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A study on respiratory disease prevention/treatment methods using aloe, aloe lactic acid bacteria and natural product complex extracts

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ABSTRACTRespiratory diseases are increasing due to the changing living conditions of modern people, air pollution, and the emergence of new viruses. In this context, we aim to prevent and improve respiratory diseases through the development of natural compositions. This study aimed to evaluate the effectiveness of Chenpi, Platycodi Radix, Red Poria, and Aloe in preventing and treating respiratory diseases. A mixed extract of Chenpi, Platycodi Radix, and Red Poria (CPRE, KJMA0002) inhibited mucin secretion in human bronchial cell lines, while Aloe gel extract inhibited weight loss, decreased viral gene copy number, inhibited inflammatory cell infiltration, and reduced IL-6 levels in response to SARS-CoV-2 and influenza viruses. Additionally, freeze-dried Aloe-derived lactic acid bacteria (ALAB, KJMA0001) powder showed inhibition of upper respiratory tract infections and treatment of SARS-CoV-2. These results demonstrate that natural product compositions are effective in preventing and treating respiratory diseases, confirming the potential for the development of natural food compositions. Compositions using natural products and Aloe-derived lactic acid bacteria were shown to be effective in preventing and improving respiratory diseases. These findings confirm the potential for the development of natural food compositions, which may serve as safe and effective alternatives for the prevention and treatment of respiratory diseases in the future.

KEYWORDS: Chenpi, Platycodi Radix, Red Poria, Aloe, Lactic acid bacteria, Respiratory disease, SARS-CoV-2

I. INTRODUCTION

Respiratory disease refers to a wide range of conditions that affect the respiratory system, including the common cold, bronchitis, pneumonia, tuberculosis, lung cancer, acute and chronic bronchitis, etc. [1]. These diseases are caused mainly by pathogens, air pollutants, or emerging viral infections that are introduced through the respiratory tract [2]. In recent years, the incidence of respiratory diseases has increased due to rapid industrialization, environmental pollution, emerging viral infections, and changing dietary patterns [3,4,5]. This has resulted in respiratory diseases accounting for three of the top 10 causes of death, and according to the World Health Organization (WHO), more than 8 million people die from respiratory diseases each year [6]. Particulate matter or yellow dust can enter and accumulate in the respiratory tract, and the coronavirus can cause inflammation inside the respiratory tract, leading to symptoms such as sore throat, high fever, cough, and shortness of breath, which can lead to pneumonia [7,8].

Natural products have a variety of bioactive properties that make them useful for health maintenance, disease prevention, and treatment, and many studies have emphasized their importance [9]. Representative natural products include Chenpi, Platycodi Radix, Red Poria and Aloe, which have been shown to be effective against respiratory diseases. Chenpi is the peel of tangerine, which has anti-inflammatory and antibacterial effects as well as phlegm thinning, making it effective for treating respiratory conditions such as bronchitis through phlegm and

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cough relief [10]. Platycodi Radix helps to clear phlegm and relieve cough, and has long been used to treat respiratory conditions including cough, excess phlegm, and sore throat [11]. Red Poria is the fungal nucleus of Bok Ling, which relieves phlegm and edema, is antibacterial and antioxidant, enhances immunity and inhibits inflammation [12].

Aloe is a natural product traditionally used to treat a variety of ailments, including skin health, and has recently been used in various studies related to viruses such as influenza virus and SARS-CoV-2, as well as respiratory diseases [13,14]. Aloe has antiviral, anti-inflammatory, antibacterial, and immunomodulatory effects, which may help reduce inflammation in the respiratory tract and improve respiratory health [15,16]. Lactic acid bacteria in particular have been shown to be effective in preventing and treating respiratory diseases, and combining aloe and lactic acid bacteria has been shown to have better antibacterial and anti-inflammatory effects [17,18].

Each of the four natural products has been used in various ways to prevent or treat respiratory diseases. However, to date, there has been a lack of research on the effects of mixed extracts of chenpi, platycodi radix, red poria(CPRE), aloe gel extract and aloe-derived lactic acid bacteria(ALAB) on respiratory diseases.

This study developed compositions for the prevention and improvement of respiratory diseases that protect, improve, and strengthen the respiratory system, overcoming the limitations of current drug therapies through each new composition, using natural products such as chenpi, platycodi radix, red poria, and aloe. We evaluated their inhibitory effects on respiratory diseases and demonstrated the potential for new preventive and therapeutic compositions.

II. MATERIAL AND METHODS

1. Materials

The fresh leaves of aloe were 3-5 years old and were collected from Kim Jeong Moon Aloe Jeju Farm, located at Seongsan-eup, Jeju Island, South Korea, and the cultivars used were Aloe vera, Aloe avorescens, and Aloe saponaria[19]. Chenpi, platycodi radix and red poria were purchased from Bibongherb Co., Ltd. (Seoul, Korea). The samples were used and purchased from companies approved by the Korea Food and Drug Administration (KFDA) and the authenticity of the samples has been verified. Individual natural products are registered with the Ministry of Food and Drug Safety of the Republic of Korea. All other reagents and media were purchased from Sigma Aldrich (USA) unless otherwise noted.

2. Preparation of Mixed Extract of Chenpi, Platycodi Radix, and Red Poria (CPRE, KJMA0002)

Chenpi, Platycodi radix and red poria were mixed at a weight ratio of 1:1:1, and the extraction method was hydrothermal extraction [20]. Water was added 10 times to the mixed material and reflux-cooled extraction was performed at 80~90℃ for 3 hours. After extraction, the mixture was filtered to obtain the primary extract. The remaining solids were further extracted via the same method and the two extracts were combined. The combined extracts were concentrated under reduced pressure at less than 60℃ and vacuum dried to obtain the final dry extract. The CPRE was then prepared. In addition, the newly prepared CPRE was named KJMA0002.

3. Preparation of aloe gel extract

Fresh aloe leaves were thoroughly washed with water to remove attached soil and other debris, and then cut into 0.5 cm thickslices, including the peel. The prepared slices were dehydrated by osmotic treatment by placing them in a circulating water bath at 25℃ for 2 hours. The obtained osmotically dehydrated slices were peeled to obtain parenchyma tissue. This mixture was homogenized in a homogenizer (T25, IKA Labortechnik, Staufen, Germany) at 24,000 rpm for 3 min, and then centrifuged (8000 rpm, 30 min) to obtain the supernatant, which was used to prepare a fiber-free aloe gel extract.

