International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1025-1038 www.ijprajournal.com ISSN: 2456-4494

Effect of Aloe vera Raw Gel on the Growth of Aloe-Derived Lactic Acid Bacteria (L. heveticus KCTC 15075BP, L. gallinarum KCTC 14824BP) and Antioxidant Activity

DongMyong Kim[1,5†], SeoHyeon Hwang[2†],ChaeYun Yang[3], YeoJin Lee[4], Hyung-Kon Lee[1], Yong-Seong Kwon[1], and Yeon-Mea Choi[5]

[1]*R&D Center, KJMBIO Ltd, Seocho-gu, Seoul, Korea*
[2]*Dept. of Biological Sciences, KAIST, Daejeon, Korea*
[3]*Dept. of Chemical & Biomolecular Engineering, KAIST, Daejeon, Korea*
[4]*Dept. of Biomedical Engineering, UNIST, Ulsan, Korea*
[5]*KimJeongMoon Aloe Ltd, Seocho-gu, Seoul, Korea*

Date of Submission: 01-08-2024 Date of Acceptance: 10-08-2024

ABSTRACT: This study was conducted to investigate the effect of Aloe vera raw gel on the growth of aloe-derived lactic acid bacteria and its antioxidant activity according to the manufacturing method. The antimicrobial activity of Aloe vera raw gel was measured using the agar diffusion method at a concentration of 50 mg/mL of Aloe vera raw gel. Aloe vera raw gel had no inhibitory effect on the tested intestinal bacteria. However, Aloe vera raw gel significantly enhanced the growth of lactic acid bacteria, especially Lactobacillus heveticus KCTC 15075BP, Lactobacillus gallinarum KCTC 14824BP,Bifidobacterium bifidumand Bifidobacteriumadolescentis. Total phenolic compounds and flavonoid compounds were higher in the first Aloe vera raw gel preparation than in the second Aloe vera raw gel preparation. The EDA values of Aloe vera raw gel preparations increased in proportion to the concentration. The first Aloe vera raw gel preparation showed the highest value among all other preparations, which was 85.60% at a concentration of 0.05 mg/mL. In addition, the first Aloe vera raw gel preparation showed stronger antioxidant activity than the other preparations with an activity of 679.91 mg AA eq/g. These results support the potential use of Aloe vera raw gel preparation as a functional food ingredient and a valuable resource for the development of health food products, which can increase the growth of Lactobacillus spp. and Bifidobacterium spp. in the human intestine.

KEYWORDS: Aloe vera raw gel, Lactic acid bacteria growth, Lactobacillus, Bifidobacterium, Antioxidant activity, Total polysaccharides, Total polyphenol compounds, Total flavonoid compounds, human intestine

I. INTRODUCTION

Aloe is a very common Liliaceae family, native to Africa, containing approximately 400 species of tropical plants. Throughout history, it has long been used for medical purposes. Meanwhile, from the 400 species of Aloe,only a few have been traditionally used as herbal medicine. In this regard,Aloe vera is the commonlyused form of Aloe for medical purposes.Aloe contains several classes of secondary metabolites such as phenolic compounds, flavonoid compounds, saponins, sterols, and several anthraquinones.Aloin and emodin are the most importantanthraquinones, and they exhibit anti-bacterial, anticancer, anti-viral, and analgesic activities [1-5].Metabolomics has been demonstrated to be an appropriate tool for the composition analysis of plants and foods [6].

There are 100 trillion bacteria in the intestines of the human body, and they account for about 30% of the solid feces (7). Intestinal microorganisms maintain a mutualistic or antagonistic relationship in the intestines of the human body, and they proliferate and excrete by using the food consumed and the biological components separated from the digestive tract. This intestinal microbial flora is greatly affected by the age and diet of the person, and the composition of this flora is known to be closely related to aging, constipation, and the occurrence of intestinal diseases (8,9).

Intestinal microorganisms can be broadly divided into beneficial bacteria such as Bifidobacterium, Lactobacillus, and Streptococcus, and harmful bacteria such as Clostridium, Eubacterium, Peptostreptococcus, Desulfotomaculum, and Veilonella. Representative beneficial bacteriaBifidobacterium genus is reported

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1025-1038 www.ijprajournal.com ISSN: 2456-4494

to produce organic acids and antibiotics in the intestines, and to suppress the growth of external pathogens and excessive growth of harmful bacteria.

On the other hand, representative harmful bacteria Clostridium perfringens produces variousBifidobacterium genus is reported to produce organic acids and antibiotics in the intestines, and to suppress the growth of external pathogens and excessive growth of harmful bacteria. On the other hand, representative harmful bacteria Clostridium perfringens producesvarious toxins, and is deeply involved in necrotizing enterocolitis, cholelithiasis, liver cancer, and viral infections in newborns,and Clostridium difficile is a pathogen that causes diarrhea and colitis related to the use of antibiotics (10,11).

In 2001, the joint expert committee of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) defined probiotics as 'live microorganisms that are beneficial to health when consumed in adequate amounts' (12), and including the genera Bifidobacterium and Clostridium described above, and Lactobacillus, Bacillus, and Saccharomyces (13). Prebiotics refer to nutrients necessary for the growth of probiotics (12), and include fructooligosoccharides, galactooligosaccharides, lactitol, lactulose, and soybean oligosaccharides.Among these, oligosaccharides are low-molecular substances with a molecular weight of 300 to 20,000, which are composed of 2 to 10 monosaccharides such as glucose or galactose, and are not decomposed by digestive enzymes in the body, but reach the large intestine and are selectively utilized by bifidobacteria, which are representative intestinal beneficial bacteria (14). Recently, many types of yogurt and health foods containing lactic acid bacteria have become popular, and products that are mixed with lactic acid bacteria and prebiotics are called synbiotics (15).

Up to now, research on Aloe vera has mainly been conducted on components that have pharmacological effects, such as antioxidant effects (16,17,18), immune system regulation, anti-inflammatory effects, wound healing promotion (19), allergic reactions, rheumatoid arthritis, rheumatic fever, and acid indigestion (20). Among natural materials, research has been conducted on extracts that can affect intestinal microorganisms, such as mugwort (21), cactus (22), dandelion (23), and mulberry leaves (24).Studies related to microorganisms have been reported, including a method for producing a fermented lactic acid bacteria powder derived from aloe and a study on an immune-enhancing composition containing the same (25), a method for producing aloe polysaccharide sandwich-coated lactic acid bacteria and a study on coated lactic acid bacteria (26), an aloe food composition for preventing and improving respiratory diseases and a method for producing the same (27), a method for producing powder containing fermented aloe mycelia and a functional food composition comprising the powder produced thereby (28), nano-exosome derived from Aloe vera bark callus as a new type of Transdermal Delivery System (29), exosomal nanoencapsulated AlabTM peptide for Nutrient Delivery System (30), antioxidation and skin barrier recovery of phyto DNA extracted from Aloe vera (31), and sleep inducing effect of L. helveticus KJMA-0001 isolated from Jeju Aloe vera (32). However, studies on the effect of Aloe vera raw gel on intestinal microorganisms are insufficient.

