Antimicrobial Effect of Extracts from Chinese Chive, Cinnamon, and Corni Fructus

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Extracts were prepared from Chinese chive (*Allium tuberosum*), cinnamon (*Cinnamomum cassia*), and corni fructus (*Cornus officinalis*) and used to evaluate their antimicrobial activity on common foodborne microorganisms, alone and in combination. The mixed extract, consisting of three extracts in equal volumes, showed an entire antimicrobial spectrum and had excellent stability to heat, pH, and storage. The mixed extract exhibited better inhibition on growth of *Escherichia coli* than potassium sorbate at 2−5 mg/mL. The mixed extract inhibited the growth of *Pichia membranaefaciens* at levels as low as 2 mg/mL. When the mixed extract was used in foods, the expected antimicrobial effect in orange juice, pork, and milk was observed. After gel filtration chromatography, each extract was partially purified into fractions, and one fraction in each extract showed enhanced antimicrobial activity. Overall, the mixed extract was of promising potential for incorporation into various food products for which a natural antimicrobial additive is desired.

Keywords: *Chinese chive; cinnamon; corni fructus; mixed extract; antimicrobial effect; gel filtration*

INTRODUCTION

The use of chemicals to enhance the safety of many foods is of great interest to the food industry. Chemical preservatives vary in their ability to kill microorganisms. Effectiveness depends on the types of microorganisms and the physical and chemical characteristics of foods (*1*); however, the presence of chemical residues in foods and labeling of preservatives on food packages are major concerns to consumers these days. Therefore, the need for naturally derived compounds and other natural products with antimicrobial properties has been explored (*2*).

The stability of some foods against attack by microorganisms is due to the fact that they contain naturally occurring substances with antimicrobial activity. Some spices are known to contain essential oils that possess antimicrobial activity, such as eugenol in cloves, allicin in garlic, and cinnamic aldehyde and eugenol in cinnamon (*3*−*6*). In addition, some vegetables and herbs also contain substances that inhibit microbial growth (*5*, *7*−*9*). Limitations in the use of naturally derived preservatives are due to associated flavors that can alter the taste of food. Understanding how these natural preservatives work and affect the growth of microorganisms can lead to new technologies for their use in maintaining the quality of foods.

Chinese chive (*Allium tuberosum* Rottler) belongs to the same family as garlic, onion, and leek and is an important ingredient in Asian cooking. The pressed juice from Chinese chive is shown to be very effective in inhibiting a wide range of microorganisms (*10*). Cinnamon, the dried bark of *Cinnamomum cassia* Blume, is used to flavor or season various foods and as a therapeutic agent for various diseases. Cinnamon is rich in essential oils and tannins, which inhibit microbial growth (*3*, *5*). Corni fructus, the fruit of *Cornus officinalis* Sieb. et Zucc., has long been used as a tonic in traditional Chinese medicine and contains tannins (*11*). After studying 105 kinds of plants (vegetables and Chinese medicine herbs), Chen (*10*) found that these three edible plants are not only potential antimicrobials but also excellent flavoring ingredients; however, the antimicrobial spectra and properties of Chinese chive, cinnamon, and corni fructus, alone and in combination, are not available.

The goal of this research was to examine a mixed extract with effective antimicrobial activity for use in various food products. Accordingly, a mixed extract was prepared from these three edible plants for evaluation of its antimicrobial effect and spectrum on common foodborne microorganisms, including bacteria, yeasts, and molds. The factors affecting its antimicrobial activity, including heat, pH values, and storage times at refrigeration and room temperatures, were investigated. Furthermore, the effect of various concentrations of the mixed extract on bacteria and yeasts was studied as compared to that of a chemical preservative, potassium sorbate. The possibility for use of the mixed extract in orange juice, pork, and milk was evaluated. Finally, after purification of each extract using gel filtration chromatography, the eluted peaks were examined for antimicrobial activities and molecular weights were determined.

MATERIALS AND METHODS

Edible Plants. Cinnamon and corni fructus were obtained from a traditional Chinese pharmacy at Neipu, Pingtung

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County, Taiwan. Chinese chive was purchased from a local market at Neipu.

Microbial Strains. Eight species of bacteria, *Bacillus subtilis*, *Escherichia coli*, *Flavobacterium* sp., *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus*, and two species of molds, *Aspergillus flavus* and *Aspergillus niger*, were obtained from stock cultures at the Department of Food Science and Technology, National Pingtung University of Science and Technology, Neipu, Taiwan. Three species of yeasts, *Kloeckera apiculata* CCRC 20539, *Pichia membranaefaciens* CCRC 20859, and *Debaryomyces hansenii* CCRC 21945, and two species of molds, *Penicillium italicum* CCRC 30567 and *Aureobasidium pullulans* CCRC 31981, were obtained from the Culture Collection and Research Center, Food Industry Research and Development Institute, Hsinchu City, Taiwan.