4. Preparation of freeze-dried aloe-derived lactic acid bacteria (ALAB, KJMA0001) powder

4-1. Isolation and analysis of ALAB

The isolation and screening of ALAB was conducted at the Department of Microbiology, Seoul National University for three months. The three aloe varieties used in the experiment were Vera, Saponaria, andAvorescens, 0.1% peptone water was used as the diluent, and the Bromocresol Purple MRS Agar Medium (BCP-MRS)and bismuth sulfite (BS) agar were used as the culture medium for lactic acid bacteria. Each variety of aloe was sampled in 30 g portions, divided into outer, inner, top, and bottom parts, and homogenized using a stomacher with 270 mL of diluent. The homogenized samples were inoculated and smeared on BS media and BCP-MRS media, which are the selective media for anaerobic lactic acid bacteria isolation, after serial dilutions. After

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incubation at 37°C for 2 to 7 days, the lactic acid bacteria that appeared were isolated. The isolated lactic acid bacteria were screened and genetically identified by whole genome sequence analysis against sequences registered in the GenBank database [21]. Afterward, the cultures were grown under anaerobic conditions using anaerobic jars for mass proliferation of anaerobic bacteria.

4-2. Cultivation of ALAB and preparation of ALAB powder

For high-concentration cultivation, Lactobacillus helveticus (KJMA0001, KCTC 15075BP, Korea), ALAB, was inoculated into MRS broth (Difco, USA) and incubated for 24 hours at 37°C using the fed-batch culture method. At this time, the lactic acid bacteria count averaged 2.58×109/mL. The recovery of bacteria from the lactic acid bacteria culture was performed using a high-speed centrifuge (8,000 rpm, 20-30 min) at 3-5°C. The recovered lactic acid bacteria were re-centrifuged using protein mixing solution to recover the protein coating. The recovered coatings were frozen in a -80°C ultralow temperature freezer for 12 hours and then lyophilized using a freeze dryer (Ilshin Bio, FD-8508, Korea) for 120 hours at a condensing temperature of -80°C and a pressure of 5 mmTorr or less. The lyophilized strains were ground to powder and stored in a refrigerator at 4℃. This ALAB powder was used for the experiments and production.

5. Evaluationof the mucin secretion inhibitory effect of CPRE

The human bronchial cell line Calu-3 was used to evaluate the inhibitory ability of CPRE prepared in Method 2 to inhibit respiratory mucin secretion. Calu-3 cells synthesize and secrete MUC5AC mucin, a mucin in the airways, and its secretion was validated using a MUC5AC Enzyme-linked immunosorbent assay (ELISA) kit [22,23]. One day before the experiment, Calu-3 cells cultured in DMEM medium were harvested with trypsin-EDTA, seeded1×10^5 into 48-well plates and incubated for 24 hours. After being washed several times with DMEM without fetal bovine serum (FBS), the extracts were diluted and treated for 3 hours. The medium was removed, washed with FBS-free DMEM, and the extracts and Phorbol 12,13-dibutyrate (PDBu, 1 μM) were diluted and re-treated for 1 hour. The amount of secreted mucin in the medium was measured using the MUC5AC ELISA kit (Novus Biologicals, USA) [24]. One hundred microliters of medium was used to measure the amount of MUC5AC, and 10 μl of medium was used to measure the amount of total protein at 660 nm. Then, the MUC5AC ELISA measurement was corrected to the total protein amount. This experiment was used to quantitatively evaluate the mucin secretion inhibitory effect of CPRE.

6. Evaluation of the ability of aloe gel extract to inhibit SARS-CoV-2 and influenza viruses

The effects of aloe gel extracts prepared in Method 3 on SARS-CoV-2 and influenza viruses were comprehensively evaluated by measuring weight loss, viral gene copy number, inflammatory cell infiltration, and interleukin 6 (IL-6) levels.

6.1 Evaluating weight loss inhibition

To evaluate the weight loss inhibition effect of aloe gel extract in the context of viral infection, 7-week-old Syrian hamsters were stabilized for 1 week and then randomized into groups with uniform average body weights. Syrian hamsters were infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, NCCP43330, 5×10^4 TCID50/50μL/head) via the left nasal cavity, and oral administration was started immediately after viral inoculation [25]. Low (200 mg/kg) and high (400 mg/kg) concentrations of 300 μL were administered orally seven times (days 0, 1, 2, 3, 4, 5, and 6) for a total of six days (n=12 each). Noninfected controls received sterile distilled water orally in the same manner (n=12) [26]. Hamsters were weighed a total of eight times from day 0 to day 7 of infection to assess the inhibition of weight loss.

6.2 Evaluating the number of viral gene copies

To evaluate the effect of aloe gel extract on viral gene replication, viral lesion sites were collected from lung tissue on days 3, 5, and 7 after SARS-CoV-2 infection. The harvested lung tissue was ground using WizolTM reagent to extract total RNA. The extracted RNA was quantified with a NanoDrop 2000, complementary DNA (cDNA) was synthesized with the WizScriptTM cDNA Synthesis Kit (Wizbiosolutions, Korea), and real-time polymerase chain reaction (PCR) was performed using the WizPureTM qPCR Master-UDG (Wizbiosolutions, Korea) [27]. The PCR conditions were initial denaturation (95°C, 10 min), denaturation (95°C, 15 s), ligation and amplification (60°C, 1 min), which were repeated 40 times, and the primers used are listed in Table 1. The RNA-dependent RNA polymerase (RdRp) and envelope (E) genes of SARS-CoV-2 were detected by 5′-hexachlorofluoresceinphosphoramidite (HEX)

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and 6-carboxyfluorescein (FAM) fluorophores, and the number of viral gene copies per ng of total RNA was calculated [28].

Table 1. Primer information used for PCR

		Sequence	Length
nCoV_IP2-12669Fw	1	ATGAGCTTAGTCCTGTTG	108bp
nCoV_IP2-12759Rv	2	CTCCCTTTGTTGTGTTGT	
nCoV_IP2-12696bProbe(+)	3	[HEX]AGATGTCTTGTGCTGCCGGTA[[BHQ1]	
E_Sarbeco_F1	4	ACAGGTACGTTAATAGTTAATAGCGT	125bp
E_Sarbeco_R2	5	ATATTGCAGCAGTACGCACACA	
E_Sarbeco_P1	6	[FAM]ACACTAGCCATCCTTACTGCGCTTCG[BHQ1]	

6.3 Evaluatingthe regulation of the inflammatory response after infection

To determine whether the extract could modulate the inflammatory response after viral infection, inflammatory cell infiltration was assessed, and the amount of IL-6 in the lung tissue was evaluated. For inflammatory cell infiltration assessment, lung tissues were fixed in 10% neutralized formalin (NBF) on days 3, 5, and 7 after SARS-CoV-2 infection and extract administration. The fixed lung tissues were observed for tissue lesions after hematoxylin and eosin (H&E) staining, and the degree of inflammatory cell infiltration was scored (not observed, 0; mild, 1; weak, 2; moderate, 3; severe, 4) [29].

