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ORIGINAL ARTICLE

The Study of Quadruple Therapy Zinc, Quercetin, Bromelain, and Vitamin C on the Clinical Outcomes of Patients Infected with COVID-19

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Abstract

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by a new strain of coronavirus, SARS-CoV-2. There are three phases of COVID-19: early infection stage, pulmonary stage, and hyper-inflammation stage. It is essential to prevent lung or other organ injuries by preventing phase-II and phase-III using pharmacological or non-pharmacological treatments. This case series study aims to evaluate the safety and efficacy of Quercetin, Bromelain, zinc, and vitamin C combination supplements in patients with COVID-19. In this study, twenty-two patients diagnosed (mean age 49.27, 68.18% male) with COVID-19 in Imam Abdulrahman Alfaisal Hospital in Riyadh have administrated Quercetin 800 mg, Bromelain 165 mg, zinc acetate 50 mg, and vitamin C 1 g once daily as supplements for 3 to 5 days. The mean levels of WBC, ANC, ALC, AMC, and AST, D dimer were measured to investigate the safety of the used treatment. we planned to measure how long stay at the hospital, Chemistry profiles like D dimer, serum ferritin, CRP, serum zinc, and interleukin 6 as planned in the protocol and measured not done for all parameters like zinc, and interleukin 6 were not done as unavailable facilities at lab of hospital Their mean values were normal among all included patients before and after taking the supplements. Therefore, the once-daily administration of these supplements was found to be safe for patients infected with SARS-CoV-2 and may prevent the poor prognosis of the disease. Randomized clinical trials are needed in the future to ensure the efficacy of Quercetin, Bromelain, zinc, and vitamin C combination. The study was registered at clinicaltrials.gov with the identifier: NCT04468139.

Key Words: *Quercetin; Bromelain; Zinc; Ascorbic acid; COVID-19; Inflammation; Safety; Clinical trial*

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Introduction

The outbreak of the pneumonic Coronavirus disease 2019 (COVID-19) in Wuhan, China, is caused by the SARS-CoV-2, a new strain of betacoronavirus [1]. About 180 million people worldwide have been infected and got COVID-19, and around four million of them died of the disease complications, including acute respiratory distress syndrome (ARDS) and cytokine storm [2-3]. There are three phases of COVID-19: phase-I (early infection stage), in which the virus starts to spread and proliferate, and the innate immunity is activated, phase-II (pulmonary stage), characterized by lung tissue injury and increased leukocyte recruitment. Phase III (hyper-inflammation stage), in which various organs could be damaged, and there is an extreme exacerbation of immune response. To treat COVID-19 patients, it is essential to prevent lung or other organ injury by preventing phase II and phase III via pharmacological or non-pharmacological treatments [4].

Quercetin is a natural flavonoid molecule distributed broadly in many fruits and vegetables, including red onion, cranberry, kale, tomatoes, Hungarian wax, and watercress [5]. It was revealed in previous studies that Quercetin has an anti-inflammatory and anti-hypersensitivity effect by preventing pro-inflammatory prostaglandins and leukotrienes by inhibiting cyclooxygenase (COX) and lipoxygenase (LOX) enzymes. Therefore, Quercetin was used as an extract in various trials to treat different infectious and non-infectious diseases. In addition, Quercetin has been shown to reduce tumor necrosis factor-alpha (TNF-α) production with chronic inflammation [7]. Reduction in the ratio of $CD4^+$:$CD8^+$ T cells and suppression of macrophages, dendritic, mast cells, and interleukin-6 (IL-6) levels were revealed after a specific tissue was treated with Quercetin in pre-clinical studies (Figure 1) [8-9]. Besides, Quercetin is expected to have an antiviral activity by acting as a zinc chelator and zinc ionophore [10]. However, because most of these studies were done using Quercetin *in vitro* with high concentration and *in vivo* studies cannot use the same doses, it showed minimum effect during clinical trials. The available data shows that Quercetin is very safe and used as a nutritional supplement with up to 1500 mg/day dose [11].

