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Inhibitory Effect of Corn Silk Extract on Growth of Food-Borne Bacterial Pathogens

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Abstract Various levels of antibacterial activity have been identified for water and ethanol extracts of corn silk, particularly against *Salmonella typhimurium* KCTC 2515. In general, the water extract was more effective than the ethanol extract. The minimum inhibitory concentration (MIC) for the water extract was 7.5 mg/disc for *S. typhimurium* KCTC 2515 and *B. cereus* KCTC 1092, as well as for the ethanol extract against *S. typhimurium* KCTC 2515 and *S. typhimurium* KCTC 1925. However, the MICs for the water extract were lower than those for the ethanol extract against all bacteria tested, except *S. typhimurium* KCTC 1925 and *B. cereus* KCTC 1014. The growth of the tested organisms in the synthesized broth medium was inhibited with the addition of 5-fold levels of MIC. Using sterilized milk as the model food system, we found that the lag phase for these microorganisms was extended up to 3 days at 20°C, but was not affected at 4°C. These results indicate that bacterial growth was strongly inhibited by corn silk extract at 20°C.

Key words: corn silk, corn silk extract, inhibitory effect, food-borne bacterial pathogens

Introduction

Outbreaks of food-borne illness tend to increase every year, in part because of improved reporting systems and in part due to an actual increase in the prevalence of such illnesses (1). These findings have been reported throughout the world (2).

Many researchers have developed techniques for releasing natural antimicrobial substances from various plant sources to control the growth of food-borne pathogens to minimize outbreaks of illness (3). Although the food industry commonly uses synthetic chemical preservatives, consumers tend to avoid them because of their questionable safety (4). The food industry is, therefore, trying to identify natural food preservatives in specific plants to replace artificial preservatives.

Antimicrobial substances isolated from plants are mostly secondary metabolites or their derivatives and include phenolic compounds, quinones, alkaloids, terpenes, and volatile aromatic compounds (5). In the food industry, the use of natural antimicrobials has been limited because their antimicrobial activity is not as strong as that of artificial synthetic preservatives. Further research is needed to determine how to use natural antimicrobial substances efficiently to combat pathogenic microorganisms that are harmful to humans and are responsible for food spoilage.

Corn silk is a by-product of harvested corn. Corn silk is a well known Chinese medicine and is used as a Korean folk remedy (6) to help reduce swellings in the body. It has also been used to treat urethral stones, kidney disease, and diabetes and is used as a diuretic (7). It has been reported that maisin, a substance that has been isolated and purified from corn silk, inhibits the growth of the larvae of insects such as earthworms and the fall armyworm, which are responsible for a tremendous loss of the corn crop and, thus, corn silk (8). Although corn silk is a very useful and plentiful corn byproduct, there are only a few scientific reports on its physicochemical properties and antimicrobial activity (9).

The objectives of this study were to determine the antibacterial activity of water and ethanol extracts of corn silk against bacteria that are known to cause food-borne illnesses and to confirm the antibacterial activity of the extract in synthetic agar media using sterilized cocoa milk as a model food system.

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Materials and Methods

Corn silk preparation Fresh corn silk was harvested from waxy corn, washed with tap water, and dried in an oven at a temperature of 50±1°C for 24 hr. The dried corn silks were sealed with polyethylene film and stored in deep freezer at -20°C until it was used.

Stock cultures The bacteria used in this study were purchased from the Korean Collection for Type Cultures and the American Type Culture Collection. The media used to grow each type of bacteria and the incubation temperature used in this study were as follows: *S. typhimurium* and *Staphylococcus aureus:* a nutrient broth at 37°C; *B. cereus:* a in nutrient broth at 30°C; *Escherichia coli:* a tryptic soy broth at 37°C; *Listeria monocytogenes:* a brain heart infusion broth at 37°C. Culture broth was solidified with the addition of 1.8% agar powder and surface-plated to be able to observe antibacterial activity. Stock cultures were maintained on selective agar slants at 4°C and transferred monthly to maintain their viability. Working cultures were prepared by inoculating a loopful of stock culture into 5 mL of broth and incubating the broth at the optimal temperatures for 18 hr.