In addition, IL-6, a cytokine that plays an important role in inducing and regulating inflammatory responses following viral infection, was used to assess the amount of IL-6 in lung tissue [30]. After SARS-CoV-2 infection and extract administration, the lung tissues harvested on days 3, 5, and 7 were placed in PBS and ground with a homogenizer, after which the supernatant was collected. The amount of the cytokine IL-6 was quantified using the Hamster IL-6 ELISA kit, and the amount of cytokine protein per g of lung tissue was calculated after quantifying the amount of lung tissue protein by the Bradford method.

7. Evaluation of ALAB powder in Defense Against Upper Respiratory Tract Infections and Treatment with SARS-CoV-2

We comprehensively evaluated the effects of ALAB powder on the defense against upper respiratory tract infection and the inhibition of viral infection in an acute Non-typeable Haemophilus influenzae (NTHi) respiratory tract infection mouse model and SARS-CoV-2 patients.

7.1 Suppression of upper respiratory tract infection in an acute NTHi respiratory infection mouse model

A mouse model of acute NTHi respiratory infection was used to evaluate the effectiveness of ALAB powder in suppressing upper respiratory tract infection. Five randomly assigned groups of mice were injected with 0.75 mL of Polybutylene succinate (PBS), 5×10^9 NTHi, 5×10^9 NTHi, and 2.5×10^{10} ALAB powder(Table 2). After 14 days, group A was injected with 50 μL of PBS, and groups B-D were injected with another 50 μL of PBS. After 21 days, they were infected with 5×10^8 of live NTHi. Four hours after infection, the mice were sacrificed and the upper airways were flushed with 10 mL of PBS to obtain bronchoalveolar lavage (BAL) fluid and lung homogenate (LH). BAL and LH were serially diluted and cultured on chocolate agar plates to determine total colony forming units (CFU) counts [31,32].

Table 2. Amounts injected into the upper airways of four groups of mice

Group	Immunization in the upper respiratory tract	Additional Dosage
A	PBS	PBS
B	NTHi $5x10^9$	NTHi $5x10^8$
C	NTHi $5x10^9$ + ALAB powder $2.5x10^{10}$	NTHi $5x10^8$
D	ALAB powder $2.5x10^{10}$	NTHi $5x10^8$

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7.2 Comparison of SARS-CoV-2 infection inhibition and patient outcomes with ALAB powder and enzyme combinations

To evaluate the ability of ALAB powder to inhibit SARS-CoV-2 infection in the upper respiratory tract, arginine deaminase (ADI) and sphingomyelinase (Smasi) enzymes were administered to SARS-CoV-2 patients in combination with 90 nmol ceramide/h/g, 120 μmol L-citrulline/h/g, and 2.5×10^{10} ALAB powder per g, respectively. Enzyme activity was assessed by fluorescence measurement. The results were presented in tables and graphs, and statistical analysis used chi-square tests and generalized linear mixed models (GLIMMIX) to compare the incidence of respiratory failure between control and treatment groups [33].

SARS-CoV-2 patients were treated with hydroxychloroquine plus antibiotics and a vaccine. [34,35]. The data collected were used to compare upper respiratory tract infections in patients who received these treatments with those who received ALAB powder. The two groups were examined for age, sex, body mass index(BMI), alanine transaminase(ALS), aspartate aminotransferase(AST), hemoglobin level(Hb), Charlson comorbidity index(CCI), and prevalence of SARS-CoV-2-associated symptoms [36]. This comparison comprehensively evaluated the therapeutic effectiveness and inhibition of SARS-CoV-2 infection by ALAB powder [37].

8. Statistical analysis

All statistical analyses, except as mentioned above, were performed using SAS v. 9.4 (SAS Institute Inc., Cary, NC, USA). p values<0.05*, 0.01**, and 0.001*** were considered statistically significant.

III. RESULT

1. ALAB screening and sequence analysis

Lactic acid bacteria from aloe were isolated and identified in lactic acid bacteria culture media. As shown in Figure 1, the ALAB sample group presented microscopic colonies in BCP-MRS and BS media on day 2 and were cultured until day 7. FE-SEM (field emission scanning electron microscope; SIGMA, Carl Zeiss, UK) analysis taken at the Seoul National University College of Agriculture and Life Sciences revealed that the isolated lactic acid bacteria exhibited typical rod-shaped morphological features, as shown in Figure 2. Furthermore, genetic identification was performed by analyzing the full-length sequence of the gene, which was performed by Accugene (Korea), and it was confirmed that the lactic acid bacteria had 99% homology with Lactobacillus helveticus when compared to the sequence registered in the GenBank database (Figure 3). On the basis of these findings, the molecular phylogenetic identification of the lactic acid bacteria revealed that they were Lactobacillus helveticus. In addition, the strain of Lactobacillus helveticus, an aloe-derived lactic acid bacteria, was named 'KJMA0001' and assigned the accession number KCTC 15075BP by the Korea Research Institute for Biotechnology and Biological Biology (KRIBB)

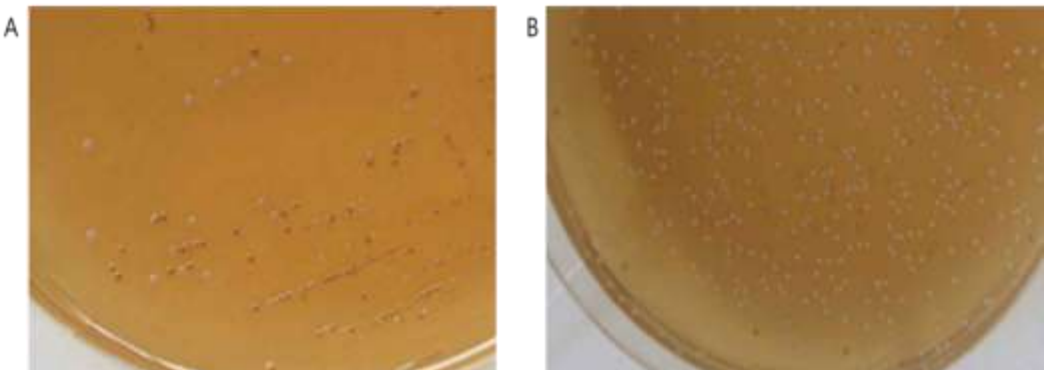

Figure 1. Lactobacillus Colonies Isolated from Aloe Vera

(A) Colonies observed after 2 days of cultivation. (B) Colonies observed after 7 days of cultivation