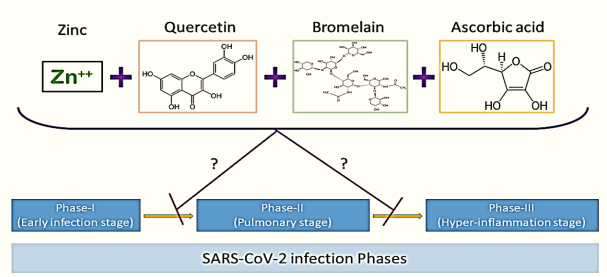

Figure 1) *Graphical abstract: Quercetin, Bromelain, zinc, and ascorbic acid combination are expected to prevent poor prognosis of COVID-19 by restraining patients from pulmonary and inflammation stages [7] [13-14].*

Bromelain is a protein enzyme found mainly in the stem of the pineapple plant. The bioavailability of Bromelain is high through the oral route and safe even when consumed with a dose of more than 11 g/day [12]. *In vitro* studies showed that Bromelain exerts an anti-inflammatory effect by reducing serum bradykinin and modulating the expression of some genes related to inflammation [13-14]. Three genes related to inflammation, including TLR4, TNF-α, and IL-8, were found to be less expressed after Bromelain treatment (Figure 2). On the other hand, PPARγ gene expression was elevated after treatment with Bromelain [15] Therefore, Bromelain may have a role in reducing inflammations during various disorders and may be used in combination with other analgesics and anti-inflammatory drugs.

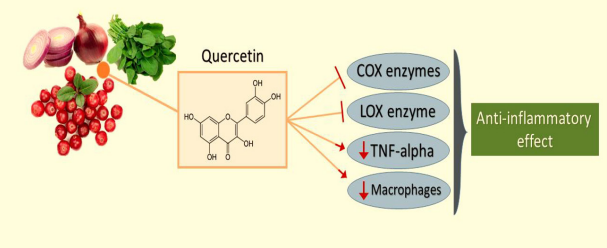

Figure 2) *Role of Quercetin in inhibiting inflammation by blocking the activity of COX (cyclooxygenase) enzymes and LOX (lipoxygenase) enzyme, in addition to reducing TNF-α (tumor necrosis factor-alpha) and macrophages levels.*

It has been found that Zn^{2+} deficiency or dysregulation leads to an exaggerated activity of Cysteine Cathepsin (CysCt), increasing the autoimmune/inflammatory response. For this purpose, Zn^{2+} metal can be safely combined with a drug that increases the anti-proteolytic effect of endogenous Zn^{2+} lowering the excessive activity of some CysCts. Also, biguanide derivatives complex with Zn^{2+} were found to be promising inhibitors of CysCts protease reactions, hence decreasing the exaggerated inflammatory response [16]. Using molecular docking, we targeted the mechanism of action of both Quercetin and Bromelain by inhibiting the Human Cathepsin L protein, thus lowering the inflammatory response and the disease's poor prognosis.

Since the inflammatory status of patients during COVID-19 may lead to severe consequences and even death if not prevented or treated adequately, it is crucial to ensure high-quality care to patients and provide evidence-based prophylaxis and treatment. This study evaluates the efficacy and safety of Quercetin, Bromelain, zinc, and ascorbic acid supplements in patients with COVID-19.

Materials and Methods

Study design and subjects

The study was conducted between June and September 2020. In this case series study, twenty-two subjects diagnosed with COVID-19 were included. The study subjects included are adults and hospitalized in Imam Abdulrahman Alfaisal Hospital in Riyadh.

Supplements and Measurements

COVID-19 patients in this study were administered Quercetin 800 mg, Bromelain 165 mg, zinc acetate 50 mg, and ascorbic acid 1 g once daily as supplements for 3 to 5 days while hospitalized. Several laboratory tests were implemented for all patients included in this study. These tests include absolute neutrophil count (ANC), absolute lymphocyte count (ALC), absolute monocyte count (AMC), hemoglobin (Hb), platelets (Plts), potassium (K), aspartate aminotransferase (AST), oxygen saturation percentage (SaO_2), D-dimer and white blood cells (WBCs). In addition, medical and medication history were reported.

Endpoint and Statistical Analysis

The primary endpoint was to ensure the efficacy of Quercetin, Bromelain, zinc, and ascorbic acid supplements by evaluating the laboratory results pre- and post-supplements. A paired t-test was used to reveal the differences between different lab tests before and after the administration of the supplements.