Preparation of the water extract A 300 g portion of prepared corn silk was ground in a blender (Model DS-8585, Dongyang Industry, Korea) and then extracted with 7.8 L of distilled water at 50°C for 2 hr; this extraction process was repeated 3 times. This extract was then filtered through cheese cloth and then filtered through a Büchner funnel lined with Whatman No. 2 filter paper under vacuum conditions. The filtrate was dried in a freeze dryer (EYELA, FDU-540, Japan) under vacuum conditions for 24 hr. The freeze-dried sample was stored in capped plastic vials at -20°C until it used.

Preparation of the ethanol extract A 300 g portion of corn silk was ground in a blender and then extracted with 1.0 L of 50% ethanol at room temperature for 24 hr; this extraction process was repeated 3 times. The extract was then filtered through cheese cloth and then through a Büchner funnel lined with Whatman No. 2 filter paper under vacuum conditions. The filtrate was evaporated in a rotary vacuum evaporator (EYELA Type N-N, Japan) equipped with a cooling aspirator (EYELA Cool Ace CA-111, Japan) at 40°C. The ethanol extract was stored at -20°C.

Antibacterial activity The paper disc method (10) was used to determine the antibacterial activity of the corn silk extracts. The plate was prepared with the selected agar media in advance. Inoculum containing 10^4 colony-forming units (cfu)/mL was poured and suspended on the plate. A specific concentration of the extract (35 mg/disc) was loaded onto a sterile paper disc (Ø 8 mm, Whatman) and air-dried. The loaded paper disc was transferred onto the surface of the inoculated agar plate, and then the extract on the paper disc was diffused into the agar plate using a 0.85% sodium chloride solution. The plate was incubated at the optimum temperature for 24 hr. After the incubation was complete, the diameter of clear zone around the disc was measured; this measurement served as a measurement of antibacterial activity. The antibacterial activity of a benzoic acid solution (0.5 g/disc), distilled water, and a 50% ethanol solution was also measured and served as the controls.

Minimum inhibitory concentration (MIC) The MIC of the extract was also determined using a paper disc method. Several concentrations of each extract were loaded onto sterile paper discs and air-dried. The discs were then placed on the surface of the appropriate inoculated agar plate. The extract on each disc was diffused into the agar using a 0.85% sodium chloride solution, and the plates were incubated at the optimum temperature for each type of bacteria for 24 hr. The MIC was determined as the lowest concentration of the extract in which bacterial growth was inhibited by 10%.

Inhibitory effect of water extract in culture media The inhibitory effect of the water extract in the synthetic culture media was determined using a broth microdilution assay (11). One mL of water extract at a concentration that is 5 times MIC was added to 8.9 mL of culture broth. This was followed by addition of 0.1 mL of the inoculum (10^2-10^3 cfu/mL) to bring the total volume up to 10 mL. The control consisted of 1 mL of the distilled water added to a test tube instead of the water extract. The prepared test tubes were incubated at the optimum temperatures for 24 hr. Then 1 mL of the culture solution was placed on the plate every 3 hr and agar medium poured onto it and mixed gently. The plates were then solidified using the method described previously and incubated to measure the inhibitory effect of the water extract on the tested bacteria in culture using the plate-count method.

Inhibitory effect of the water extract in a model food system The inhibitory effect of the water extract in a model food system was determined according to the method described by Davidson (12). Sterilized cocoa milk was selected as a model food system for evaluating the antibacterial activity of the water extract of corn silk. We dissolved 5 g of the freeze-dried powder of the water extract in 10 mL of distilled water and sterilized this solution by filtering it through a 0.45 μm membrane filter. We then transferred 1 mL of the filtrate into a test tube containing 3.9 mL of sterilized cocoa milk and adjusted the final concentration with 100 mg/mL of water extract. The control tube contained 1 mL of distilled water instead of the water extract. The prepared test tubes were inoculated with *S. typhimurium* KCTC 2515, *E. coli* ATCC 43888, *Listeria monocytogenes* KCTC 3569, *S. aureus* KCTC 13566, and *B. cereus* KCTC 1092 to an initial level of 10^2 to 10^3 cfu/mL and stored at 4°C or 20°C for 7 days. Viable cells in milk were enumerated every 24 hr using the pour-plate method. Each experiment was carried out in triplicate.

Statistical analysis The data obtained in this study were analyzed using the analysis of variance technique. Significant differences among means were determined by *t*-test, and significance was set at $p<0.05$.