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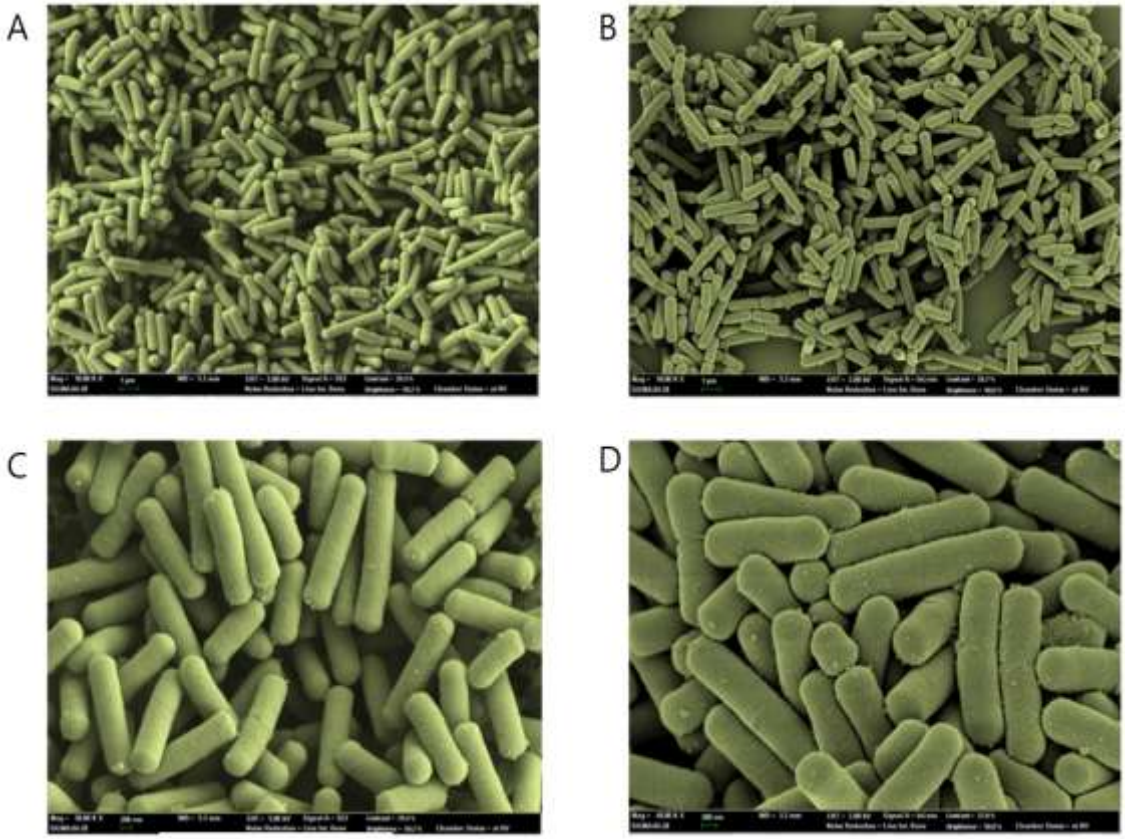

Figure 2. Morphological Characterization of Aloe-Derived Lactobacillus via FE-SEM
(A)-(B) Images captured at 10,000x magnification. (C) Image captured at 30,000x magnification. (D) Image captured at 40,000x magnification.

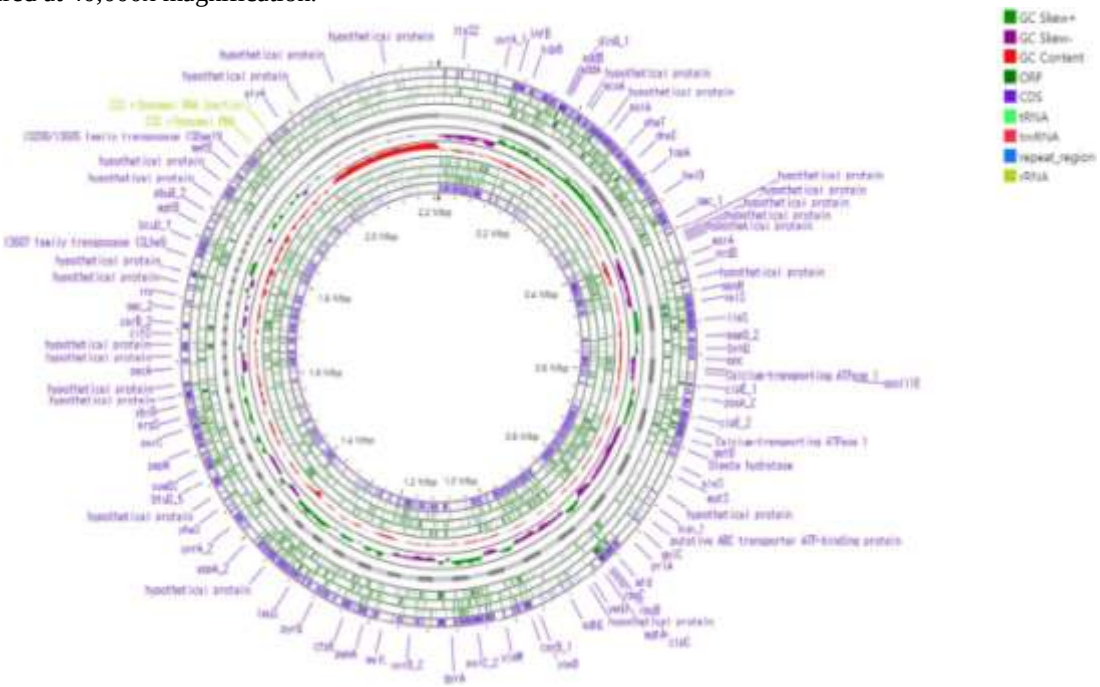

Figure 3. Genome Map of Aloe-Derived Lactobacillus (Lactobacillus helveticus)

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2.Evaluating the ability of CPRE to isolate mucin and prevent acute respiratory disease

The mucin secretion levels of PDBu, Chenpi, Platycodi Radix, Red Poria and CPRE were 1±0.13, 0.69±0.11, 0.56±0.13, 0.52±0.13 and 0.33±0.07, respectively. Compared with the single extracts of chenpi, platycodiradi and red poria, CPRE significantly reduced PDBu-induced mucin secretion, as assessed by the MUC5AC ELISA kit (Figure 4). Although each of the single extracts of chenpi, platycodiradi and red poria showed some inhibitory effect on mucin secretion, the crude extract (KJMA0002) had a greater inhibitory effect than the single extracts did. These findings suggest that, compared with single extracts CPRE has a more potent inhibitory effect on respiratory diseases through synergistic effects. KJMA0002 significantly reduced PDBu-induced mucin secretion, confirming its effectiveness in preventing and improving acute and chronic respiratory diseases.