Therefore, in this study, we aimed to investigate the effect of Aloe vera gel on the growth of lactic acid bacteria, among intestinal beneficial bacteria, in vitro in order to combine it with lactic acid bacteria as probiotics to prevent intestinal putrefaction by regulating the growth of intestinal beneficial bacteria. In addition, in order to investigate the effect of polysaccharides present in Aloe vera gel as growth factors on the growth of lactic acid bacteria, primary and secondary hot water and ethanol extracts of Aloe vera gel with different polysaccharide contents were manufactured by different Aloe vera gel manufacturing methods. In addition, we aimed to secure basic data for commercializing Aloe vera, produced by KimJungMoon Aloe Co., Ltd., which was the first in Korea to successfully cultivate aloe in Jeju Island, as a functional food material by searching for phenolic compounds and antioxidant properties.

II. EXPERIMENTATION

Materials and strains

The Aloe vera used in this experiment was purchased from KimJungMoon AloeCo.,LTD., Jeju Agricultural Factory. The peel of the Aloe vera fresh leaf was removed by hand filleting, and only the inner gel was taken, which was then homogenized with a mixer (EBR 400, Electrolux, Sweden). The homogenized inner gel was centrifuged (10,000 rpm, 30 min) to remove fibres, and the supernatant was taken to obtain aloe vera gel.

The strains used were those provided by the Korean Collection for Type Cultures (KCTC, Daejeon, Korea) and those isolated by the Mitsuoka method (33). Clostridium difficile KCTC 5009,

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1025-1038 www.ijprajournal.com ISSN: 2456-4494

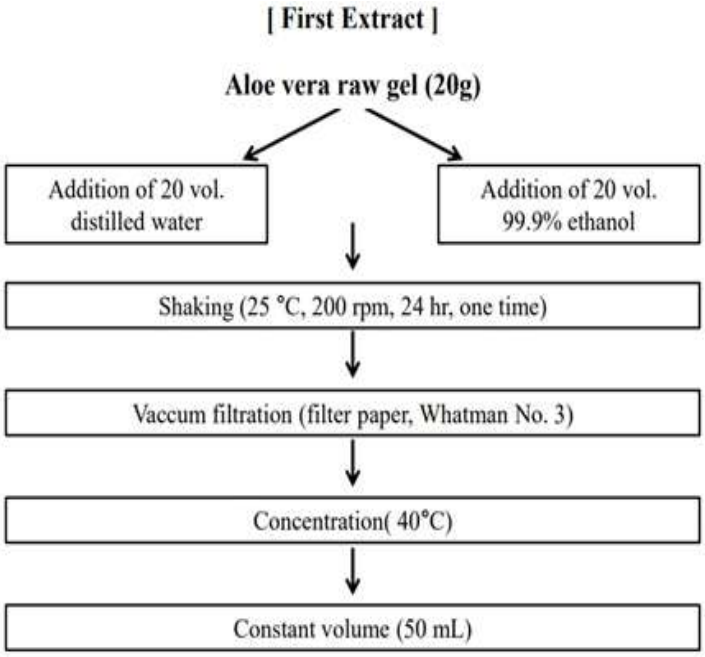
Clostridium perfringens KCTC 3269, Eubacterium limosum KCTC 3266, and Bacteroides fragilis KCTC 3688, which were distributed from the Korea Biological Resource Centre, were used in the experiment. Lactobacillus helveticus KJMA0001 KCTC 15075BP, Bifidobacterium adolescentis, Bifidobacterium bifidum KCTC 3472, Lactobacillus acidophilus KCTC 3164, and Streptococcus thermophilus KCTC 3658, which were isolated from aloe vera fresh leaves by the Mitsuoka method (33) in the laboratory of KJMBio Co., Ltd., were used as beneficial intestinal bacteria. Reinforced Clostridial Medium (RCM) broth (Difco, Detroit, MI, USA) was used as the growth medium for preculture and main culture for intestinal harmful bacteria, and Lactobacilli MRS broth (Difco) was used for intestinal beneficial bacteria. These strains were stored in a -85℃deep freezer (Ultra-low temperature freezer, MDF-192, SANYO Electric Biomedical Co., Ltd., Osaka, Japan) in a medium containing 50% glycerol, and used after activation by subculture at least three times before use in the experiment.

Preparation of ethanol extract and water extract

In order to find out how polysaccharides present in Aloe vera raw gel as growth factors affect the growth of lactic acid bacteria, primary and secondary extracts of aloe vera raw gel with different polysaccharide contents were prepared by changing the initial extraction method (Fig. 1).

First extract: 20 g of Aloe vera raw gel was added 20 times more 99.9% ethanol and distilled water, respectively, and extracted at 25℃ for 24 hours at 200 rpm using a stirring extractor (VS-8480, Vision Scientific Co., Ltd., Seoul, Korea). The extract was filtered through a filter paper (Whatman No. 3, Bukinghamshire, UK) and the solvent was removed using a rotary vacuum concentrator (EYELA CCA1110, Tokyo, Japan), which was used as the first extract. Each of the prepared ethanol and hot water extracts was diluted to 50 mL with distilled water and used in the experiment.

Second extract: In order to remove the sugar component present in the Aloe vera raw gel, 20 g of the Aloe vera raw gel was extracted at 25℃ for 24 hours at 200 rpm using a stirring extractor (VS-8480, Vision Scientific Co.) with 3.5 times the amount of distilled water. After filtering through a filter paper (Whatman No. 3), the solvent was removed, and 20 times the amount of ethanol and distilled water were added to the remaining solids, and extracted in the same way as the first extract preparation method. The extract was filtered, and the solvent was removed using a rotary vacuum concentrator (EYELA CCA-1110), and then used as the second extract. Each of the prepared ethanol and water extracts was diluted to 50 mL with distilled water and used in the experiment.



International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1025-1038 www.ijprajournal.com ISSN: 2456-4494

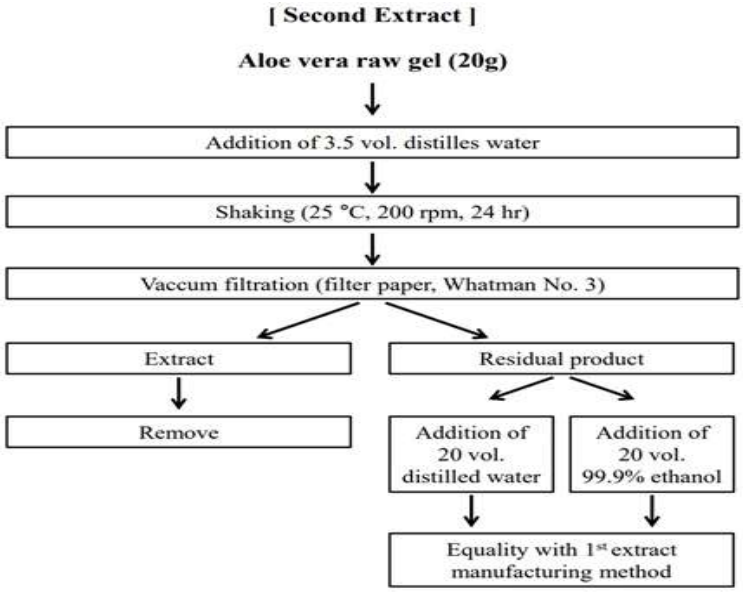


Fig. 1. Schematic diagram of the extraction process of Aloe veraraw gel

Analysis of total polysaccharides in Aloe vera gel

TLC:1 µL each of standard (1% glucomannan, acemannan, galactose, mannose) and first and second Aloe vera raw gel extracts were placed 1.5 cm above the bottom of a silica gel TLC plate (20×10 cm, Whatman), developed three times with acetonitrile:water (85:15-v/v) solvent, and then colour was developed using a colour developing reagent (0.5% α-naphtal, 5% H_2SO_4 in EtOH solution) to qualitatively analyse the polysaccharide in the extracts.