Preparation of Extracts. Dry samples were extracted with ethanol, and fresh samples were blended with water according to the method given in Chen (*10*). Extraction of dry samples with ethanol gave rise to the extract with better antimicrobial activities (*10*). Cinnamon or corni fructus (10 g each) was extracted with an equal amount of 95% ethanol (w/v) in a water bath at 50 °C for 1 h. After filtration, the residue was re-extracted with an additional volume of ethanol and filtered. The combined filtrate was then concentrated on a rotary evaporator (Tokyo Rikakikai Co., Tokyo, Japan, Type N-N) at 45 °C to dryness and made up to 10 mL with 0.1% Tween 80 aqueous solution. Fresh Chinese chive (100 g) was blended with an equal amount of deionized water in a Waring blender. After homogenization, the mixture was pressed through cheesecloth, then concentrated, and adjusted to a volume of 100 mL. The extract from cinnamon, Chinese chive, or corni fructus was finally centrifuged at 11200*g* for 20 min to precipitate suspending particles, and the supernatant thus obtained was used as a stock solution for antimicrobial tests.

Preparation of Test Microorganisms. Bacteria were grown on plate count agar (PCA) or in broth (PCB), pH 7.0 at 30 °C, except for *P. aeruginosa* in Luria−Bertani medium and *V. parahaemolyticus* in Bouillon medium. Yeasts and molds were grown on potato dextrose agar (PDA) or in broth (PDB), pH 7.0 at 25 °C. All media were obtained from Difco Laboratories, Detroit, MI. Media were distributed into test tubes (5 mL each) and autoclaved at 121 °C for 15 min. Each microbial culture was activated by transferring a loopful of the slant culture into tubes containing PCB or PDB, incubating at 30 °C for 12 h, and adjusting the optical density to 0.1 at 660 nm with sterile 0.85% physiological saline prior to use in tests (*12*). For molds, the spore suspension was prepared by washing the slant culture with sterile 0.1% Tween 80 aqueous solution and similarly adjusting the optical density to 0.1.

Antimicrobial Test. The antimicrobial tests were carried out according to disk diffusion tests (*13*, *14*). Sterile filter paper (Whatman No. 1, diameter = 6 mm) was impregnated with 10 μL of the individual or mixed extract and placed in the center of the agar plate, on which the test microorganism was uniformly inoculated by transferring inoculum to solidified agar plates and spreading the plates with an L-shaped glass rod. After 20 min, the plate was inverted and incubated at 30 °C for 12, 16, and 24 h, for bacteria, yeasts, and molds, respectively. The diameter of the clear zone shown on plates was measured using calipers and expressed in millimeters as its zone of antimicrobial activity.

For tests of heat effect, 5 mL of each extract from cinnamon, Chinese chive, and corni fructus were mixed, and the mixed extract (1:1:1, v/v/v) was processed at 100 or 121 °C for 15 min before use. For tests of pH effect, the mixed extract was adjusted to pH 4.5, 5.5, or 6.5 with 4.5 N NaOH and stored at 4 °C for 24 h, and then pH values were readjusted to the original value before use as described in Yu (*15*). For tests of storage temperature effect, the mixed extract was stored at either 4 °C (refrigeration temperature) or 25 °C (room temperature). The samples were taken from tubes at weeks 1, 4, 7, and 10 for antimicrobial tests.

Growth of *E. coli* and *P. membranaefaciens*. *E. coli* or *P. membranaefaciens* was transferred from the slant culture to test tubes containing 5 mL of PCB or PDB, respectively, and incubated at 30 °C for 12 h. After dilution, 0.1 mL of the culture was inoculated into 10 mL of PCB containing various concentrations of the mixed extract and incubated at 30 °C. In addition, various concentrations of potassium sorbate were added into PCB or PDB as controls of an antimicrobial agent for *E. coli* or *P. membranaefaciens*, respectively. The samples were taken from the incubated tubes at predetermined periods of time, followed by serial dilution, pour-plating onto PCA or PDA, and incubation at 30 °C for 24−48 h. The viable microbial counts were expressed as colony-forming units (CFU) per milliliter.

