Research Note

Antibacterial Activity of Xanthorrhizol Isolated from Curcuma xanthorrhiza Roxb. against Foodborne Pathogens

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ABSTRACT

Xanthorrhizol, isolated from the ethanol extract of Curcuma xanthorrhiza Roxb., is a sesquiterpene compound with a molecular weight of 218. The aim of this study was to investigate the antibacterial activity of xanthorrhizol against foodborne pathogens. The antibacterial activity of xanthorrhizol was measured in terms of the MIC and the MBC. MICs and MBCs of xanthorrhizol against Bacillus cereus, Clostridium perfringens, Listeria monocytogenes, Staphylococcus aureus, Salmonella Typhimurium, and Vibrio parahaemolyticus were 8, 16, 8, 8, 16, 8 μg/ml and 16, 32, 16, 16, 16 μg/ml, respectively. The bactericidal study, as determined by the viable cell count method, revealed that xanthorrhizol treatment at 4 × MIC reduced viable cells by at least 6 to 8 log for all six foodborne pathogens in 4 h. Xanthorrhizol maintained its antibacterial activity after thermal treatments (121°C, 15 min) under various pH ranges (pH 3.0, 7.0, and 11.0). These results strongly suggest that xanthorrhizol, conferring strong antibacterial activity with thermal and pH stability, can be effectively used as a natural preservative to prevent the growth of foodborne pathogens.

The number of reported foodborne illnesses caused by a variety of foodborne pathogenic bacteria has increased throughout the world. When new food products are introduced in the market, they require a longer shelf life and a higher degree of protection against pathogenic microorganisms (19, 20). There have been many attempts to reduce the incidence of food poisoning and spoilage with antibacterial synthetic chemicals (28). Unfortunately, the use of synthetic chemical preservatives with various levels of killing effectiveness against foodborne pathogens has been controversial due to concerns over the presence of synthetic chemical residues in foods and labeling of preservatives on food packages (2). Thus, the search for natural antimicrobial agents, mainly originating from plants, animals, and microorganisms, is of primary importance, since food products free of synthetic chemicals or synthetic preservatives have been increasingly demanded (9, 18).

The stability of some foods against attack by foodborne pathogens can be attributed to the fact that they contain naturally occurring components with antimicrobial activity. Spices and herbs are widely used in foods primarily because they impart desirable flavors and aromas. Moreover, they also exhibit antimicrobial activity and help preserve the foods (9). For example, thymol (in thyme and oregano), cinnamic aldehyde (in cinnamon), eugenol (in cloves), and vanillin (in vanilla beans) are well known as natural main antimicrobial components (7, 13, 15).

Curcuma xanthorrhiza Roxb., an edible medicinal plant known as temulawak or Javanese turmeric in Indonesia, has been traditionally used for culinary and folk medicinal purposes. It possesses a variety of biological activities including anti-inflammatory, anticarcinogenic, wound-healing, and serum cholesterol-lowering effects (1, 8, 24). Xanthorrhizol (Fig. 1), a sesquiterpene compound isolated from the rhizome of C. xanthorrhiza Roxb., has been reported to confer strong antimicrobial and antibiofilm activities against oral pathogens (11, 12, 25, 26). However, its efficacy in preventing foodborne bacteria has not as yet been reported. The present research was undertaken to investigate the antibacterial activity of xanthorrhizol against some foodborne pathogens.

MATERIALS AND METHODS

Plant materials. Dried rhizomes of C. xanthorrhiza Roxb. were collected in Semarang, West Java, Indonesia, and identified by Dr. Nam-In Baek, Department of Oriental Medicinal Materials and Processing, Kyunghee University (Yongin, Korea). A voucher specimen has been deposited in the Department of Biotechnology, Yonsei University (Seoul, Korea).