HPLC : The pretreatment of the sample used in this experiment was to denature and precipitate the protein in the extract, boil it for 10 minutes, cool it on ice, centrifuge it at 13,000 rpm for 10 minutes, filter it with a 0.45 µm filter, and remove the pigment from the extract using Sep-pak (Sep-Pak® Vac 3 cc C18 Cartridges, Waters, Dublin, Ireland), and then use it as an HPLC injection sample. HPLC was performed using Acme 9000 (Acme 9000 HPLC, Young Lin Instrument Co., Anyang, Korea), and the column was NH2P-50 4E (Asahipak NH2P-50 4E, 5 µm, 4.6×250 mm, Shodex, Tokyo, Japan). The mobile phase was acetonitrile: water = 73:27(v/v%), the flow rate was 1 mL/min, and the detector was Refractive Index (RI, Acme 9000, Young Lin Instrument Co.). A calibration curve was prepared using standard solutions to quantify the individual polysaccharide contents in the extract (34).

Antibacterial activity of Aloe veraraw gel

Antibacterial activity was tested using the agar diffusion test (35).The test strains cultured at $10^{4\sim5}$ CFU/mL were inoculated at 2% on each sterilized RCM (Difco) and MRS (Difco) agar medium, and then poured into a petri dish to create a medium. A paper disc (ADVANTEC 8 mm, TOYO ROSHI Kashia Ltd., Tokyo, Japan) was attached so as not to fall on the medium, and 30 µL of Aloe vera raw gel extract [dissolved in 30% DMSO (solid content 50 mg/mL) after nitrogen concentration and decompression concentration to obtain the desired extract concentration and reduce weight error)] was injected into an anaerobic culture device (general gravity convection incubator anaerobic culture device MART Microbiology, ANOXOMAT WS80, Lichtenvoorde, Netherlands) and cultured at 37℃ for 24~48 hours. The presence or absence of growth inhibition zone formation was confirmed.To determine the effect of the solvent in which the Aloe veraraw gel was dissolved, 30% DMSO (dimethyl sulfoxide, Sigma Chemical Co., St. Louis, MO, USA), which is a stable substance for the test strain and can dissolve the solute of the Aloe veraraw gel, was used as a control.

Effect of Aloe veraraw gel on growth of beneficial bacteria

The growth curve of beneficial bacteria in the human intestine was tested for four species, B. adolescentis, B. bifidum, L. acidophilus, and S. thermophilus. 2.5 mL of the test bacterial solution (a bacterial suspension made with sterile saline and the

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1025-1038 www.ijprajournal.com ISSN: 2456-4494

bacterial concentration was adjusted to an optical density value of 0.4 at 660 nm) was inoculated into 42.5 mL of sterilized MRS broth, and 5 mL each of the first and second extracts of Aloe veraraw gel [dissolved in 30% DMSO after nitrogen concentration and decompression concentration to obtain the desired extract concentration and reduce weight error (solid content 50 mg/mL)] was added and cultured at 37°C for 72 hours. The degree of fungal growth was observed by measuring the O.D. value at 660 nm using a spectrophotometer (UV-1650PC, Shimadzu, Kyoto, Japan) every 4 hours for the first 24 hours and then at 24-hour intervals (0, 4, 8, 12, 16, 20, 24, 48, and 72 hours) for 3 days. As a control, 5 mL of 30% DMSO containing the extract was added instead of the extract and the same measurement was made.

Functional analysis of Aloe veraraw gel

Total polyphenol content: The total polyphenol content was analysed according to the method of Dewanto et al. (36) based on the principle that the Folin-Ciocalteu reagent is reduced by the polyphenol compounds of the extract and develops molybdenum blue colour. 2 mL of 2% Na_2CO_3 solution was added to 100 µL of the aloe vera gel extract, left for 3 minutes, then 100 µL of 50% Folin-Ciocalteu reagent was added, and the reaction solution was reacted for 30 minutes. The absorbance value of the reaction solution was measured at 750 nm. Flavonoids (Sigma Chemical Co.), which are representative polyphenol substances with two or more phenolic hydroxyl (OH) groups in one molecule and various physiological activities, were used as standard substances to create a calibration curve, and the total polyphenol content was expressed as mg flavonoids per 100 g of the sample. All experiments were repeated three times, and significance was tested using Duncan's multiple range test (p=0.05).

Total flavonoid content: The total flavonoid content was measured by modifying the method of Dewanto et al. (36) and Choi et al. (37). 1 mL of distilled water and 75 µL of 5% $NaNO_2$ were added to 250 µL of aloe vera raw gel extract, and the mixture was reacted for 5 minutes. Then, 150 µL of 10% $AlCl_3$. $6H_2O$ was added, left for 6 minutes, 500 µL of 1 N NaOH was added, and the mixture was reacted for 11 minutes. The absorbance value of the reaction solution was measured at 510 nm. (+)-catechin hydrate (Sigma Chemical Co.) was used as a standard substance, and a calibration curve was created. All experiments were repeated three times, and significance was tested using Duncan's multiple range test (p=0.05).

DPPH: In order to measure the antioxidant activity of the first and second extracts of Aloe vera raw gel, the electron donating ability (EDA(%)) was measured by modifying the Blois method (38). 0.2 mL of Aloe vera raw gel extract was added to 0.8 mL of 2×10-4 M DPPH (0.2 mM DPPH) (Sigma Chemical Co.) solution (0.00788 g dissolved in 100 mL of 99.9% ethanol), and the decrease in absorbance at 520 nm was measured after leaving it at room temperature for 30 minutes. When measuring absorbance, the difference in absorbance due to each sample dispensed into the cell was compensated by measuring the absorbance of ethanol alone, and the electron donating ability was expressed as a percentage (%) of the absorbance difference between the sample-added and -unadded groups.

$$EDA\ (\%) = B - A / B \times 100$$

A: Absorbance value when extract was added
B: Absorbance value when the same amount of ethanol was added instead of the extract

In addition, the absorbance was measured by varying the concentration of the extract into three sections, and the IC_{50} (inhibition concentration) that reduces the EDA (%) value of the extract by 50% was calculated and expressed as a concentration for the yield of each extract. This experiment was repeated twice and the significance was tested using Duncan's multiple range test (p=0.05).

$ABTS^+$ decolorization assay: The antioxidant activity of the first and second extracts of Aloe vera raw gel was measured by the $ABTS^+$decolorization assay method of Dewanto et al. (36). 2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)(ABTS, Sigma Chemical Co.) 7.4 mM and potassium persulphate 2.6 mM were mixed 1:1 and left in the dark for one day to form $ABTS^+$. Then, the solution was diluted with distilled water using the molar absorption coefficient ($\varepsilon = 3.6\times10^4\ M^{-1}cm^{-1}$) so that the O.D. value was 1.4~1.5 at 735 nm. 50 µL of the extract was added to 1 mL of diluted $ABTS^+$ solution, and the absorbance change was measured exactly 60 minutes later, and an equal amount of L-ascorbic acid was added as a standard substance. The total antioxidant power was calculated using the following formula, and the experiment was repeated twice and the significance was tested using Duncan's multiple range test (p=0.05).