Results and Discussion

Antibacterial activity of the water extract of corn silk The water extract of corn silk inhibited the growth of 8 bacterial strains that cause food-borne illnesses (Table 1). The growth of *S. typhimurium* KCTC 2515 was most strongly inhibited, as indicated by a clear zone with a diameter of 21.0 mm. The next most strongly inhibited organism was *B. cereus* KCTC 1092, with a clear zone diameter of 17.0 mm, then *S. aureus* KCTC 13566, with a clear zone of approximately 14.0 mm. By comparison, the weakest antibacterial activity was shown against *E. coli* ATCC 43888, which formed a clear zone with a diameter of 10.5 mm. These results suggest that the antibacterial activity of water extract of corn silk is stronger against gram-positive bacteria than against gram-negative bacteria. It also suggests that the water extract of corn silk has almost same degree of antibacterial activity as benzoic acid control, which is a common antimicrobial substance.

Antibacterial activity of the ethanol extract of corn silk The ethanol extract of corn silk also inhibited the growth of 8 bacterial strains (Table 2). The growth of *S. typhimurium* KCTC 2515 was most strongly inhibited, forming the largest clear zone (diameter: 17.0 mm). This was followed by *B. cereus* KCTC 1092 and *S. aureus*

Table 1. Antibacterial activities of water extract from corn silk against the tested bacteria

Tested Bacteria	Inhibitory Zone (diameter, mm)	
	Water extract* (35 mg/disc)	Benzoic acid* (0.5 mg/disc)
S. typhimurium KCTC 1925	17.0±0.4^{bc}	14.5±0.1^{b}
S. typhimurium KCTC 2515	21.0±0.3^{c}	23.0±0.3^{ac}
S. aureus KCTC 13566	14.0±0.2^{a}	25.0±0.2^{ac}
B. cereus KCTC 1014	10.0±0.1^{a}	9.0±0.2^{a}
B. cereus KCTC 1092	17.0±0.2^{bc}	23.0±0.4^{ac}
E. coli ATCC 43888	10.5±0.2^{a}	18.0±0.1^{b}
E. coli ATCC 10536	11.5±0.1^{a}	17.0±0.1^{b}
L. monocytogenes KCTC 3569	12.0±0.2^{a}	13.0±0.2^{ab}

*Mean ± standard deviation of triplicate determinations.
$^{a-c}$Means within the same column with different superscript letters are significantly different ($p<0.05$).

Table 2. Antibacterial activities of ethanol extract from corn silk against the tested bacteria

Tested Bacteria	Inhibitory Zone (diameter, mm)	
	Ethanol extract* (35 mg/disc)	Benzoic acid* (0.5 mg/disc)
S. typhimurium KCTC 1925	16.0±0.3^{b}	14.5±0.2^{b}
S. typhimurium KCTC 2515	17.0±0.1^{b}	23.0±0.2^{c}
S. aureus KCTC 13566	15.0±0.2^{b}	25.0±0.4^{c}
B. cereus KCTC 1014	14.0±0.1^{ab}	9.0±0.2^{a}
B. cereus KCTC 1092	16.0±0.1^{b}	23.0±0.3^{c}
E. coli ATCC 43888	9.0±0.2^{a}	18.0±0.1^{b}
E. coli ATCC 10536	9.0±0.1^{a}	17.0±0.1^{b}
L. monocytogenes KCTC 3569	10.0±0.3^{a}	13.0±0.2^{a}

*Mean±standard deviation of triplicate determinations.
$^{a-c}$Means within the same column with different superscript letters are significantly different ($p<0.05$).

KCTC 13566 (clear zone diameters: 16.0 mm and 15.0 mm, respectively). However, the antibacterial activity of the ethanol extract was comparatively weaker than that of water extract.