Isolation of xanthorrhizol. Xanthorrhizol was isolated from the rhizome of C. xanthorrhiza Roxb. as described previously (11) with slight modification. Briefly, the dried rhizomes of C. xanthorrhiza (100 g) were ground and extracted with 100% ethyl alcohol (vol/vol; 400 ml), and further fractionations were carried out consecutively with ethyl acetate, n-butanol, and water. The ethyl acetate fraction was applied to a silica gel column chromatography (70-230 mesh; Merck & Co., Whitehouse Station, N.J.) by eluting with an n-hexane–ethyl acetate solution (10:1, vol/vol),...
and xanthorrhizol (2.5 g) was finally obtained as a single compound.

**Bacterial strains.** A total of 10 foodborne bacteria were used: *Bacillus cereus* ATCC 21772, *Campylobacter jejuni* ATCC 49943, *Clostridium perfringens* ATCC 3624, *Escherichia coli* O157:H7 ATCC 43894, *Listeria monocytogenes* ATCC 15313, *Shigella sonnei* ATCC 11060, *Vibrio parahaemolyticus* ATCC 17802, and *Yersinia enterocolitica* ATCC 23715 were purchased from the American Type Culture Collection (ATCC; Manassas, Va.); and *Salmonella* Typhimurium KCCM 11862 and *Staphylococcus aureus* KCCM 11764 were purchased from Korean Culture Center of Microorganisms (KCCM; Seoul, Korea). Mueller-Hinton broth (MHB; Difco, Becton Dickinson, Sparks, Md.) was used for the aerobic culture of *B. cereus*, *E. coli* O157:H7, *L. monocytogenes*, *Salmonella Typhimurium*, *S. sonnei*, *S. aureus*, *V. parahaemolyticus*, and *Y. enterocolitica* at 37°C for 24 h. *C. perfringens* and *C. jejuni* and *C. perfringens* were cultured anaerobically in supplemented brucella broth (SBB; Difco, Becton Dickinson) with 5% lysed horse blood, 5 μg of hemin per ml and 1 μg of vitamin K<sub>1</sub> solution per ml (Sigma-Aldrich Co., St. Louis, Mo.) at 37°C for 48 h in an anaerobic jar with anaero-pack (Mitsubishi Gas Chemical Co., Inc, Tokyo, Japan).

**Susceptibility testing.** The MICs and the MBCs were determined using the Clinical and Laboratory Standards Institute (formerly NCCLS)-recommended broth microdilution assay (4). The experiments were performed in duplicate. Colonies were suspended in MHB and SBB to a density equal to a 0.5 McFarland standard (5). The final concentration of bacteria in each broth microdilution well was approximately 5 x 10<sup>8</sup> CFU/ml of MHB and 10<sup>6</sup> CFU/ml of SBB. Twofold serial dilutions of xanthorrhizol were used to determine MICs and MBCs. MIC was defined as the lowest concentration of xanthorrhizol that resulted in no visible growth compared with the control after 24 h of incubation at 37°C.

**Time-kill assays.** The bacteria used for time-kill assays were *B. cereus*, *L. monocytogenes*, *S. aureus*, *Salmonella Typhimurium*, *V. parahaemolyticus*, and *C. perfringens*, which were significantly susceptible to xanthorrhizol. Aliquots of 10 ml of MHB and SBB and an initial inoculum of 10<sup>8</sup> CFU of each strain per ml were prepared for the time-kill assay. Concentrations of antimicrobial agents used were 0.25 x MIC, 0.5 x MIC, 1 x MIC, 2 x MIC, and 4 x MIC for each strain. Bacterial growth was measured at 0, 4, 8, 12, and 24 h after incubation. A 0.1-ml sample was collected from each culture tube and serially diluted with sterile water. Aliquots of 50 μl were spread on MH agar plates for *B. cereus*, *L. monocytogenes*, *S. aureus*, *Salmonella Typhimurium*, and *V. parahaemolyticus* or on SB agar plates for *C. perfringens* and then were incubated at 37°C for 24 h to determine the number of viable cells (in CFU per milliliter). Bactericidal activity was defined as a ≥3-log reduction in the bacterial population after 24 h of incubation (3). All time-kill experiments were conducted in duplicate.