Molecular Docking

To prepare the Human Cathepsin L protein for molecular docking, the binding site was generated from the co-crystallized ligand within the protein's crystal structure (PDB codes: 1MHW) by using MOE 19.0901 Software. At first, all water molecules were removed from the complex. Then, crystallographic disorders and unfilled valence atoms were corrected using protein reports and utility and clean protein options. The protein energy was minimized by applying MMFF94 force fields. The rigid binding site of the protein was obtained by applying a fixed atom constraint. The protein essential amino acids were defined and prepared for the docking process. 2D structures of the tested compounds were drawn using Chem-Bio Draw Ultra16.0 and saved in MDL-SD file format. Using MOE 19.0901 software, the saved file was opened, 3D structures were protonated, and energy was minimized by applying 0.05 RMSD kcal/mol MMFF94 force field.

Then, the minimized structures were prepared for docking according to the standard procedure. The molecular docking process was implemented according to the

CDOCKER protocol. The receptor was held rigid while the ligands were allowed to be flexible. During the refinement, each molecule was allowed to produce ten different interaction poses with the protein. Then, the docking scores (-CDOCKER interactions energies) of the best-fitted poses with the active site were recorded and viewed as 3D using Discovery Studio 2019 Client software.

Results

Twenty-two hospitalized patients diagnosed with COVID-19 was enrolled in this study. All of them were taking Quercetin, Bromelain, zinc, and ascorbic acid as supplements. The mean age of patients was 49.27 years, and 59% were older than 50. The percentage of male patients was 68.18%. More than 13% of the patients had chronic diseases. About half of the patients received antibacterial and antiviral medications during hospitalization, and 63.63% of total patients were on anticoagulants. Days of stay, average was nine days (Table 1)

The mean D-dimer level at admission was elevated (1.0082 mcg/ml). Mean WBC levels at admission and discharge were 7440 and 8550 cells/mm^3, respectively (P-value=0.34). Mean ANC at admission and discharge were 5570 and 5800 cells/microliter, respectively (P-value=0.86). O_2 sat% mean was less than 94% at admission and was more than 94% at discharge (P-value=0.83). AST mean levels were slightly elevated at admission and discharge (46 and 44.8 U/L, respectively (P-value=0.9)). Mean ALC was 1240 at admission and was 1740 cells/microliter at discharge (P-value=0.11). Mean platelets count at admission and at discharge were 243830 cells/microliters and 304200 cells/microliter, respectively (P-value=0.45). The mean AMC was 456 cells/microliters at admission and 587 cells/microliters (P-value=0.09). Regarding the mean hemoglobin levels, it was 13.68 at admission and 13.24 g/dl at discharge (P-value=0.78). Mean potassium concentrations at admission and discharge were 4.53 and 4.38 mmol/l, respectively (P-value=0.45) (Table 2).

Our docking studies show that Quercetin and Bromelain are potential inhibitors of the Human Cathepsin L. In (Table 3) the free energy of binding (ΔG) of each compound at the protein's active site and the interactions with the residues and the backbone are shown. Quercetin and Bromelain interact with their target with free energies of -5.65 and -5.15 kcal/ mol, respectively, indicating a potential inhibitory action against the Human Cathepsin L.

TABLE 1
Patients Baseline Characteristics

Variables	All COVID-19 cases (N=22)
Mean age (in years)	49.27
Older than 50 years (%)	59.09
Male (%)	68.18
Female (%)	31.82
Diabetes (%)	22.72
Hypertension (%)	13.63
Antibacterial use (%)	54.54
Antiviral use (%)	40.9
Anti-platelet or anti-coagulant use (%)	63.63
Mean Days of hospital stay	9
Mortality	0

TABLE 2

Laboratory Tests Pre and Post Supplements

Lab tests-mean values-	Pre-supplements (N=22)	Post-supplements (N=22)	P-value
WBCs (cells/mm3)	7440	8550	0.34
Hb (g/dl)	13.68	13.24	0.78
K (mmol/L)	4.53	4.38	0.45
Platelets (cells/microliter)	243.83	304.2	0.45
AST (Units/L)	46.08	44.8	0.9
ANC (cells/microliter)	5.57	5.8	0.86
ALC (cells/microliter)	1.24	1.74	0.11
AMC (cells/microliter)	0.46	0.59	0.09