Application in Foods. Commercial orange juice at 12 °Brix was adjusted to pH 6.0 with 4.5 N NaOH and mixed with the mixed extract to final concentrations of 0.1 and 0.2% (w/v) with deionized water as the control. After autoclaving at 121 °C for 15 min, the tubes were uncapped and exposed to the atmosphere in a room at 30 °C. Fresh lean pork with minimal fat tissue was selected, ground, and mixed with the mixed extract to final concentrations of 0, 0.1, and 0.2% (w/v); 0.2% (w/v) potassium sorbate was used as the control. The samples were stored at 4 °C. Commercial whole milk was distributed into sterile containers in which various concentrations of the mixed extract (0, 0.1, and 0.2%, w/v) were added. Sample containers were inoculated with 0.1 mL of *E. coli* liquid culture that was incubated for 24 h and stored at 4 °C. The samples from orange juice, pork, and milk were taken every 24 h, followed by serial dilution, pour-plating onto PCA, and incubation at 30 °C for 24−48 h.

Purification Using Gel Filtration Chromatography. Extracts were purified according to the method of Andrews (*16*). Extract from cinnamon, Chinese chive, or corni fructus was loaded into a Pharmacia gel filtration column (1.6 × 90 cm) packed with Sephadex G-50 (40−120 μm, Pharmacia, Washington, DC) and eluted with 0.1 M sodium acetate buffer, pH 5.0, at a flow rate of 0.25 mL/min. The eluate was collected in tubes as fractions using a fraction collector (Pharmacia, FRAC-100), with 3.75 mL per fraction. The absorbance of each fraction was measured at 400 nm in a spectrophotometer. The fractions with high absorbance were combined, concentrated, and used for antimicrobial tests.

Molecular Weight Determination. Commercial markers were used for molecular weight determination of eluted fractions, including carbonic anhydrase (MW 29000, Sigma Chemical Co., St. Louis, MO), cytochrome *c* (MW 12400, Sigma), aprotinin (MW 6500, Sigma), and vitamin B_{12} (MW 1355.4, Sigma). Each compound (7 mg) was dissolved in 0.1 M sodium acetate buffer, loaded onto the Sephadex G-50 column, and eluted as described above. The fractions collected were measured at 280 nm in a spectrophotometer. The molecular weights versus their elution volume of the peaked absorbance were plotted for markers. From the plot, the molecular weight of fractions from corni fructus, cinnamon, and Chinese chive was determined.

Statistical Analysis. The triplicate data were subjected to an analysis of variance for a completely random design using Statistical Analysis System (*17*) programs. Duncan's new multiple-range test was used to compare the difference among means at the level of 0.05.

RESULTS AND DISCUSSION

Antimicrobial Effect of Mixed Extract. Fifteen species of common foodborne microorganisms were used to evaluate the antimicrobial effect of extracts and extract mixtures from Chinese chive, cinnamon, and corni fructus (Table 1). Inhibition zones of 6 and 12 mm are considered as indicators for good and better inhibitory effects, respectively (*10*). Extracts from Chinese chive (CC) showed a broad antimicrobial spectrum (12 of 15 microorganisms, better; 2/15 good). Extract from cinnamon (CI) possessed extensive inhibitory effect on

Table 1. Antimicrobial Effect of Extracts and Extract Mixtures from Three Edible Plants

strain	inhibition zone diameter[a] (mm)						
	CC[b]	CI[b]	CF[b]	M1[b]	M2[b]	M3[b]	M4[b]
Bacillus subtilis	11[b]	16[a]	13[ab]	–[c]	11[b]	11[b]	15[a]
Escherichia coli	15[c]	18[b]	18[b]	–	19[b]	22[a]	19[b]
Flavobacterium sp.	15[bc]	17[ab]	20[a]	–	13[c]	18[ab]	17[ab]
Listeria monocytogenes	10[d]	15[c]	15[c]	–	17[bc]	20[a]	19[ab]
Pseudomonas aeruginosa	12[bc]	–	16[a]	9[c]	15[ab]	15[ab]	14[ab]
Salmonella typhimurium	20[a]	9[d]	17[b]	10[d]	15[bc]	15[bc]	14[c]
Staphylococcus aureus	–	–	18[a]	8[b]	9[b]	–	9[b]
Vibrio parahaemolyticus	14[c]	20[ab]	21[a]	12[c]	12[c]	20[ab]	18[b]
Debaryomyces hansenii	16[bc]	18[ab]	–	14[c]	–	–	21[a]
Kloeckera apiculata	25[b]	28[a]	–	15[d]	20[c]	–	23[b]
Pichia membranaefaciens	18[b]	21[a]	–	16[b]	12[c]	–	10[c]
Aspergillus flavus	21[b]	28[a]	–	10[c]	–	–	10[c]
Aspergillus niger	35[a]	28[b]	–	18[d]	15[d]	–	23[c]
Aureobasidium pullulans	30[a]	21[b]	–	20[b]	12[c]	–	20[b]
Penicillium italicum	25[a]	20[b]	–	15[c]	12[c]	–	12[c]