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1025-1038 www.ijprajournal.com ISSN: 2456-4494

AEAC (mg AA eq) = ΔA/ΔAaa × Caa × V × 100/W
ΔA: Change in O.D. when extract was added
ΔAaa: Change in O.D. when the same amount of AA std. soln. was added instead of the extract
Caa: Concentration of AA std. soln. (mg/mL)

V: Volume of extract (mL)
W: Weight of sample homogenate (g)

III. RESULTS AND DISCUSSION

Total Polysaccharide analysis of Aloe vera raw gel extract using TLC

Standard was prepared by mixing Sigma's standard glucomannan, acemannan, galactose, and mannose to make 1%, and the first and second ethanol/water extracts of Aloe vera raw gel with a concentration of 0.4 g/mL were diluted 5-fold and 10-fold, respectively, and 1 μL was spotted, and it was found that the sugar composition of the Aloe vera raw gel extract was glucomannan, acemannan, galactose, sucrose and mannose.

Lee Jin-hee et al. (39) reported that the content of total polysaccharides in Aloe vera raw gel is about 5 to 40 wt%, preferably 20 to 30 wt%, and more preferably 23 to 25%, based on the dry weight (weight cut-off, 3,500 Daltons) of the residual fraction in the dialysis net during extensive dialysis against distilled water. In addition, it was consistent with the fact that mannose and glucomannan were contained in an average molar ratio of 5~40:1.

In addition, the colour development of the second extract spot was reduced compared to the first extract, confirming that the sugar content in the jujube extract as a growth factor for intestinal beneficial bacteria differed depending on the extraction method (Fig. 2).

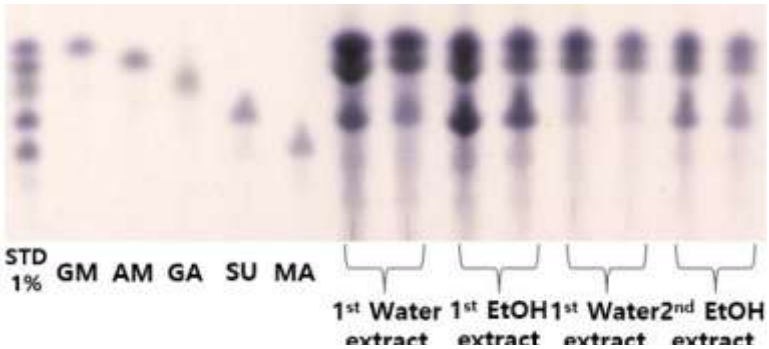


Fig. 2.TLC of polysaccharide in Aloe vera raw gel extracts. Solvent; acetonitrile : water (85:15-v/v), GM: glucomannan, AM: acemannan, GA: galactose, SU: sucrose MA: mannose

Analysis of total polysaccharides of Aloe veraraw gel using HPLC

Through TLC analysis, it was confirmed that glucomannan, acemannan, galactose, mannose, sucrose, and fructose were present in the Aloe veraraw gel extract, among which glucomannan, acemannan, and galactose was present. Based on this, the standard solution calibration curves of glucomannan, acemannan, and galactose, which are the main individual polysaccharides in the Aloe veraraw gel extract, were analysed (Fig. 3). As a result of the calibration curve analysis for each individual polysaccharide, the R value of the galactose calibration curve was 0.9997, the R value of the glucomannan calibration curve was 0.9993, and the R value of the acemannan calibration curve was 0.999, showing linearity. The individual polysaccharide contents of the Aloe vera raw gel extract was analysed in three replicate experiments, and the glucomannan, acemannan, and galactose contents were 124.71 mg/g, 145.13 mg/g, and 116.05 mg/g in the first ethanol extract, and 197.12 mg/g, 254.75 mg/g, and 52.77 mg/g in the first water extract, respectively. In the second ethanol extract, glucomannan, acemannan, and galactose were 46.45 mg/g, 55.16 mg/g, and 19.27 mg/g, respectively, and in the second water extract, they were 61.72 mg/g, 65.37 mg/g, and 6.05 mg/g, respectively. Compared to the primary extract, glucomannan, acemannan, and galactose in the ethanol extract decreased by 63%, 62%, and 83%, respectively, and in the water extract, they decreased by 69%, 74%, and 89%, respectively (Table 1). This was consistent with the report by Lee et al. (MFDS paper) that the polysaccharide content detected in water is higher than in the ethanol extract because the polysaccharide is water-soluble. However, Lee et al. (MFDS paper) showed a large difference from our results, with polysaccharide content of Aloe veraraw gel with the peel removed being glucomannan 25%, acemannan 21.2%, and galactose 20.7%. This difference in results indicates

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1025-1038 www.ijprajournal.com ISSN: 2456-4494

that there may be a large difference in polysaccharide content depending on the extraction method in each part of Aloe vera.

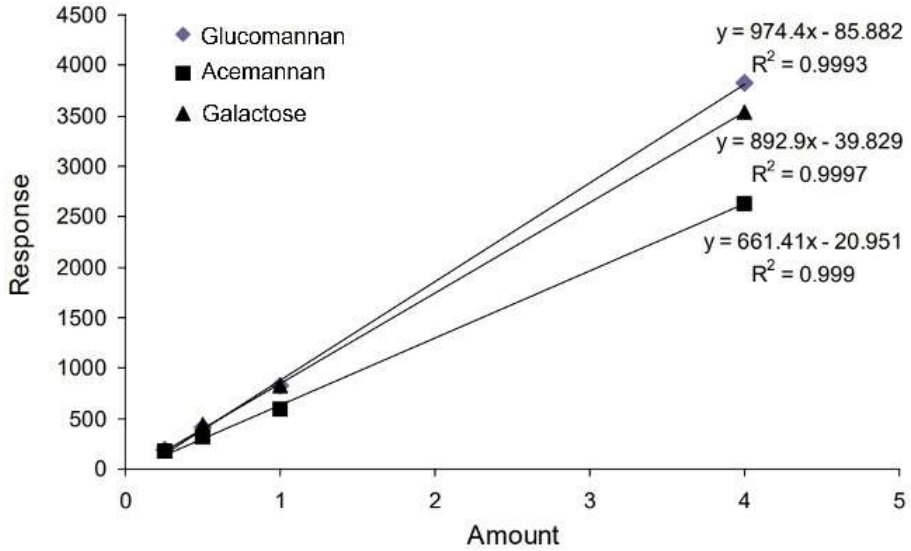


Fig. 3. Calibration curve of polysaccharide standards

Antibacterial activity of Aloe veraraw gel

Aloe veraraw gel first and second extracts were each tested for antibacterial activity at a concentration of 50 mg/mL using an agardiffusion test. As a result, no inhibitory zones were formed on the intestinal harmful bacteria Cl. difficile, Cl. perfringens, Eu. limosum, B. fragilis, and intestinal beneficial bacteria B. bifidum, B. adolescentis, L. acidophilus, and S. thermophilus, indicating no antibacterial activity.

Effect on the growth of beneficial intestinal bacteria

Although the antibacterial effect through the agar diffusion test did not show antibacterial activity against harmful intestinal bacteria, the polysaccharide present in the extract of Aloe veraraw gel was judged to affect the growth of beneficial intestinal bacteria, so the promotion effect was analysed.

When compared to the control group in which 30% DMSO was added instead of the extract of Aloe veraraw gel, L. heveticus, L. gallinarum, B. bifidum and B. adolescentis in the first extract of Aloe veraraw gel were dissolved in the extract of Aloe veraraw gel, the time to reach the logarithmic phase for each strain in the ethanol extract was similar to that of the control group, and the absorbance values were lower or similar to those of the control group, indicating that there was no significant effect on growth. However, when B. bifidum and B. adolescentis were compared to other test strains in the water extract, the absorbance values when reaching the logarithmic phase were greater than those of the control group, indicating that growth was promoted (Fig. 4).