TABLE 3

The (ΔG) kcal/mol of the tested compounds against Human Cathepsin L active site (PDB ID: 1MHW)

Ligand	RMSD value (Å)	Docking score (kcal/mol)	Interactions	
			H-bonds	Pi-interactions
Bromelain	**1.64**	**-5.65**	**7**	**0**
Quercetin	**1.32**	**-5.15**	**5**	**1**

Discussion

Quercetin supplement was and still is considered attractive by many researchers globally since various studies focused on it. Regarding studies about infectious diseases, Quercetin was tested with Zika virus, Ebola virus, murine coronavirus, dengue virus, SARS-CoV-2, and influenza A virus [17-22]. Most of these studies conclude that Quercetin may have a substantial role as a prophylactic or treatment of different types of viruses. Unlike Quercetin, Bromelain was not widely studied in terms of efficacy against infections. However, few types of research claimed that Bromelain could prevent or eradicate some microorganisms, including *Escherichia coli* and SARS-CoV-2 [23-24].

In this study, the mean D-dimer level of patients diagnosed with COVID-19 was more than 0.5 mcg/ml, which indicates that their condition was not mild and needed hospitalization. In addition to Quercetin and Bromelain supplements, most of the twenty-two patients were on hospital medications, including vitamin C, zinc, enoxaparin, and drugs expected to have an anti-SARS-CoV-2 effect (ribavirin, hydroxychloroquine, or lopinavir-ritonavir) and antibacterial drugs. As shown in the results, all the patients' lab tests at admission and discharge were not significantly different, and the mean hospitalization period was nine days.

These results reveal that Quercetin 800 mg once daily with Bromelain 165 mg in addition to zinc acetate 50 mg and vitamin C 1 g supplements are safe with COVID-19 patients who were on multiple therapies, including antivirals and

antibacterial medications. In (Figures 3 and 4), bromelain is shown in the active site of its target protein. With free energy of binding of -5.56 kcal/ mol that results from seven hydrogen bonds between the ligand and its target, bromelain can be a potent inhibitor of the Human Cathepsin L. Similarly, Quercetin docking shows five hydrogen bonds and a pi-interaction with the active site of the protein (Figures 5 and 6), indicating a potential antagonistic activity against the Human Cathepsin L.

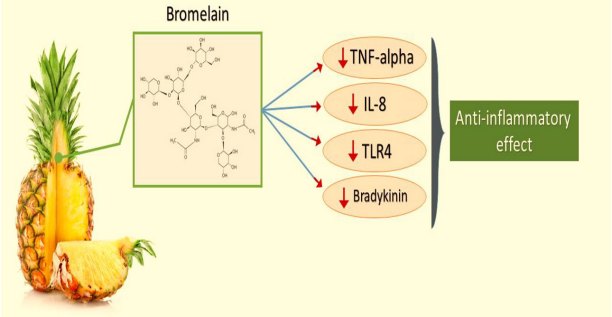

Figure 3) *Role of Bromelain in preventing inflammation by lowering TNF-α (tumor necrosis factor-alpha), IL-8 (interleu kin-8), TLR4 (toll-like receptor-4), and bradykinin levels.*