[a] Means with different superscripts within the same row are significantly different ($p < 0.05$). [b] CC, Chinese chive, *Allium tuberosum* Rottler; CI, cinnamon, *Cinnamomum cassia* Blume; CF, corni fructus, *Cornus officinalis* Sieb. et Zucc.; M1, CC + CI (1:1, v/v); M2, CC + CF (1:1, v/v); M3, CI + CF (1:1, v/v); M4, CC + CI + CF (1:1:1, v/v/v). [c] No inhibition.

growth of test microorganisms (12/15 better, 1/15 good). Extract from corni fructus (CF) showed an inhibitory effect on growth of test bacteria (8/8 bacteria, better), whereas no effect was observed on growth of yeasts and molds.

When two extracts were combined in equal volume, only the extract mixture of Chinese chive and corni fructus (M2, 1:1, v/v) exhibited an extensive spectrum of antimicrobial effect (11/15 better, 2/15 good) (Table 1). When three extracts were mixed, the extract mixture (M4, 1:1:1, v/v/v, referred to as the mixed extract hereafter) exhibited nearly an entire spectrum of antimicrobial effect (14/15 better, 1/15 good). The slightly low antimicrobial effect observed in the extract mixtures from two or three plants might be due to the fact that the antimicrobial components from each extract were diluted upon combination. However, the inhibitory effect of the mixed extract was comparable to that of Chinese chive extract (CC, Table 1) and the pressed juice from Chinese chive in Chen (*10*). For the purpose of seasoning and antimicrobial effects, the mixed extract is of great interest for use as a natural additive in various food products. Therefore, the subsequent experiments were carried out using the mixed extract of Chinese chive, cinnamon, and corni fructus (1:1:1, v/v/v).

Heat Effect. To study its thermal stability, the mixed extract was processed at 100 or 121 °C for 15 min and examined for antimicrobial activity (Table 2). Generally, after heating, the antimicrobial activity of the mixed extract remained stable or was even slightly enhanced. This enhanced effect might be due to the slight concentration of the mixed extract as a result of evaporation. Hsieh (*18*) also found that cinnamon extract was stable after heat treatments up to 100 °C for 20 min, and its antimicrobial effect was not significantly affected. In this study, its antimicrobial activity was not significantly affected by autoclaving. Accordingly, it is anticipated that its thermal stability will allow the mixed extract to be a potential food additive for use in food processing when heat treatment is used.

pH Effect. The pH of processed foods usually ranges from acid to neutral. The mixed extract with the original pH of 3.5 was adjusted to pH 4.5, 5.5, or 6.5 with 4.5 N NaOH and stored at 4 °C for 24 h. The pH values were readjusted to the original value and used for antimicrobial tests (Table 3). The pH values of extracts of corni fructus, cinnamon, and Chinese chive were 3.3, 4.9, and 6.5, respectively. The better inhibitory effects of the mixed extract were 12/15, 12/15, 10/15, and 9/15 microorganisms for original pH, 4.5, 5.5, and 6.5, respectively.

Table 2. Antimicrobial Activity of Mixed Extract after Various Heat Treatments[a]

strain	inhibition zone diameter[b] (mm)		
	no heat treatment	100 °C for 15 min	121 °C for 15 min
Bacillus subtilis	12[b]	15[ab]	16[a]
Escherichia coli	20[a]	22[a]	24[a]
Flavobacterium sp.	18[a]	17[a]	21[a]
Listeria monocytogenes	19[ab]	16[b]	21[a]
Pseudomonas aeruginosa	14[a]	15[a]	13[a]
Salmonella typhimurium	14[a]	14[a]	13[a]
Staphylococcus aureus	10[b]	12[ab]	14[a]
Vibrio parahaemolyticus	19[b]	21[ab]	24[a]
Debaryomyces hansenii	21[a]	21[a]	20[a]
Kloeckera apiculata	23[a]	18[b]	15[b]
Pichia membranaefaciens	10[a]	10[a]	10[a]
Aspergillus flavus	10[a]	10[a]	10[a]
Aspergillus niger	23[a]	22[a]	21[a]
Aureobasidium pullulans	20[b]	25[a]	22[ab]
Penicillium italicum	125[ab]	15[a]	10[b]

[a] Mixed extract: Chinese chive + cinnamon + corni fructus (1:1:1, v/v). [b] Means with different superscripts within the same row are significantly different ($p < 0.05$).