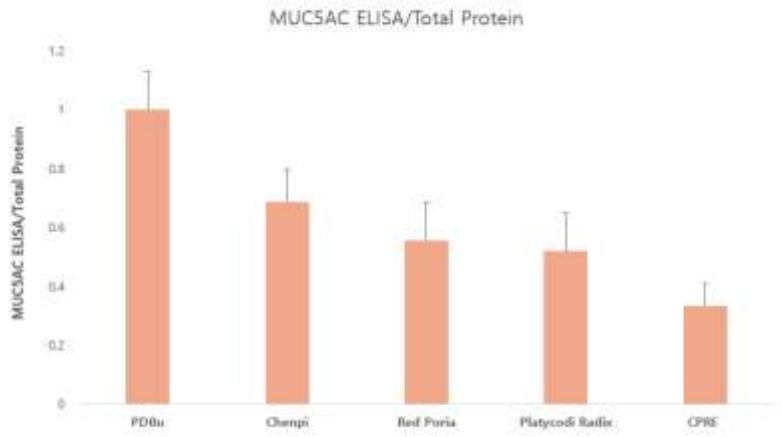

Figure 4. MUC5AC ELISA/Total Protein Analysis of Mucin Secretion in Calu-3 Cells

3. Evaluation of the effects of aloe gel extract on in SARS-CoV-2 infection

3.1 Weight change

Figure 5 showed the effects of aloe gel extract on viral infection through changes in the body weights of Syrian hamsters. The noninfected excipient control group presented a gradual increase in body weight, whereas the infected excipient group showed a decrease in body weight from day 1 to day 7 of infection. After infection with SARS-CoV-2, the low concentration group of aloe gel extract (200 mg/kg) significantly inhibited body weight loss compared with the infection excipient control from day 1 to day 7 ($p<0.01$, $P<0.001$). A high concentration of aloe gel extract (400 mg/kg) also significantly inhibited body weight loss from day 1 to day 4 ($p<0.01$, $p<0.001$), and the body weight gradually recovered from day 5.

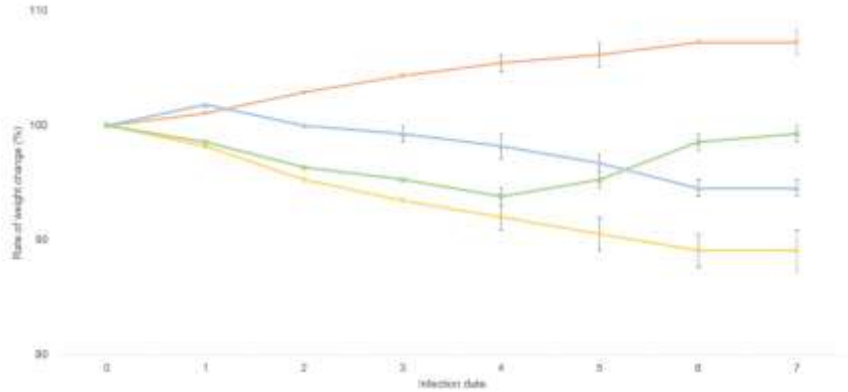

Figure 5. Body Weight Changes in Hamsters Following Aloe Gel Extract Administration

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Orange: Non-infectious excipient, Yellow: Infectious excipient, Blue: Infection + Aloe gel extract 200 mg/kg, Green: Infection + Aloe gel extract 400 mg/kg.

3.2 Reducing the number of viral gene copies

The viral gene copy number assessment of the extracts was performed by calculating the gene copy number per RNA of the RdRp and E genes of SARS-CoV-2. On day 3 after SARS-CoV-2 infection, the levels of the RdRp and E genes of the virus in the low (200 mg/kg) and high (400 mg/kg) aloe gel extract groups tended to decrease compared with those in the infectious excipient control group. The levels of RdRp and E genes were also significantly reduced on days 5 and 7. (Figure 6)