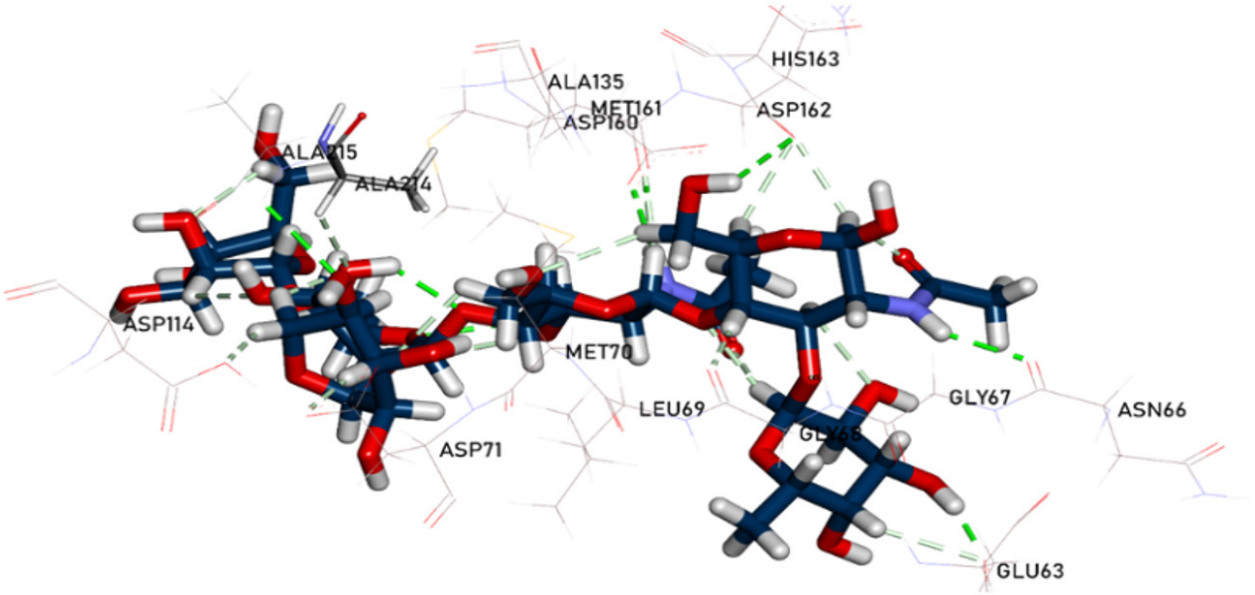

Figure 4) *The binding mode of the (Bromelain) exhibited an energy binding of -5.65 kcal/ mol-1 against Human Cathepsin L. As shown, the hydroxyl groups formed seven hydrogen bonds with Asp160, Met161, Gly68, Glu63, Asn66, Asp162, and Ala215 with a distance of 1.69, 1.36, 1.96, 1.78, 2.06, 1.25, and 1.64 Å.*