Table 3. Antimicrobial Activities of Mixed Extract at Various pH Values[a]

strain	inhibition zone diameter[b] (mm) at pH			
	original[c]	4.5	5.5	6.5
Bacillus subtilis	16[a]	13[ab]	11[b]	11[b]
Escherichia coli	18[a]	17[ab]	14[b]	16[ab]
Flavobacterium sp.	15[a]	15[a]	16[a]	14[a]
Listeria monocytogenes	20[a]	17[b]	13[c]	14[c]
Pseudomonas aeruginosa	15[a]	13[a]	13[a]	14[a]
Salmonella typhimurium	16[a]	14[a]	14[a]	10[b]
Staphylococcus aureus	10[a]	9[a]	10[a]	11[a]
Vibrio parahaemolyticus	18[a]	16[a]	15[a]	15[a]
Debaryomyces hansenii	21[a]	20[a]	21[a]	19[a]
Kloeckera apiculata	24[a]	20[b]	22[ab]	23[a]
Pichia membranaefaciens	10[a]	9[a]	9[a]	8[a]
Aspergillus flavus	10[a]	10[a]	10[a]	9[a]
Aspergillus niger	25[a]	22[ab]	20[bc]	17[c]
Aureobasidium pullulans	21[a]	16[b]	20[a]	20[a]
Penicillium italicum	12[a]	11[a]	10[a]	10[a]

[a] Mixed extract: Chinese chive + cinnamon + corni fructus (1:1:1, v/v). [b] Means with different superscripts within the same row are significantly different ($p < 0.05$). [c] pH 3.5.

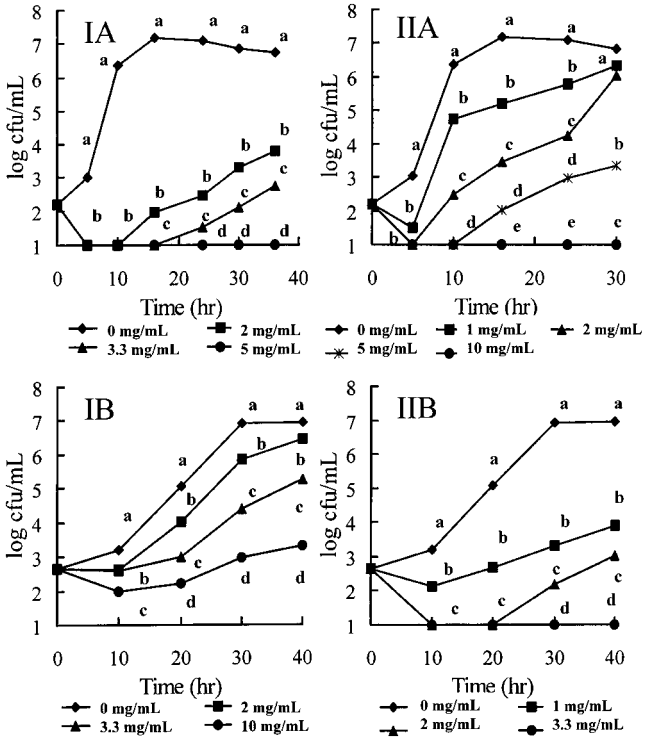

Figure 1. Effect of various concentrations of mixed extract (I) or potassium sorbate (II) on growth of *E. coli* (A) or *P. membranaefaciens* (B). Values with different letters within a time are significantly different ($p < 0.05$). Mixed extract: Chinese chives + cinnamon + corni fructus (1:1:1, v/v).

Therefore, the inhibitory effect of the mixed extract was better at more acidic pH values but slightly lowered at increased pH values. This phenomenon is consistent with the findings of Hsieh and Den (*19*) and Hsieh (*18*). Although the mixed extract showed better inhibitory effect for 9/15 microorganisms at pH 6.5, it remained sufficient to inhibit microbial growth (9/15 better, 6/15 good). Accordingly, the thermal and pH stability of the mixed extract revealed that it was particularly applicable to processed food products.