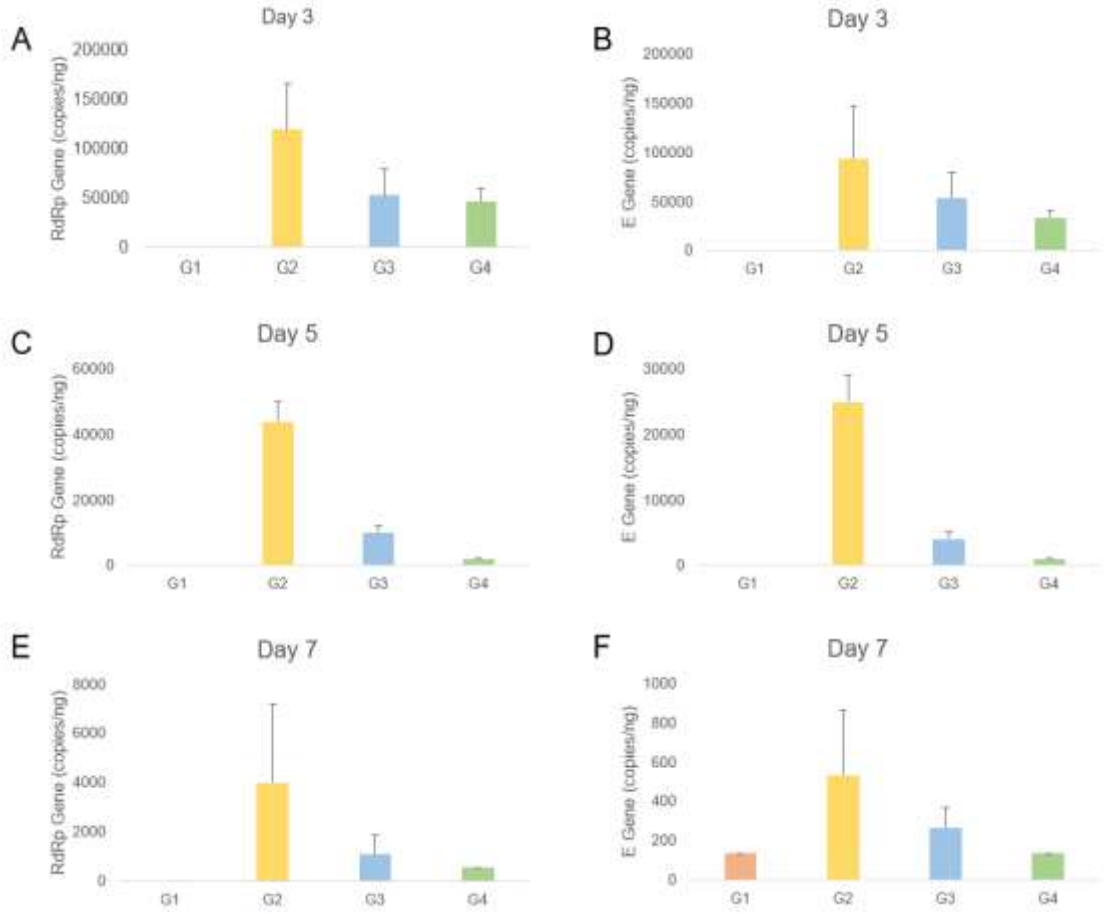

Figure 6. RdRp and E Gene Levels in Hamster Lung Tissue Following Aloe Gel Extract Administration
RdRp Gene levels (copies/ng) on Day 3 (A), Day 5 (C), and Day 7 (E), E Gene levels (copies/ng) on Day 3 (B), Day 5 (D), and Day 7 (F). G1(orange): Non-infectious excipients, G2 (yellow): Infectious excipients, G3 (blue): Infectious excipients + Aloe gel extract 200 mg/kg, G4 (green): Infectious excipients + Aloe gel extract 400 mg/kg.

3.3 Regulating the Inflammatory Response

The degree of infiltration of inflammatory cells and the amount of IL-6 in the lung tissue were determined to assess the regulation of the inflammatory response after infection. Figure 7 shows the scores for the degree of inflammatory cell infiltration in the lung tissue. Histopathologic inflammation was observed in all groups on day 3 after SARS-CoV-2 infection, with no differences between the groups. On day 5, the inflammation levels in the low (200 mg/kg) and high (400 mg/kg) aloe gel extract treatment groups were significantly lower ($p<0.05$) than those in the vehicle control group. At day 7, inflammation levels were also significantly reduced in both groups ($p<0.05$), indicating that inflammation levels were reduced over time in the aloe gel extract treatment group compared with those in the control group.

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Furthermore, when the Hamster IL-6 ELISA kit was used to determine the amount of IL-6 in the lung tissue, the levels of the proinflammatory IL-6 did not differ among the groups on day 3 after SARS-CoV-2 infection. However, on day 5, the IL-6 levels were significantly reduced in the low (200 mg/kg) and high (400 mg/kg) aloe gel extract treatment groups ($p<0.01$). Significantly, the infection excipient control group presented higher IL-6 levels than did the aloe gel extract treatment group on day 5. On day 7, the IL-6 levels decreased in all the groups, but the difference was not statistically significant. (Figure 8)

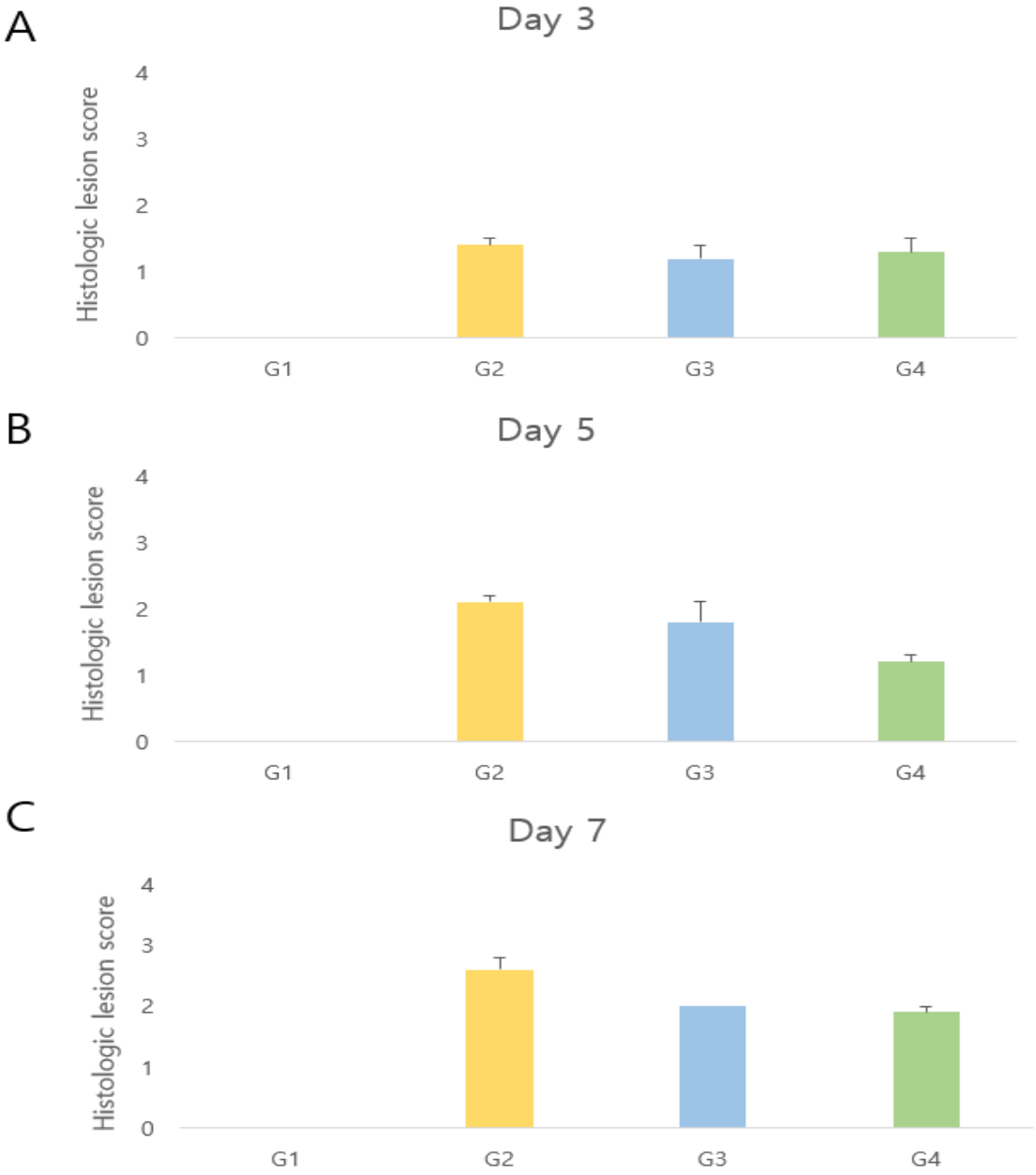

Figure 7. Aloe Gel Extract's Effect on Lung Lesions in SARS-CoV-2 Infected Hamsters
G1: Non-infectious excipients, G2 (yellow): Infectious excipients, G3 (blue): Infectious excipients + Aloe gel extract 200 mg/kg, G4 (green): Infectious excipients + Aloe gel extract 400 mg/kg.