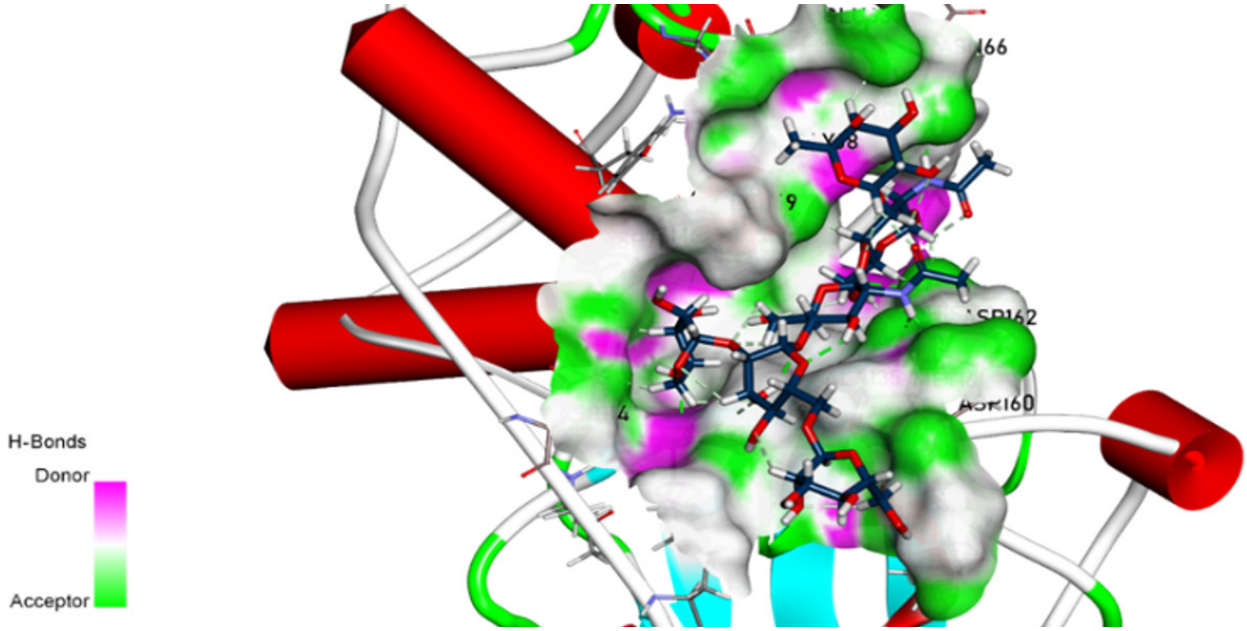

Figure 5) *Bromelain docked in Human Cathepsin L. Hydrogen bonds (green), and the pi interactions (purple lines) with the mapping surface show bromelain occupying the active pocket of Human Cathepsin L.*

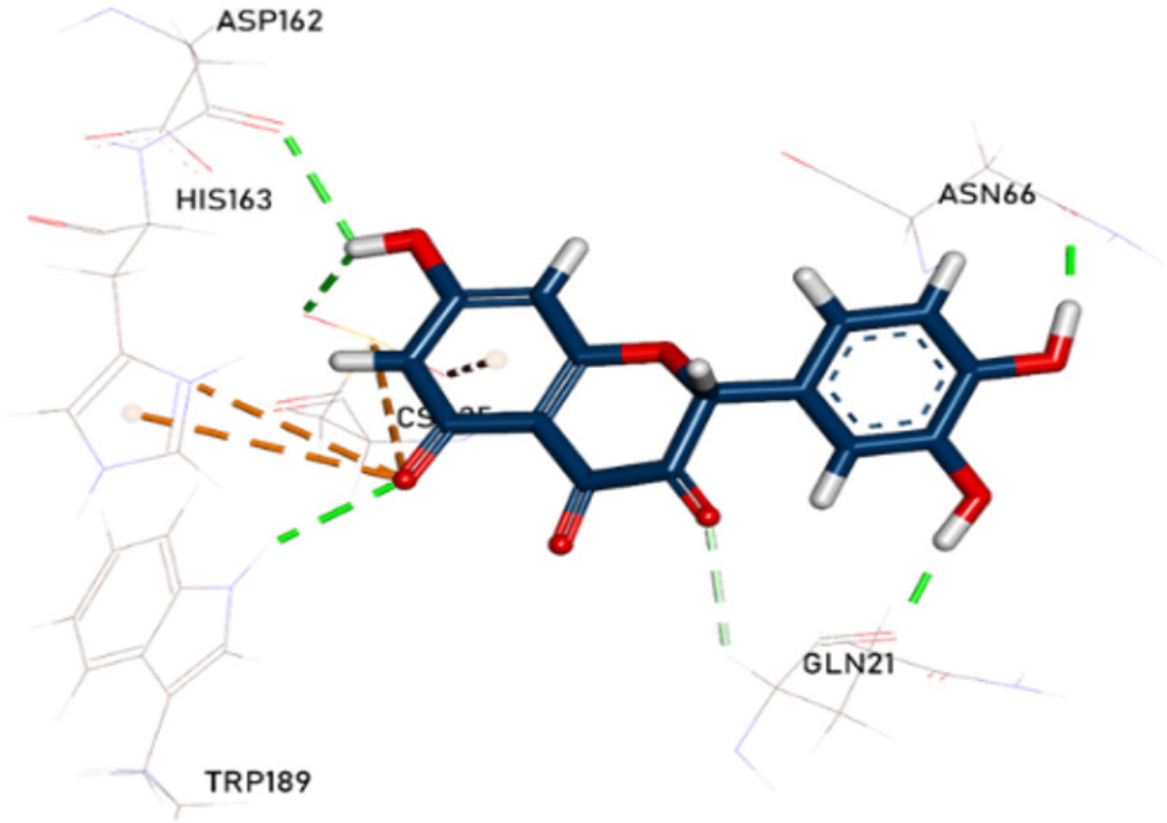

Figure 6) *The binding mode of the tested candidate (Quercetin) exhibited an energy binding of -5.15 kcal/ mol-1 against Human Cathepsin L. The 4H-chromen-4-one ring formed a pi-anion interaction with CSD25, attractive interactions with His163, and three hydrogen bonds with Trp198, Asp162, and CSD25 with distances of 1.56 and 1.85 Å. Additionally, the 3,4-dihydroxyphenyl interacted with Asn66 and Gln21 by hydrogen bonding with distances of 1.98 and 1.94 Å.*