MIC The MICs of the water and ethanol extracts of corn silk against the tested bacteria are shown in Table 3. The MICs of the water extract against *S. typhimurium* KCTC 1925 and *B. cereus* KCTC 1014 were higher than those of the ethanol extract, while the MICs of ethanol extract against *S. aureus* ATCC 13566, *B. cereus* KCTC 1092, *E. coli* ATCC 43888, and *L. monocytogenes* KCTC 3569 were higher than those of the water extract. The MICs of the water extract against *S. typhimurium* KCTC 2515 and *E. coli* ATCC 10536 were about same as those of the ethanol extract. The MIC of the water extract against *S. typhimurium* KCTC 2515 and *B. cereus* KCTC 1092 and the MIC of the ethanol extract against *S. typhimurium* KCTC 1925 and *S. typhimurium* KCTC 2515 were both 7.5 mg/disc; this was the lowest among all the tested

Table 3. Minimum inhibitory concentration (MIC) of water and ethanol extracts from corn silk against the tested bacteria

Tested Bacteria	MIC (mg/disc)	
	Water extract*	Ethanol extract*
S. typhimurium KCTC 1925	15.0±0.2^{b}	7.5±0.2^{a}
S. typhimurium KCTC 2515	7.5±0.1^{a}	7.5±0.1^{a}
S. aureus KCTC 13566	17.5±0.2^{b}	20.0±0.1^{c}
B. cereus KCTC 1014	15.0±0.2^{b}	10.0±0.2^{ab}
B. cereus KCTC 1092	7.5±0.2^{a}	12.5±0.3^{b}
E. coli ATCC 43888	25.0±0.3^{c}	27.5±0.3^{c}
E. coli ATCC 10536	20.0±0.1^{c}	20.0±0.4^{c}
L. monocytogenes KCTC 3569	20.0±0.3^{c}	25.0±0.2^{c}

*Mean±standard deviation of triplicate determinations.
$^{a-c}$Means within the same column with different superscript letters are significantly different ($p<0.05$).

bacteria. Thus, it was clear that antibacterial activities of water extract against the tested bacteria were stronger than those of ethanol extract. Oh *et al.* (13) reported that antimicrobial activity tends to increase as the purity of the substance increases. In this experiment, the MICs of the water and ethanol extracts were slightly higher than we expected, because the extracts were not purified completely. However, our results still suggest that the water extract of corn silk may useful for extending the shelf life of foods, because antibacterial activities in water extract were higher than those in ethanol extract.

Inhibitory effect of the water extract in culture In the culture broth, the inhibitory effects of the water extract of corn silk on the growth of 5 bacteria that had relatively low MICs are shown in Fig. 1 to 5. The patterns of inhibition demonstrated in the growth curve were very similar for both extracts, but the lag phases for the tested bacteria were extended by up to 3 to 5 hr, indicating that the water extract had a significant inhibitory effect on bacterial growth ($p<0.05$). The fact that the total number of the cells incubated for 24 hr at each optimum temperature was much lower for organisms exposed to the water extract of corn silk than those in the control media.

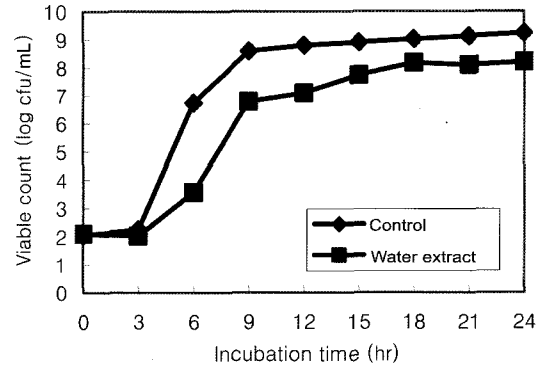

Fig. 1. Inhibition of *B. cereus* KCTC 1092 growth by water extract of corn silk in nutrient broth at 30°C.

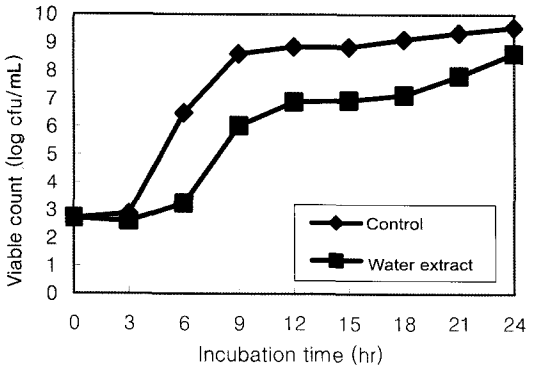

Fig. 2. Inhibition of *S. typhimurium* KCTC 2515 growth by water extract of corn silk in nutrient broth at 37°C.