In the second extract of Aloe vera raw gel, the water extract had a higher turbidity and initial absorbance value compared to the control and ethanol extracts, but when L. heveticus, L. gallinarum, B. bifidum and B. adolescentis all reached the logarithmic phase, the absorbance values of the control, ethanol extract, and water extract were all similar, indicating that the second extract of Aloe vera raw gel did not have much effect on the growth of beneficial intestinal bacteria.In the first extract of Aloe vera raw gel, B. bifidum and B. adolescentis showed higher absorbance than the second extract, indicating that growth was promoted. This result was consistent with the study by Oh et al. (Tetrachloride Aloe Fermentation No. 37), which reported that the higher the content of free polysaccharides such as glucomannan, acemannan, and galactose, the more fermentation by lactic acid bacteria was promoted.It is believed that the polysaccharides present in the extract of Aloe vera raw gel acted as prebiotics and showed the promoting effect.

Meanwhile, Dong-Myong Kimet al (40,41) reported that when he inoculated Aloe vera-derived plant-based lactic acid bacteria L. heveticus and L. gallinarum into amedium containing Aloe vera raw gel extract extracted with 70% ethanol and

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1025-1038 www.ijprajournal.com ISSN: 2456-4494

measured the O.D. value,the growth of lactic acid bacteria

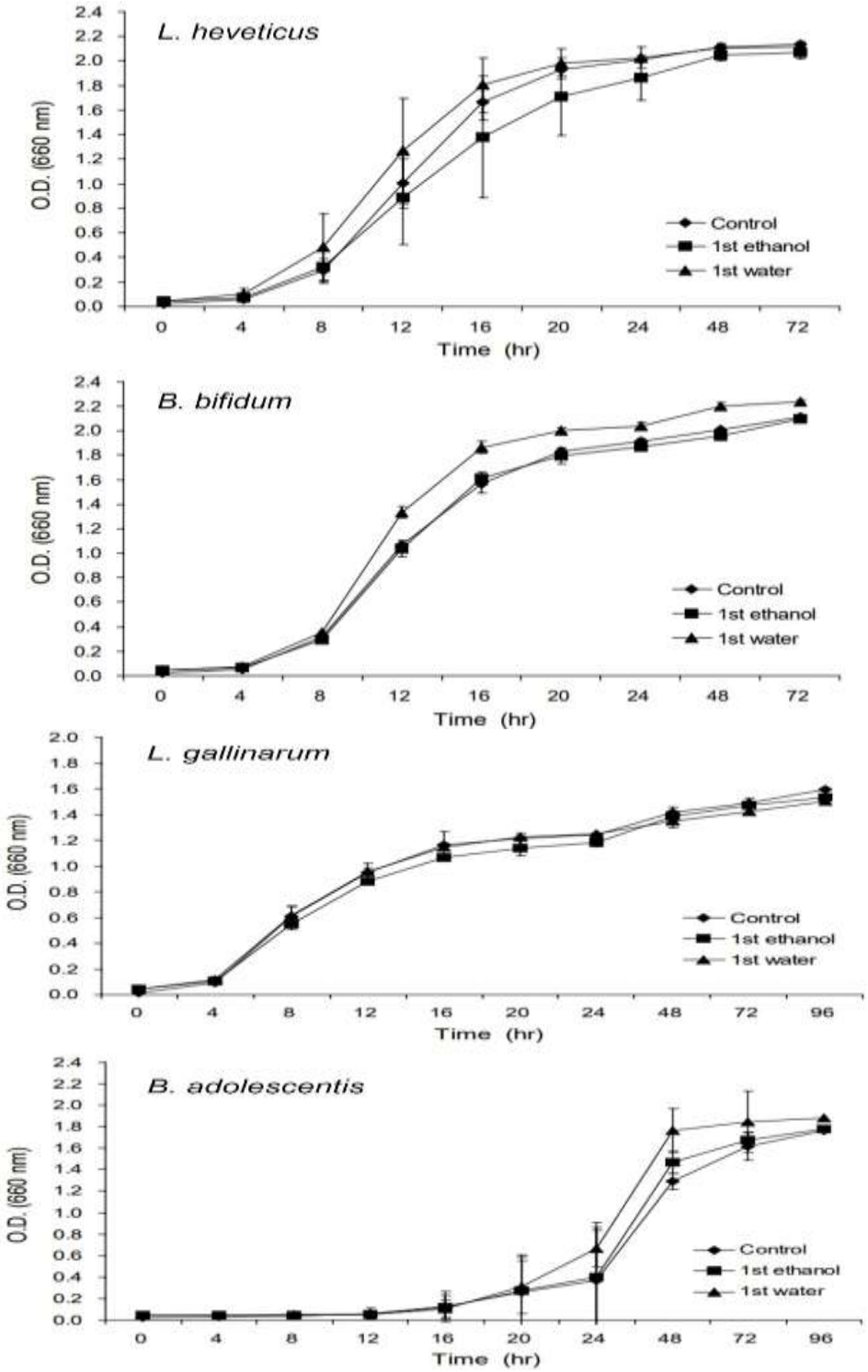


Fig. 4. Effects of first Aloe vera raw gel extract on the growth of L. heveticus, L. gallinarum, B. bifidum and B. adolescentis was promoted in a concentration-dependent manner. This suggests that there may be differences in results depending on the extraction solvent and extraction method.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1025-1038 www.ijprajournal.com ISSN: 2456-4494

Total polyphenol and flavonoid content

To investigate the correlation between the functional components other than polysaccharides and the growth-promoting components of beneficial bacteria according to the extraction method of aloe vera raw gel, the total polyphenol content and the total flavonoid content were investigated. The results are shown in Table 2. The polyphenol content of aloe vera raw gel extract was prepared using flavonoid as a standard substance to create a calibration curve, and the total flavonoid content was prepared using (+)-catechin hydrate as a standard substance.

The total polyphenol content was the highest at 11.63 mg/g in the first hot water extract, and the polyphenol content was 10.41 mg/g in the first ethanol extract, 4.37 mg/g in the second hot water extract, and 3.77 mg/g in the second ethanol extract. Min and Lee (42) reported that the polyphenol content of the water extract of Angelica dahurica was 37.92 mg/g, the water extract of Acanthopanax japonica, the ethanol extract of Acanthopanax japonica, and the ethanol extract of Acanthopanax japonica were 29.23 mg/g, and 22.86 mg/g, respectively, in the antioxidant properties of the extracts of medicinal plants from Jeju, Korea. This is consistent with the present experiment, which showed that the water extract had a higher polyphenol content than the ethanol extract. In addition, Ju et al. (43) reported that the water extract had a higher polyphenol content than the ethanol extract in the polyphenol experiment of bamboo extracts according to the extraction method.

The total flavonoid content of the Aloe vera raw gel extract was the highest in the first water extract at 247.45 μg/g, similar to the polyphenol content, and the flavonoid content was 76.39 μg/g in the first ethanol extract, 83.90 μg/g in the second water extract, and 45.70 μg/g in the second ethanol extract. Similar to the study by Kim et al. (44), which measured the polyphenol and flavonoid contents of water extracts of about 20 medicinal plants, the polyphenol content was higher than the flavonoid content.