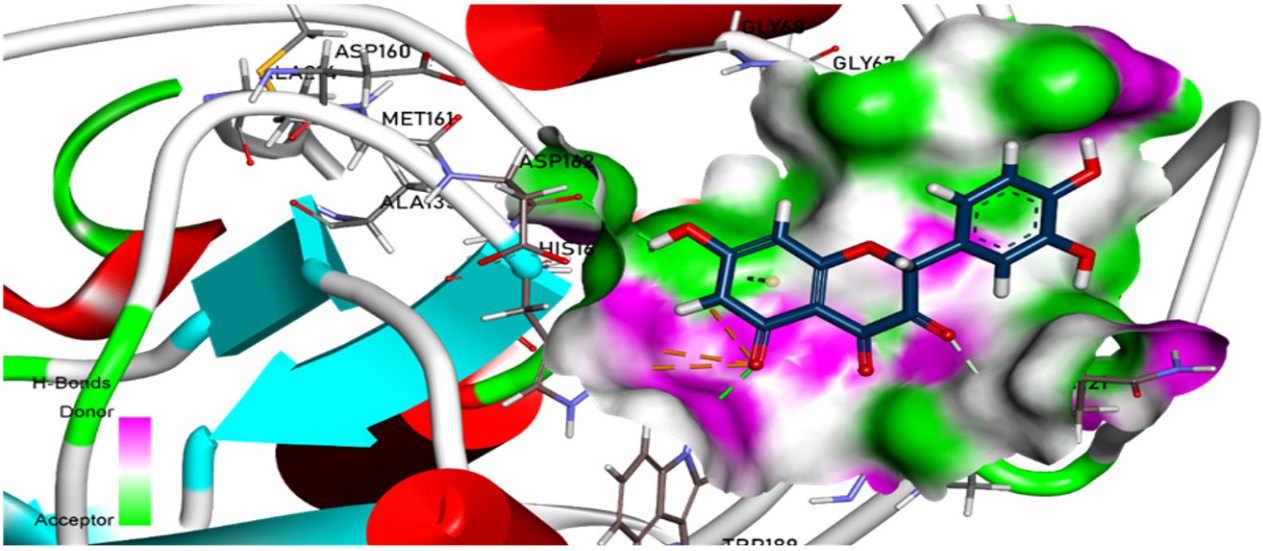

Figure 7) *Quercetin docked in Human Cathepsin L. Hydrogen bonds (green) and the pi interactions (purple lines) with the mapping surface show Quercetin occupying the active pocket of Human Cathepsin L.*

The efficacy of Quercetin, Bromelain, zinc, and vitamin C combination was not evident in this study because of the lack of a placebo or comparable group. However, their effectiveness in preventing severe consequences of SARS-CoV-2 infections cannot be ruled out based on previous studies. Extensive comparative studies need to be done about Quercetin and Bromelain to confirm their efficacy in treating COVID-19 cases. Using quercetin intravenously in patients with COVID-19 resulted in a positive outcome [25].

Conclusion

Quercetin 800 mg, Bromelain 165 mg, zinc acetate 50 mg, and ascorbic acid 1 g once daily as supplements for 3 to 5 days is safe for patients infected with SARS-CoV-2 and may prevent poor prognosis through restraining from hyper-inflammation and cytokine storm by inhibiting the Human Cathepsin L, a proposed by docking investigation. Randomized clinical trials are needed in the future to ensure the efficacy of Quercetin, Bromelain, zinc, and ascorbic acid combination.

Ethical Approval

Institutional review board (IRB) was obtained from the Saudi Ministry of Health on June 7, 2020, with the central IRB log number: 20-95M.

Authors' Contributions

(Amr Ahmed wrote the protocol of the study, and follow-up of cases at the hospital for patients which was included in the study to finish the study and follow-up of treatment, Heba Abdelseed taking consent from patients, Dr. Abdullah Alkattan, lab results, and statistics, Eman Aslsalameen, lab results collection). Yousef Albalawi follows up on cases during the study and files cases, and Hassan Shoura revises the protocol)

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