Effect on *E. coli* and *P. membranaefaciens*. To study its antimicrobial efficiency, various concentrations of the mixed extract were added to cultures of *E. coli* or *P. membranaefaciens* and compared with that of the commonly used preservative potassium sorbate (Figure 1). The mixed extract inhibited the growth of *E. coli*, and the inhibition was much more effective with increased concentrations (Figure 1-IA). At 2 mg/mL, the mixed extract inhibited the growth of *E. coli* more effectively than potassium sorbate did (Figure 1-IIA). At 5 mg/mL, the mixed extract inhibited the growth of *E. coli* completely, whereas a similar result was observed with potassium sorbate at 10 mg/mL; however, the inhibition was achieved at an early incubation time. Starting from 20 h on, growth of *E. coli* was observed at a much lower rate. This pattern was also observed with the addition of cinnamon extract (*18*). This might be due to the fact that the effective antimicrobial components in the mixed extract were depleted.

For *P. membranaefaciens*, the mixed extract affected the microbial growth at a concentration as low as 2 mg/mL (Figure 1-IB). However, at 10 mg/mL, the mixed extract could not repress the growth. Conversely, potassium sorbate was more effective in inhibiting the growth of *P. membranaefaciens*. Because corni fructus could not inhibit the growth of yeasts (Table 1), the inhibition might be effected by Chinese chive and cinnamon. The mixed extract and potassium sorbate affected their antimicrobial activity by delaying microbial growth (Figure 1). To avoid using synthetic preservatives in foods, the mixed extract might be an alternative of chemical reagents.

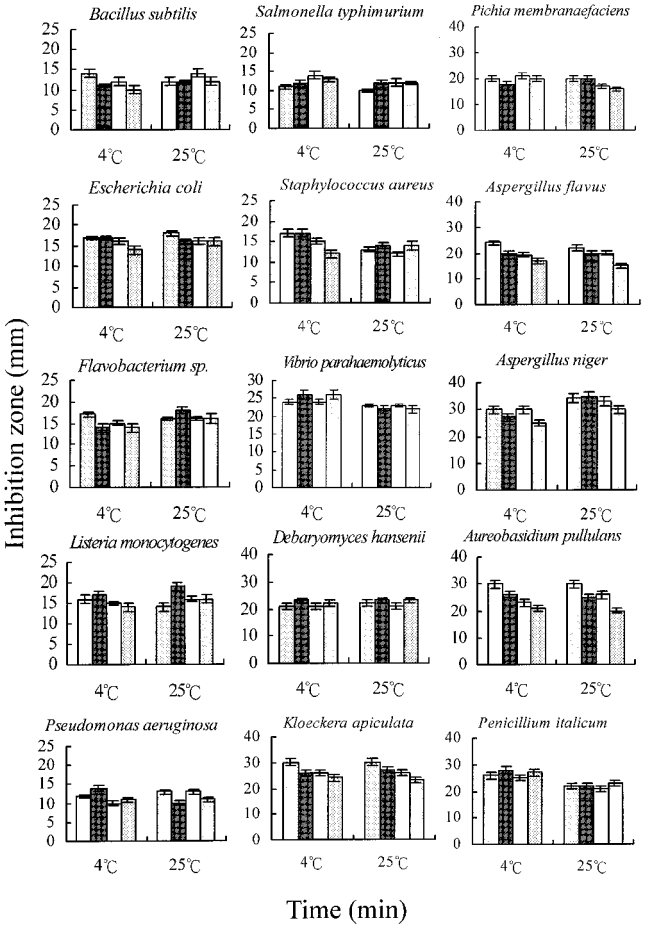

Figure 2. Effect of storage temperature on antimicrobial activities of mixed extract. Four bars from left to right represent the storage times of 1, 4, 7, and 10 weeks, respectively. Mixed extract: Chinese chives + cinnamon + corni fructus (1:1:1, v/v).

Storage Temperature Effect. To study its storage stability, the mixed extract was stored at either 4 or 25 °C for 10 weeks (Figure 2). Overall, the antimicrobial activity of the mixed extract was moderately stable during storage at 4 or 25 °C. Although the inhibitory activity was slightly reduced at prolonged storage time, its activity remained sufficient to inhibit the growth of test microorganisms. Therefore, this result will allow the mixed extract to be massively produced and stored at room temperature for as long as 10 weeks.

Application in Foods. To study its application in foods, the mixed extract was added to orange juice, pork, and milk to examine its antimicrobial activity (Figure 3). The mixed extract showed significant antimicrobial effect in orange juice at 0.1% (w/v). No microbial growth was observed until 24 h (Figure 3A). At 48 h, the orange juice without the mixed extract spoiled, whereas at the same time the orange juice with 0.1% mixed extract showed apparent microbial growth; however, with 0.2% mixed extract added, the orange juice stored at 30 °C for 4 days exhibited extremely low microbial growth (~10 CFU/mL). It is obvious that the mixed extract can be added into orange juice to inhibit microbial growth.