Therefore, the Aloe vera raw gel first water extract, which contains a large number of flavonoids, one of the largest groups of naturally occurring polyphenols and phenol compounds with various functions, compared to other extracts, is thought to not only promote the growth of L. helveticus, L. galinarum, B. bifidum and B. adolescentisbut also have excellent antioxidant activity and physiological functions.However, it is thought that many effective ingredients other than polyphenols and flavonoids are involved in the antioxidant properties, so it is thought that the experiment should be continued.

Table 1. Polysaccharidecontents inAloe vera raw extracts

Sample		Polysaccharide[1] (mg/g)			Reduction rate[2] (%)		
		Glucomannan	Acemannan	Galactose	Glucomannan	Acemannan	Galactose
Frist extract	Water	197.12±9.61[a]	254.75±13.25[a]	52.77±8.63[b]	-	-	-
	EtOH	124.71±20.39[b]	145.13±16.18[b]	116.05±27.06[a]	-	-	-
Second extract	Water	61.72±31.41[c]	65.37±24.85[c]	6.05±0.42[c]	62.76	61.99	83.40
	EtOH	46.45±21.45[c]	55.16±28.66[c]	19.27±8.78[c]	68.69	74.34	88.54

[1)]Values are the mean±standard deviation of triplicate experiments. Means with different letters in same column are significantly different from the others at $p<0.05$.
[2)]Reduction rate (%)＝(contents of first extracts－contents of second extracts/contents of first extracts)×100.

Table 2. Concentration of total polyphenol compound and flavonoid compound in Aloe vera raw extracts

Sample		Total polyphenol compound[1] (mg/g)	Total flavonoid compound[2] (μg/g)
First extracts	Water	11.63±0.78[a][3)]	247.45±0.46[a]
	EtOH	10.41±1.32[a]	76.39±19.85[b]
Second extracts	Water	4.37±1.52[b]	83.90±9.23[b]
	EtOH	3.77±0.96[b]	45.70±30.01[b]

[1)]Milligrams of total polyphenol content/g of plants based ongallic acid as standard.
[2)]Milligrams of total flavonoid content/g ofplants based on (+)-catechin hydrate as standard.
[3)]Valuesare the mean±standard deviation of triplicate experiments. Means with different letters in same column are significantly different from the others at $p<0.05$.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1025-1038 www.ijprajournal.com ISSN: 2456-4494

Table 3.EDAs and IC50ofAloe vera raw extracts

Sample		Electron donating ability (%)		IC_{50} (mg/mL)
		0.05 mg/mL	0.01 mg/mL	
Frist extract	Water	85.60±5.82[a1)]	24.68±0.35[a]	0.024±0.002[b]
	EtOH	48.66±1.89[b]	11.18±0.34[b]	0.051±0.002[b]
Second extract	Water	37.43±3.19[d]	8.05±0.01[d]	0.068±0.006[b]
	EtOH	12.36±1.53[c]	4.19±0.66[c]	0.243±0.060[a]
Ascorbic acid		95.92	19.74	0.022±0.005

[1)]Values are the mean±standard deviation of triplicate experiments. Means with different letters in same column are significantly different from the others at $p<0.05$.

DPPH and ABTS free radical scavenging activity

To increase the validity of the radical scavenging activity results of the Aloe vera raw gel extract,we used two measurement methods using DPPH and ABTS to examine the antioxidant activity of the first and second Aloe vera raw gel extracts.As a control, ascorbic acid (95.92%, 0.05mg/mL), a natural antioxidant, was used to examine the antioxidant activity by DPPH free radical scavenging activity (Table 3). The higher the concentration of the Aloe vera raw gel extract, the better the electron donating ability was shown. The Aloe vera raw gel primary water extract showed the highest electron donating ability of 85.60% compared to other extracts, confirming its functionality comparable to that of the natural antioxidant ascorbic acid. At a concentration of 0.05 mg/mL, the first ethanol extract of Aloe vera raw gel showed 48.66%, the second water extract of Aloe veraraw gel showed 37.43%, and the second ethanol extract of Aloe veraraw gel showed 12.36% of electron donating ability. In addition, the IC50 value was 0.03 mg/mL for the first water extract of Aloe veraraw gel, 0.05 mg/mL for the first ethanol extract, 0.07 mg/mL for the second water extract, and 0.24 mg/mL for the second ethanol extract, showing that the first water extract of Aloe veraraw gel showed the best antioxidant activity compared to the other extracts. The results of this experiment showed that the first water extract of Aloe veraraw gel showed a higher tendency than the electron donating ability of chrysanthemum water extract (63.0%), angelica root (49.6%), and peony root (49.6%) when compared with the study of Park (45) who studied the polyphenol compounds and antioxidant activity of herbal extracts. The results of calculating the antioxidant activity as an AEAC value using water-soluble ascorbic acid as a standard substance are shown in Table 4. Aloe vera raw gelthe first water extract had the highest antioxidant power at 679.91 mg AA eq/g, the first ethanol extract had 343.18 mg AA eq/g, the second water extract had 327.30 mg AA eq/g, and the second ethanol extract had 176.65 mg AA eq/g.

Table 4. AEAC ofAloe vera raw extracts

Sample		AEAC (mg AA eq/g)
Frist extract	Water	679.91±33.35[a1)]
	EtOH	343.18±1.14[b]
Second extract	Water	327.30±31.70[b]
	EtOH	176.65±15.22[c]

[1)]Values are the mean±standard deviation of triplicate experiments. Means with different letters in same column are significantly different from the others at $p<0.05$.

ABTS radical scavenging activity was higher than DPPH, which is consistent with the study by Re et al. (46) that ABTS radical scavenging activity was more sensitive because the ABTS method can measure both hydrogen-donating antioxidants and chain-breaking antioxidants and can be applied to both aqueous phase and organic phase.

IV. CONCLUSION

In this study, we investigated the effect of Aloe vera raw gel extract on the growth of lactic acid bacteria among intestinal beneficial bacteria in vitro in order to combine Aloe vera raw gel extract with lactic acid bacteria as probiotics to control the growth of intestinal beneficial bacteria and prevent intestinal putrefaction. In addition, in order to investigate the effect of polysaccharides present in Aloe vera raw gel extract as growth factors on the growth of lactic acid bacteria, first and second water

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1025-1038 www.ijprajournal.com ISSN: 2456-4494