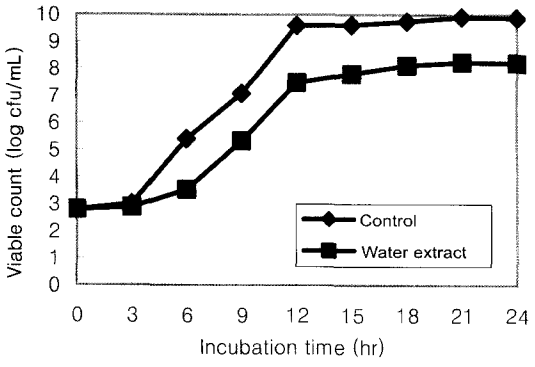

Fig. 3. Inhibition of *S. aureus* KCTC 13566 growth by water extract of corn silk in nutrient broth at 37°C.

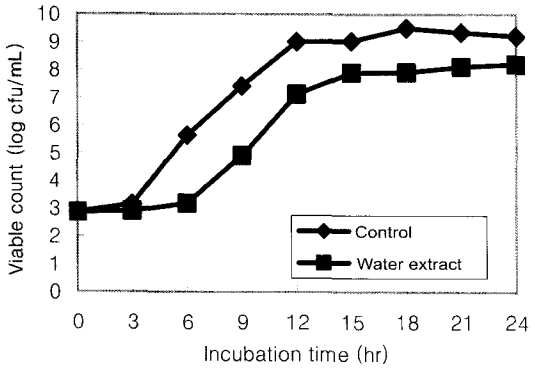

Fig. 4. Inhibition of *L. monocytogenes* KCTC 3569 growth by water extract of corn silk in brain heart infusion broth at 37°C.

Inhibitory effect of the water extract in a model food system At 4°C, there were no differences in antibacterial activity against *S. typhimurium* KCTC 2515, *B. cereus* KCTC 1092, and *S. aureus* ATCC 13566 for the water extract of corn silk compared with controls (Fig. 6, 7 and 10) when the water extract was added to the sterilized cocoa milk and stored for 7 days. The growth of *E. coli* ATCC 43888 and *L. monocytogenes* KCTC 3569 was slightly inhibited, but growth inhibition in *L. monocytogenes* KCTC 3569 was more severe. Considering that *L. monocytogenes* (14) is a heat- and acid-resistant organism and occasionally causes a food-borne illness in milk that has been sterilized at low temperature, our findings suggest that the water extract of corn silk may be useful as

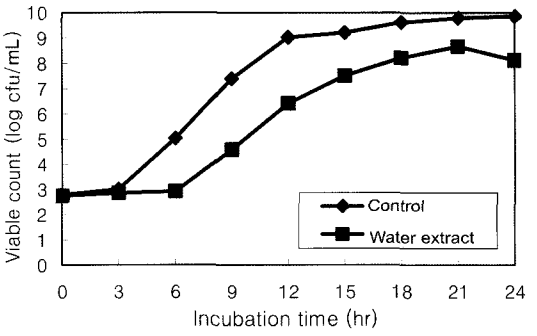

Fig. 5. Inhibition of *E. coli* ATCC 43888 growth by water extract of corn silk in tryptic soy broth at 37°C.

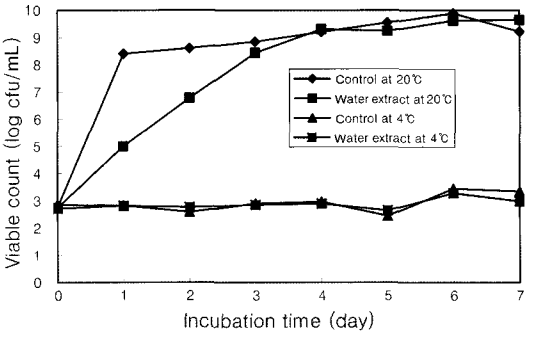

Fig. 6. Inhibition of *B. cereus* KCTC 1092 growth by water extract of corn silk in cocoa milk at 4 and 20°C.

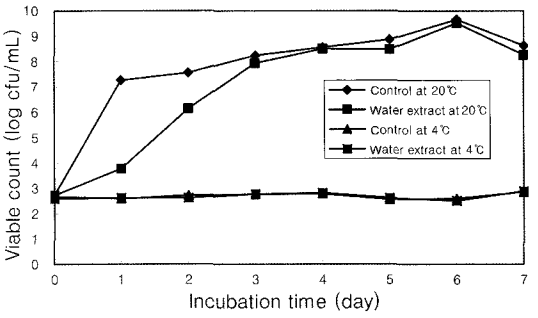

Fig. 7. Inhibition of *S. typhimurium* KCTC 2515 growth of water extract of corn silk in cocoa milk at 4 and 20°C.