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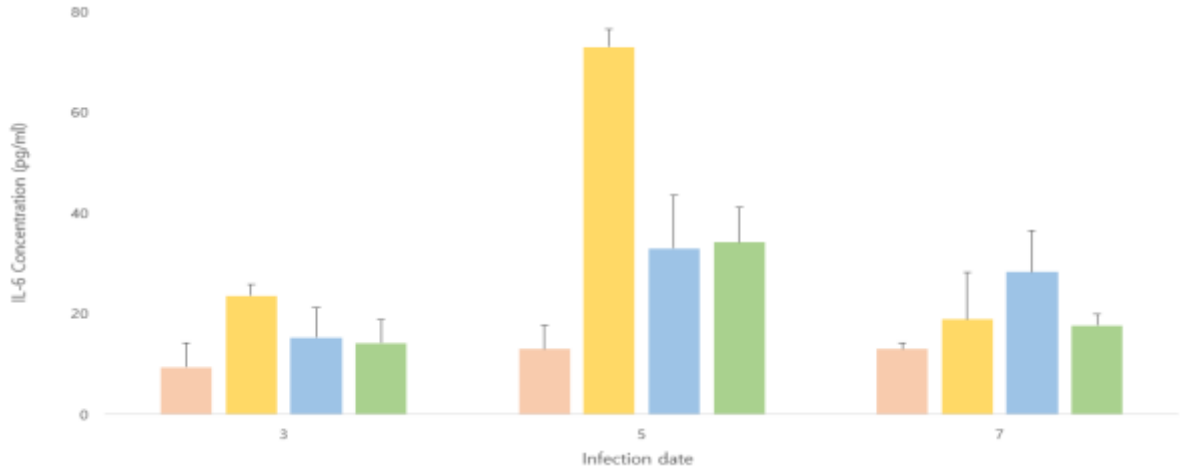

Figure 8. IL-6 Concentration (pg/ml) in Lung Tissue Following Aloe Gel Extract Administration
G1(orange): Non-infectious excipients, G2 (yellow): Infectious excipients, G3 (blue): Infectious excipients + Aloe gel extract 200 mg/kg, G4 (green): Infectious excipients + Aloe gel extract 400 mg/kg.

4. Effectiveness of ALAB powder in defense against upper respiratory tract infections and treatment of SARS-CoV-2

4.1 Suppressive effect on acute respiratory infections in a mouse model of acute NTHi respiratory infection:

The total CFU count was used to evaluate the inhibitory activity of ALAB powder against upper respiratory tract infections. As shown in Table 3, group B containing NTHi and ALAB powder had a lower total number of CFUs than did the control group C, which was treated with NTHi aloneThese findings indicate that ALAB powder has a good inhibitory effect on upper respiratory tract infections, suggesting the potential of ALAB powder as a preventive agent for acute respiratory tract infections.

Table 3. Total number of CFCs in the BAL and LH in the four groups of mice

Group	BAL CFU (10^8)	LH CFU (10^6)
A	3.67±1.30	103.5±34.2
B	0.4±0.22 p=0.038*	17.4±6.8 p=0.039*
C	0.27±0.11 p=0.032*	5.7±1.5 p=0.021*
D	0.49±0.19 p=0.042*	0.82±0.37 p=0.017*

The total number of CFCs was determined by counting colonies after 12 hours of incubation in a 37℃ incubator. A-D represent the four groups of mice injected into the upper respiratory tract in Table 2.

4.2 Comparison of SARS-CoV-2 infection inhibition and patient outcomes with ALAB powder and enzyme combinations:

The analysis of the characteristics of the patient groups according to the administration of ALAB powder in Table 4 showed that there was variation in the amount of ALAB powder administered, but there were no significant differences between the groups in the number, type, or combination of medications used during the observation period. As shown in Table 5, the outcomes of the two groups of patients were significantly different..

In addition, with respect to the resolution of SARS-CoV-2-related symptoms, the gap between the two groups increased at 24 hours after the first dose of ALAB powder and was maintained for up to 7 days, as shown in Figure 9A. By day 3 of treatment, more than 90% of the patients taking ALAB powder were clear of upper respiratory tract

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infections, and by day 7, this number had expanded to all the subjects. In contrast, the proportion of subjects treated with conventional therapy was lower than that of the aloe lyophilized group at day 24. Other symptoms showed a similar trend, but with a more limited effect within 24 hours of treatment with ALAB powder (Figure 9B).\

Table 4. Characterization of patient groups according to ALAB administration

	Treated groups	Control	p-value
Age (Mean±SD)	59.0±14.4	60.5.7±14.2	0.764
Gender (Male;%)	17; 60.7%	24; 57.1%	0.766
BMI(Mean±SD)- kg/㎡	24.7±3.5	23.4 ±3.5	0.084
ALT(Mean±SD)- IU/1	22.7±10.5	30.5±22.2	0.337
AST(Mean±SD)- IU/1	37.0±33.5	40.5±33.0	0.749
Hb(Mean±SD)- g/dl	12.6±1.5	12.8±2.0	0.578
CCI(Mean±SD)	1.9±1.6	2.3±2.3	0.817
Symptom			
Fever(No.; %)	27; 96.4%	39; 92.9%	0.528
Cough(No.; %)	22; 78.6%	32; 76.2%	0.816
Dyspnea(No.; %)	20; 71.4%	24; 57.1%	0.226
Asthenia(No.; %)	6; 21.4%	9; 21.4%	1
Headache(No.; %)	3; 10.7%	8; 19.0%	0.348
Muscle pain(No.; %)	2; 7.1%	2; 4.8%	0.674
Diarrhea(No.; %)	14; 50.0%	19; 45.2%	0.696

BMI: body mass index; ALT: alanine transaminase; AST: aspartate aminotransferase; Hb: hemoglobin level; CCI: Charlson comorbidity index

Table 5. Results for patient groups after administration of aloe vera.