and ethanol extracts of Aloe vera raw gel with different polysaccharide contents were prepared by different extraction methods. In addition, we searched for phenolic compounds and antioxidant properties of Aloe vera raw gel from KimJungMoon Aloe Co., LTD., which was successfully cultivated for the first time in Jeju Island,in Korea, to secure basic data for commercializing it as a functional food material. Through TLC and HPLC analysis, the polysaccharide composition of the Aloe vera raw gel extract, which can act as a growth factor for intestinal beneficial bacteria, was confirmed to be glucomannan, acemannan, and galactose. Compared to the first extract, the second hot water extract showed a decrease of 69, 74, and 89% in glucomannan, acemannan, and galactose, respectively, and the second ethanol extract showed a decrease of 63, 62, and 83%. As a result of analyzing the growth promotion effect of glucomannan, acemannan, and galactose extracts at a concentration of 50 mg/mL on human intestinal bacteria, the first water extract of Aloe vera raw gel showed thatL. helveticus, L. galinarum, B. bifidum and B. adolescentis reached the log phase at a similar time to the control group compared to other test strains, but showed a higher absorbance value than the control group, indicating that growth was promoted. It is thought that the polysaccharidepresents in the Aloe vera raw gel extract acts as a prebiotic and promotes the growth of Lactobacillus and Bifidobacterium, and it is expected to improve intestinal function by improving the composition of the human intestinal flora in a desirable direction. In addition, the content and antioxidant activity of phenols, a functional substance, were analysed to determine whether growth is promoted by functional ingredients other than polysaccharides. As a result of measuring the total polyphenol and total flavonoid contents, theAloe vera raw gel first water extract showed higher contents than other extracts, at 11.63 mg/g and 247.45 μg/g, respectively. As a result of examining the radical scavenging activity by DPPH, the higher the concentration of the Aloe vera raw gel extract, the better the electron donating ability was shown, and the Aloe vera raw gel first water extract showed the highest electron donating ability of 85.60% compared to other extracts, confirming that it has functionality comparable to ascorbic acid, a natural antioxidant. Through ABTS free radical scavenging activity, the Aloe vera raw gel first water extract showed the antioxidant power of 679.91 mg AA eq/g, showing the best antioxidant activity compared to other extracts. Therefore, it is thought that the first water extract of Aloe vera raw gel contains not only polysaccharides but also high antioxidant activity, polyphenol, and flavonoid contents, which promoted the growth of L. helveticus, L. galinarum, B. bifidum and B. adolescentis. It is judged that the Aloe vera raw gel extract containing physiologically active ingredients as a functional food material can be applied to the development of functional fermented beverages, health functional foods, and pharmaceuticals by carrying out lactic acid fermentation using L. helveticus, L. galinarum, B. bifidum and B. adolescentis.

V. ACKNOWLEDGEMENTS

The research was supported by the Korea Technology and Information Promotion Agency for SMEs through the “Commercialization of brain-enhancing health functional food from swallow sialic acid based on affinity bead technology“(grant number: S2953529). Also, the research was supported by the Korea Institute for Advancement of Technology through the “Industry-academia focused technology development field training support project “(grant number: N0001395)

REFERENCES

[1]. Dong-Myong Kim, Ju-Yeong Jung, Hyung-Kon Lee, Yong-Seong Kwon and Yeon-Mea Choi (2021) Determination and Profiling Of Secondary Metabolites in Aloe Vera, Aloe Arborescens and Aloe Saponaria. Biomed J Sci & Tech Res. Vol. 40. No. 4, 32555~32563

[2]. Muhammad Asif, Tehreem Zahid, Baila Ahmad and Syeda Noor (2023) Therapeutics Characteristics and Application of Aloe vera: A Review. RADS J. of Food Biosci. Vol. 2. No. 1, 395~404

[3]. Hamman JH (2008) Composition and applications of Aloe vera leaf gel. Molecules 13(8): 1599-1616.

[4]. Reynolds T (1985) The compounds in Aloe leaf exudates: A Review. Botanical Journal of the Linnean society 90(3): 157~177.

[5]. Shelton RM (1991) Aloe vera: Its chemical and therapeutic properties. International journal of dermatology 30(10): 679~683

[6]. Qi Xiao, Xinlu Mu, Jiushi Liu, Bin Li, Haitao Liu, Bengang Zhang and Peigen Xiao (2022) Plant metabolomics: a new strategy and tool for quality evaluation of Chinese medicinal materials. Chinese Medicine. Vo1. 17. No. 45, 1~19

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1025-1038 www.ijprajournal.com ISSN: 2456-4494

[7]. Cindy D. Davis (2016) The Gut Microbiome and Its Role in Obesity. Nutrition Today. Vol. 51. No. 4, 167-174
[8]. Yikai Shao, Simon S. Evers, Jae Hoon Shin, Sadeesh K. Ramakrishnan, Nadejda Bozadjieva-Kramer, Qiyuan Yao, Yatrik M. Shah, Darleen A. Sandoval and Randy J. Seeley (2022) Vertical sleeve gastrectomy increases duodenal Lactobacillus spp. richness associated with theactivation of intestinal HIF2a signaling and metabolic benefits. Molecular Metabolism. Vol. 57, 1~10
[9]. Teng Ma, Xin Shen, Xuan Shi, Hafiz Arbab Sakandar, Keyu Quan, Yalin Li, Hao Jin, Lai-Yu Kwok, Heping Zhang and Zhihong Sun (2023) Targeting gut microbiota and metabolism as the major probiotic mechanism - An evidence-based review. Trends in Food Science & Technology. Vol. 138, 178~198
[10]. Cinara R. A. V. Monteiro, Monique S. do Carmo, Bruna O. Melo, Matheus S. Alves, Camilla I. dos Santos, Sílvio G. Monteiro, Maria Rosa Q. Bomfim, Elizabeth S. Fernandes and Valério Monteiro-Neto (2019) In Vitro Antimicrobial Activity and Probiotic Potential of Bifidobacterium and Lactobacillus against Species of Clostridium. Vol. 11. 448~453
[11]. Oana-Alina Petrariu, Ilda Czobor Barbu, AdelinaGabriela Niculescu, Marian Constantin, Georgiana Alexandra Grigore, RoxanaElena Cristian, Grigore Mihaescu and Corneliu Ovidiu Vrancianu (2024) Role of probiotics in managing various human diseases, from oral pathology to cancer and gastrointestinal diseases. Front. Microbiol. Vol. 14. 1296447~1296453
[12]. N.K. Ganguly, S.K. Bhattacharya, B. Sesikeran, G.B Nair, B.S. Ramakrishna, H.P.S. Sachdev, V.K. Batish, A.S. Kanagasabapathy, Vasantha Muthuswamy, S.C Kathuria, V.M. Katoch and K. Satyanarayana (2011) ICMR-DBT Guidelines for Evaluation of Probiotics in Food. Indian J Med. Res. Vol. 134. 22~25
[13]. Sejong Oh (2008) Probiotics and prolongation of life. Korean J Dairy Sci Technol. Vol. 26. 31~37
[14]. Yan Ma, Xingzhuang Wu, Vigna Giovanni and Xianjun Meng (1997) Effects of soybean oligosaccharides on intestinal microbial communities and immune modulation in mice. Saudi J Biological Sciences. Vol. 24. 114~121
[15]. Choi Jong-Bum, Shin Yong-Woo, Paek Nam-Soo and Kim Young-Man (2004) Influence of herbal extract on lactic acid bacteria growth and cyoprotectants. Korean J Food & Nutr. Vol. 17. 286~293
[16]. Marta Sánchez, Elena González-Burgos, Irene Iglesias and M. Pilar Gómez-Serranillos (2020) Pharmacological Update Properties of Aloe Vera and its Major Active Constituents. Molecules. Vol. 25. 1324~1361
[17]. Modupeola Dada and Patricia Popoola (2024) Aloe vera hydrogel for supercooling applications: a review. Discover Materials. Vol. 4. No. 10, 24~45
[18]. Aisha Saleem, Irum Naureen, Muhammad Naeem and Hafiza Safoora Murad (2022) Aloe Vera Gel Effect on Skin and Pharmacological Properties. Scholars Inter. J Anatomy and Physiology. Vol. 5. No. 1. 1~8
[19]. Sang-Hyun Kim, Kyu-Suk Shim, Youngcheon Song, Kyungjae Kim, Chan-Su Park and Chong-Kil Lee (2023) Pharmacological and Therapeutic Activities of Aloe vera and Its Major Active Constituent Acemannan. Food Suppl Biomater Health. Vol. 3. No. 2. 36-45
[20]. Priyanka Sharma, Amit Kharkwal and Harsha Kharkwal (2014) A Review on Pharmacological Properties of Aloe vera. Int. J Pharma. Sci. Rev. Res. Vol. 29. No. 2. 31~37
[21]. Mi Hyun Oh and Kwang Yup Kim (2010) Effects of Artemisia capillaris Extracts on Intestinal Microflora In vitro and In vivo. J Korean Soc. Food Sci. Nutr. Vol. 39. No. 11. 1587~1594
[22]. Bo-Hyun Ra, Woon-Jong Lee, Yun-Won Cho and Kwang-Yup Kim (2009) Effect of cactus extracts on human intestinal microflora. J Agric. Life Sci. Vol. 43. 45~54
[23]. Ji-Yun Park, Mi-Kyung Lee, Myung-Ju Kim and Su-Yel Cho (2003). Effect of dandelion (Taraxacum officinale) extracts on the intestinal microorganisms of streptozotocin-induced diabetic rats. J Korean Soc. Food Sci. Nutr. Vol. 31. 1112~1118
[24]. Heui-Sam Lee, Ho-Jung Jeon, Sang-Duk Lee and Jae-Yu Moo (2001) Effect of dietary mulberry leaf on the composition of intestinal microflora in SD rats. Korean J Food Sci. Technol. Vol. 33. 252~255
[25]. Dong-Myong Kim, Hyung-Kon Lee, Yong-Seong Kwon, Se-ho Kim, Nam-Hong Lee,