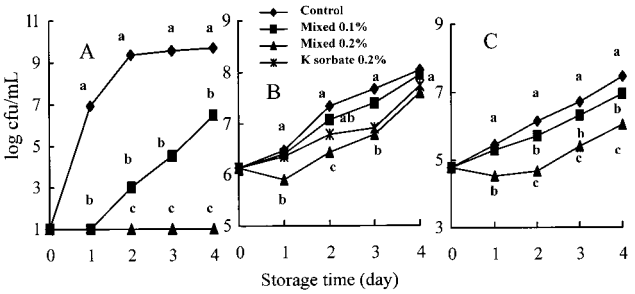

Figure 3. Effects of mixed extract on total plate counts of orange juice (A) and pork (B) and the growth of *E. coli* in milk (C). Values with different letters within a time are significantly different ($p < 0.05$). Mixed extract: Chinese chives + cinnamon + corni fructus (1:1:1, v/v).

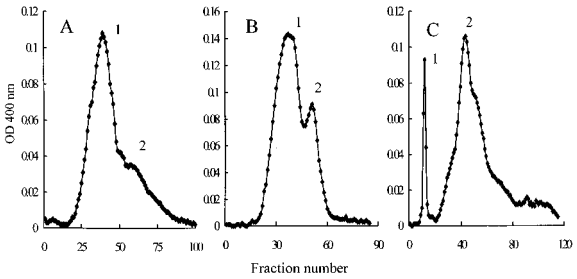

Figure 4. Gel filtration chromatograms of cinnamon (A), Chinese chive (B), and corni fructus (C) using a Sephadex G-50 column.

Chen (*20*) mentioned that during the slaughtering and cutting process, the surface of meat was always contaminated with microorganisms. The total plate counts were found to be 3.1×10^8, 2.9×10^9, 2.9×10^9, and 5.2×10^6 CFU/g of meat for raw pork obtained from Pingtung, Kaohsiung, Chiayi, and Taipei, Taiwan, respectively (*20*). The low initial plate counts of 1.4×10^6 CFU/g shown in Figure 3B indicated that the fresh pork in this study was less contaminated than that in Chen (*20*). During storage at 4 °C, the total plate counts increased with prolonged storage time, whereas the increase rate was higher for the control. Noticeably, the inhibitory effect of 0.2% (w/v) mixed extract was more effective than that of 0.2% (w/v) potassium sorbate, with the decrease in total plate counts being observed at 24 h.

The growth inhibition of *E. coli* in milk by 0.2% mixed extract added was more significant than that by 0.2% potassium sorbate (Figure 3C). With the mixed extract added, this inhibition pattern in milk was similar to that in pork but less than that in orange juice. This discrepancy might be due to the acidic condition in orange juice, which enhanced the antimicrobial effect of the mixed extract.

Purification Using Gel Filtration Chromatography. Extracts from cinnamon, Chinese chive, and corni fructus were purified using Sephadex G-50 gel filtration chromatography (Figure 4). Chromatograms from three edible plants each consisted of two peaks: fractions 23−46 (63%, w/w) and 47−60 (37%) for Chinese chive, fractions 25−48 (51%) and 48−70 (49%) for cinnamon, and fractions 10−14 (19%) and 35−55 (81%) for corni fructus. The peak fractions of each plant were combined, concentrated, and used for antimicrobial tests (Table 4). The first peak in Chinese chive extract showed enhanced inhibitory effect on bacteria (8/8 bacteria, better), especially with the inhibitory effect on *S. aureus* doubled, and reduced inhibitory effect on yeasts and molds (1/7 better, 6/7 good) as compared to the Chinese chive extract. The second peak in the Chinese chive extract also exhibited inhibitory effect on bacteria (5/8 bacteria, better; 2/8 good) and slightly less inhibitory effect on yeasts and molds (2/7 better, 5/7 good). Both peaks showed a better inhibitory effect on bacteria less effective than the first peak. These results indicated that the antimicrobial substances in Chinese chive extract might be two different substances; however, the first peak contained more substances (63%) than the second peak (37%), as evidenced by the higher absorbance.

