetin to an extent large enough for quercetin's inhibitory effects to be adequate. More research in the future can further investigate this question by applying quercetin in combination with other materials, such as Vitamin C, to observe if any significant curative effects will occur, which will continue to benefit the combat against COVID-19 in the long term.

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