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 4 July-Aug 2024, pp: 1025-1038 www.ijprajournal.com ISSN: 2456-4494

In-Suk Han and Yeon-Mea Choi (2022) Effect of Exosomal Nanoencapsulated Alab^{TM} peptide on the IL-8, COX-2, NF-kB, and Angiogenesis in vitro/in vivo Against Human Gastric Cancer for Nutrient Delivery System. J Pharmacy. Vol. 12. No. 11. 33-45

[26]. Dong-Myong Kim, Chae-Yun Yang, Yeo-Jin Lee, Seo-Hyeon Hwang, Hyung-Kon Lee, Yong Seong Kwon and Yeon Mea Choi (2024) Improvement of the Gut Environment of Sandwich Coated Lactic Acid Bacteria with Aloe Polysaccharides. Inter. J Pharm. Res. App. Vol. 9. No. 4. 281~290

[27]. Alessandra Durazzo, Siân Astley, Maria Kapsokefalou, Helena Soares Costa, Angelika Mantur-Vierendeel, Loek Pijls, Luca Bucchini, Marija Glibetic, Karl Presser and Paul Finglas (2022) Food Composition Data and Tools Online and Their Use in Research and Policy: EuroFIR AISBL Contribution in 2022. Nutrients. Vol. 14. 4788~4795

[28]. Dong-Myong Kim, Ju-Yeong Jung, Hyung-Kon Lee, Yong-Seong Kwon, Yeon-Mea Choi, HyoKyu Lee, Youn Hee Nam and Tong Ho Kang (2022) In Vitro and in Vivo Antimicrobial Effects of Aloe Vera Fermented Hericium Erinaceum KU-1 for Food Borne Pathogens and Helicobacter Pylori. Biomed. J Sci. & Tech. Res. Vol. 41. No. 1. 32341~32349

[29]. Dong-Myong Kim, Won-Jin Kim, Hyung-Kon Lee, Yong-Seong Kwon and Yeon-Mea Choi (2023) Skin improvement of the composition containing nano-exosome derived from Aloe vera bark callus as new type of Transdermal Delivery System. Asian J Beauty Cosmetol. Vol. 21. No. 1. 117~130

[30]. Dong-Myong Kim, Ju-Yeong Jung, Hyung-Kon Lee, Yong-Seong Kwon, In Suk Han (2020) Improvements in Cognitive and Motor Function by a Nutrient Delivery System Containing Sialic Acid from Edible Bird's Nest. Korean J Food Nutri. Vol. 33. No. 6. 614~623

[31]. Dong-Myong Kim, Won-Jin Kim, Hyung-Kon Lee, Yong-Seong Kwon and Yeon-Mea Choi (2023) Efficacy in vitro Antioxidation and in vivo Skin Barrier Recovery of Composition Containing Mineral-cation-phyto DNA Extracted from Aloe vera Adventitious Root. Asian J Beauty Cosmetol. Vol. 21. No. 2. 231~246

[32]. Dong-Myong Kim, Hyung-Kon Lee, Yong-Seong Kwon and Yeon-Mea Choi (2023) A novel study on the identification and sleep inducing effect of plant lactic acid bacteria (Lactobacillus helveticus KJMA-0001) isolated from Jeju Aloe vera (Aloe Barbadensis Miller). J Pharmacy. Vol. 13, No. 8. 61~75

[33]. Mitsuoka Takamine (1980) A color Atlas of anaerobic bacteria. Chongmoon Publishing Co., Tokyo, Japan. 69~89

[34]. Kwon YJ, Jang GC, Rah HH, Kim YH, Rhee MS (2005) The study of sugar analysis in licorice extract by HPLC. J Korean Soc. Tobacco. Sci. Vol. 27. 114~119

[35]. Moon YG, Her MS. (2007) Screening of antioxidative and antibacterial activity from methanol extracts of indigenous plants Jeju-island. Korean J Biotechnol. Bileng. Vol. 22. 78~83

[36]. Dewanto V, Xianzhong W, Liu RH (2002) Processed sweet corn has higher antioxidant activity. J Agric. Food Chem. Vol. 50. 4959~4964

[37]. Choi Y, Kim M, Shin JJ, Park JM, Lee J (2003) The antioxidant activities of the some chemical test. J Korean Soc. Food Sci. Nutr. Vol. 32. 723~727

[38]. Blois MS (1958) Antioxidant determinations by the use a stable free radical. Nature. Vol. 181. 1199~1203

[39]. Jin-Hee Lee and Ju-Mi Lee (2015) Jelly-type health food composition containing aloe polysaccharide as an effective ingredient and its manufacturing method. Kor. Patent. 10-2015-0119753

[40]. Dong-Myong Kim, Hyung-Kon Lee and Yong-Seong Kwon (2023) Manufacturing method of sandwich-coated lactic acid bacteria containing aloe polysaccharide and sandwich-coated lactic acid bacteria containing Aloe polysaccharide prepared thereby. Kor. Patent. 10-2589163

[41]. Dong-Myong Kim, Hyung-Kon Lee and Yong-Seong Kwon (2022) Method for producing fermented tindallization aloe lactic acid bacteria powder and immunity enhancing composition containing the same. Kor. Patent. 10-2022-0144005

[42]. Min SH and Lee BR (2007) Antioxidant activity of medicinal plant extracts cultivated in Jecheon. Korean J Food Culture. Vol. 22. 336~341

[43]. Ju Io, Jung GT, Ryu J, Choi JS and Choi YG (2005) Chemical components and physiological activities of bamboo (Phyllostachys bambusoides starf) extracts

prepared with different methods. Korean J Food Sci. Technol. Vol. 37. 542~548

[44]. Kim EY, Baik IH, Kim JH, Kim SR and Rhyu MR (2004) Screening of the antioxidant activity of some medicinal plants. Korean J Food Sci. Technol. Vol. 36. 333~338

[45]. Park YS (2002) Antioxidative activities and contents of polyphenolic compound of medicinal herb extracts. J East Asian Soc. Dietary Life. Vol. 12. 23~31

[46]. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cationdecolorizationassay. Free Radic. Biol. Med. Vol. 26. 1231~1237