The first peak in the cinnamon extract exhibited strong enhanced inhibitory effect on bacteria (8/8 better), whereas the inhibitory effect on yeasts and molds (4/7 better, 3/7 good) was comparable to that of cinnamon extract (Table 4). The second peak in the cinnamon extract showed low inhibitory effect (1/15 better, 7/15 good) and its antimicrobial activity was much less than that of cinnamon extract (5/15 better, 7/15 good). It is indicated that the antimicrobial substances were primarily concentrated in the first peak.

The first peak in the corni fructus extract showed good inhibitory effect only on *B. subtilis* and *S. aureus* (Table 4). The second peak in the corni fructus extract exhibited increased inhibitory effect on bacteria (8/8 better);

Table 4. Antimicrobial Activities of Individual Extracts after Sephadex G-50 Gel Filtration

	inhibition zone diameter[a] (mm)								
	Chinese chive			cinnamon			corni fructus		
strain	extract[b]	peak 1	peak 2	extract[b]	peak 1	peak 2	extract[b]	peak 1	peak 2
Bacillus subtilis	8[c]	15[a]	11[b]	10[b]	15[a]	–[c]	14[b]	9[c]	18[a]
Escherichia coli	9[b]	17[a]	10[b]	9[b]	18[a]	–	12[b]	–	23[a]
Flavobacterium sp.	9[c]	18[a]	12[b]	13[a]	15[a]	9[b]	14[b]	–	28[a]
Listeria monocytogenes	–	25	–	7[b]	18[a]	8[b]	14[b]	–	23[a]
Pseudomonas aeruginosa	11[b]	22[a]	23[a]	14[b]	22[a]	16[b]	16[b]	–	25[a]
Salmonella typhimurium	16[b]	22[a]	23[a]	–	18[a]	8[b]	13[b]	–	25[a]
Staphylococcus aureus	13[b]	35[a]	35[a]	10[b]	28[a]	10[b]	20[b]	12[c]	30[a]
Vibrio parahaemolyticus	11[b]	26[a]	27[a]	–	25	–	17[b]	–	24[a]
Debaryomyces hansenii	16[a]	9[b]	11[b]	–	11	–	–	–	–
Kloeckera apiculata	29[a]	10[b]	10[b]	14[a]	12[a]	8[b]	–	–	–
Pichia membranaefaciens	16[a]	9[b]	11[b]	11[b]	13[a]	–	–	–	–
Aspergillus flavus	–	8[b]	10[a]	10[a]	11[a]	–	–	–	–
Aspergillus niger	26[a]	12[c]	15[b]	12[a]	10[b]	–	–	–	–
Aureobasidium pullulans	22[a]	10[b]	10[b]	12[ab]	14[a]	11[b]	–	–	–
Penicillium italicum	9[b]	10[ab]	12[a]	10[b]	15[a]	9[b]	–	–	–

[a] Means with different superscripts within the same row for a specific plant are significantly different ($p < 0.05$). [b] Before Sephadex G-50 gel filtration chromatography. [c] No inhibition.

however, like the corni fructus extract, no inhibitory effect on yeasts and molds was observed with the second peak. Obviously, after gel filtration, the antimicrobial substances were mainly concentrated in the second peak.

Using molecular weight markers, including carbonic anhydrase, cytochrome *c*, aprotinin, and vitamin B_{12}, a linear curve was established for molecular weight determination of eluted fractions. From the plot, the molecular weights of the first peak in the cinnamon extract, the first peak in the Chinese chive extract, and the second peak in the corni fructus extract were determined to be 4100, 5600, and 1100 Da, respectively. However, the purification of these fractions and identification of antimicrobial components need to be investigated further.

In brief, when extracts from cinnamon, Chinese chive, and corni fructus were combined in equal volume, the mixed extract were found to possess an entire antimicrobial spectrum and to show excellent heat treatment, pH, and storage stabilities. The mixed extract exhibited better inhibition on growth of *E. coli* than potassium sorbate at 2−5 mg/mL. For *P. membranaefaciens*, the mixed extract inhibited its growth at levels as low as 2 mg/mL. When the mixed extract was used in foods, the expected antimicrobial effect in orange juice, pork, and milk was observed. After gel filtration chromatography, each extract was partially purified into fractions, which showed enhanced antimicrobial activity. On the basis of the results above, the mixed extract was of promising potential for incorporation into various food products for which a natural antimicrobial additive is desired. However, further studies are needed to examine how the mixed extract exhibits its antimicrobial activity in practical food systems and to identify the compounds and their structures in each purified fraction. In addition, sensory evaluation studies on the food with the mixed extract added are areas of investigation.

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