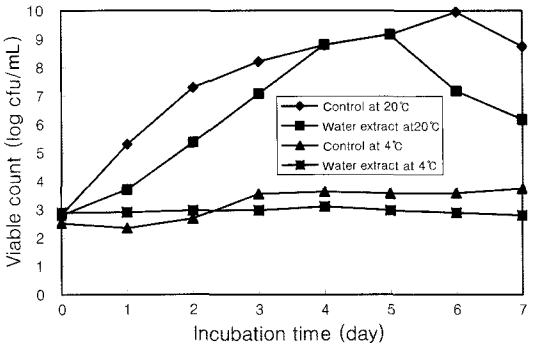

Fig. 8. Inhibition of *Escherichia coli* ATCC 43888 growth by water extract of corn silk in cocoa milk at 4 and 20°C.

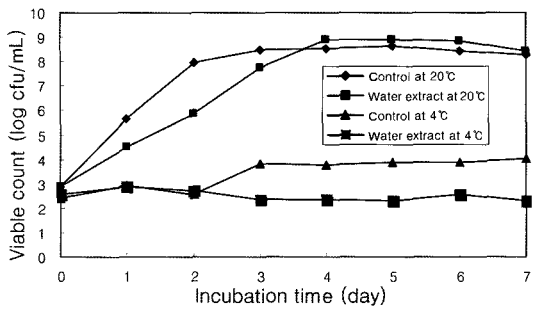

Fig. 9. Inhibition of *L. monocytogenes* KCTC 3569 growth of water extract of corn silk in cocoa milk at 4 and 20°C.

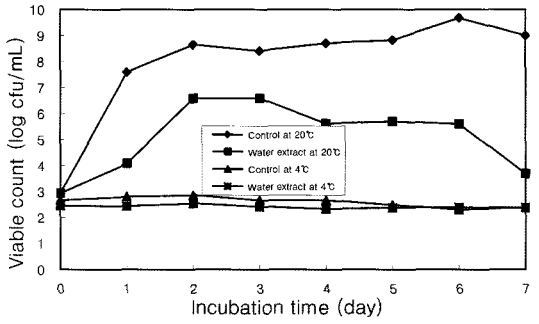

Fig. 10. Inhibition of *S. aureus* KCTC 13566 growth of water extract of corn silk in cocoa milk at 4 and 20°C.

an antibacterial agent to prevent food spoilage caused by *L. monocytogenes* at a 4°C refrigeration temperature.

By comparison, the water extract most strongly inhibited the growth of *S. aureus* ATCC 13566 in the model food system at 20°C for 7 days. This extract also inhibited the growth of *B. cereus* KCTC 1092, *S. typhimurium* KCTC 2515, *E. coli* ATCC 43888, and *L. monocytogenes* KCTC 3569 significantly ($p<0.05$ for each organism) in a model food system during first 4 days when the milk kept at a temperature of 20°C, but thereafter the extract did not have a considerable effect on their growth and their cell counts were similar to those for the controls. Therefore, it is meaningless to examine the antibacterial activity of the water extract after 4 days.

The differences in antimicrobial activity at each optimal temperature are presumably due to the lower solubility of the water extract in food systems at 4°C than at 20°C. Antimicrobial activity generally declines in food systems, because the proteins and lipids contained in foods may be affected as the inhibition of microbial growth decreases (15). Payman and Frank (16) reported that *L. monocytogenes* produces a biofilm on the surface of stainless steel that may protect these microbes against the effects of detergents, antibiotics, and antibodies and, thus, eventually reduce any antimicrobial effects. Allyl isothiocyanate and the root bark extract of *Morus alba* L. (17) destroys the cell wall of *L. monocytogenes* and then inhibits cell growth by destroying all of its ATP. A seed extract of grapefruit (18) also inhibits the growth of *S. typhimurium* by destroying its cell wall.

Corn silk has much potential for the development of antimicrobial substances to control some food-borne pathogenic bacteria. Further studies are needed to enhance the activity of its active ingredients in the food system by screening its water extract for its active component(s) and obtaining a purified form of the compound(s).

Acknowledgments

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