ALAB Therapy - No.; %.	Treated groups	Control	p-value
HCQ	25; 89.3%	40; 95.2%	0.343
TCZ	7; 25.0%	16; 38%	0.253
ABX	11;39.3%	21; 50.0%	0.378
Number of ALAB - No.; %.			
0	2; 7.1%	1; 2.4%	0.163
1	13; 46.4%	11; 26.2%	
2	9; 32.1%	24; 57.1%	
3	4; 14.3%	6; 14.3	
ALAB Combination - No.; %.			
HCQ/TCZ/ABX	4; 14.3%	6; 14.3%	1
HCQ/TCZ	2; 7.1%	9; 21.4%	0.108
HCQ/ABX	6; 21.4%	15; 35.7%	0.201
TCZ/ABX	1; 3.6%	0; 0.0%	0.217

ABX: antibiotics; HCQ: hydroxychloroquine; TCZ:tocilizumab

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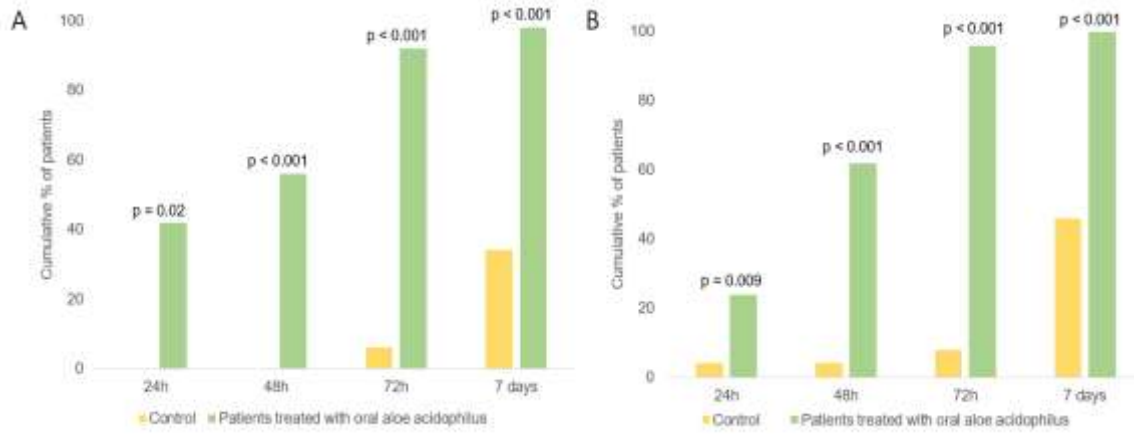

Figure 9. Cumulative Incidence of Respiratory Conditions in Subjects

(A) Cumulative percentage of patients with suppressed upper respiratory tract infections, (B) Cumulative percentage of patients with resolved SARS-CoV-2 symptoms. Yellow: control subjects administered with aloe-derived probiotics, Green: subjects not administered with probiotics

IV. DISCUSSION

Natural products, especially Chenpi, Platycodi Radix, Red Poria and Aloe, which are widely used as therapeutic agents, have many benefits for the prevention and improvement of respiratory diseases. In this study, we evaluated the effects of extracts of these natural products and aloe-derived lactic acid bacteria on respiratory diseases and SARS-CoV-2 infection.

CPRE significantly reduced PDBu-induced mucin secretion. These findings indicate that the mixed extract, CPRE, was more effective at suppressing acute respiratory illness than the single extract, and that the three extracts synergized with each other to produce more potent anti-inflammatory and antiviral effects. Compared with the efficacy of each single extract reported in previous studies, the synergistic effect of the mixed extracts shows that they are more effective in inhibiting mucin secretion and reducing inflammatory responses [38]. These findings suggest that CPRE is a potential therapeutic option to alleviate the symptoms of respiratory diseases and prevent disease progression.

Aloe gel extract has been shown to have a variety of inhibitory effects on SARS-CoV-2 and influenza viruses. Weight loss is also directly related to infection, and hamsters suffer significant weight loss and viral and immune response problems when inoculated with SARS-CoV-2 [39]. However, in our hamster model, viral infection-induced weight loss was significantly suppressed after the administration of low or high doses of the extract, as well as lower levels of SARS-CoV-2 gene replication. These findings suggest that aloe gel extract may reduce infection-induced weight loss and have antiviral effects, and previous studies have also reported anti-inflammatory and immunomodulatory effects of aloe constituents [40 Importantly, aloe gel extract can reduce inflammatory cell infiltration, which may help minimize tissue damage and reduce the risk of chronic inflammation. Additionally, aloe gel extract reduced the amount of IL-6, suggesting that aloe may be useful in suppressing inflammatory responses and alleviating symptoms of inflammation-related conditions [41]. Notably, high IL-6 levels are associated with increased mortality in Coronavirus disease 2019(COVID-19) patients, so our finding that the amount of IL-6 was reduced shows the potential to also reduce mortality in COVID-19 patients [42].

A previous report revealed that Lactic Acid Bacteria was also effective in treating SARS-CoV-2 infection by preventing or reducing the duration of upper respiratory tract infections and increasing vaccine immunogenicity [43]. In an evaluation of the ability of ALAB powder to inhibit upper respiratory tract infections, the treatment containing NTHi and ALAB powder resulted in a significantly lower total CFU count than did the control treatment with NTHi alone [44]. These results indicate the potential of ALAB powder as a prophylactic agent against acute respiratory tract infections. In addition, in an evaluation of SARS-CoV-2 infection suppression and patient outcomes, patients treated with ALAB experienced faster resolution of upper respiratory tract infection

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symptoms and reduced levels of inflammation. These findings indicate that ALABsuppress viral infections through immunomodulatory and anti-inflammatory effects.

Therefore, the results of this study confirmed that CPRE, aloe gel extract, and ALAB powder have significant effects on the prevention and treatment of respiratory diseases, including SARS-CoV-2. In particular, CPRE had more potent effects than the individual extracts did, while aloe gel extract and ALAB powder had antiviral and anti-inflammatory effects, suggesting that they may be useful in preventing and ameliorating respiratory diseases. However, the research remains limited to animal models and preliminary clinical experience. Further studies are needed to validate their efficacy in larger clinical trials and to evaluate their effectiveness against blends of natural products and different viral strains

V. CONCLUSION

This study revealed that a natural product composed of natural products and aloe-derived lactic acid bacteria was effective in preventing and improving respiratory diseases. The combination of mixed extract of chenpi, platycodi radix and red poria (CPRE, KJMA0002), aloe gel extract and freeze-dried aloe-derived lactic acid bacteria (ALAB, KJMA0001) powder resulted in stronger antiviral and anti-inflammatory effects. These results suggest that natural compositions have the potential to be safe and effective preventive and therapeutic alternatives for respiratory diseases, which are on the rise in modern society. This study is an important step in confirming the potential utilization of natural product compositions in the prevention and treatment of respiratory diseases, and their effectiveness and safety need to be further established in future large-scale clinical trials and further studies on different viral strains.

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