**pH and thermal stability.** Xanthorrhizol (10%) in dimethyl sulfoxide (Sigma-Aldrich Co.) was tested for its stability at various pHs under the thermal condition (121°C, 15 min). Xanthorrhizol was adjusted at pH 3.0, 7.0, and 11.0 using 0.1% HCl and 0.1% NaOH. After autoclave treatment, the pH was neutralized to 7.0. To determine the antibacterial activity of xanthorrhizol, the susceptibility test was performed by using *L. monocytogenes* and *V. parahaemolyticus* as representatives for gram-positive and gram-negative bacteria, respectively. A twofold serial dilution method was used to determine MIC and MBC (4, 5).

**Statistics.** Each experiment was performed at least in duplicate. All data are presented as the mean ± standard deviation. The data analysis was performed using one-way analysis of variance. The difference between treated and control groups was also analyzed by the Duncan test (SPSS 12.0). P values of <0.01 were considered statistically significant.

## RESULTS

**MIC and MBC assays.** Table 1 shows that both gram-positive (*B. cereus*, *C. perfringens*, *L. monocytogenes*, and *S. aureus*) and gram-negative (*Salmonella Typhimurium* and *V. parahaemolyticus*) bacteria are susceptible to xanthorrhizol. Xanthorrhizol strongly inhibited the growth of *B. cereus*, *C. perfringens*, *L. monocytogenes*, *S. aureus*, *Salmonella Typhimurium*, and *V. parahaemolyticus* with MICs of 8, 16, 8, 16, 8, and 8 μg/ml, respectively. These strains were killed by xanthorrhizol at MBCs of 16, 32, 16, 16, 16, and 16 μg/ml, respectively. In contrast, no susceptible activity of xanthorrhizol was observed against *C. jejuni*, *E. coli* O157:H7, *S. sonnei*, and *Y. enterocolitica*.

### TABLE 1. MICs and MBCs of xanthorrhizol against foodborne pathogens

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>MIC (μg/ml)</th>
<th>MBC (μg/ml)</th>
</tr>
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<tbody>
<tr>
<td><em>Bacillus cereus</em></td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O157:H7</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em></td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td><em>Shigella sonnei</em></td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td><em>Vibrio parahaemolyticus</em></td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

**Time-kill assays.** Time-kill curves were assessed using *B. cereus*, *C. perfringens*, *L. monocytogenes*, *Salmonella Typhimurium*, *S. aureus*, and *V. parahaemolyticus* in the presence of xanthorrhizol at its sub- and super-MICs (0.25 x MIC, 0.5 x MIC, 1 x MIC, 2 x MIC, and 4 x MIC), as shown in Figure 2. From an initial number of 5.0 to 6.0 log CFU/ml, each bacterial strain grew well, reaching 9.0 log CFU/ml, with no visible growth compared with the control after 24 h of incubation.
(2A) Viable count (log₁₀ CFU/ml) vs. Time (h)
(2B) Viable count (log₁₀ CFU/ml) vs. Time (h)
(2C) Viable count (log₁₀ CFU/ml) vs. Time (h)
(2D) Viable count (log₁₀ CFU/ml) vs. Time (h)
(2E) Viable count (log₁₀ CFU/ml) vs. Time (h)
(2F) Viable count (log₁₀ CFU/ml) vs. Time (h)
to 10.0 log CFU/ml in 24 h. At sub-MICs, xanthorrhizol did not inhibit the growth of all foodborne bacteria. At MIC points, xanthorrhizol showed bacteriostatic effects during the growth of *C. perfringens*, *L. monocytogenes*, *S. aureus*, and *V. parahaemolyticus* for 24 h. In contrast, viable cells of *B. cereus* were completely absent after 8 h at the MIC (8 µg/ml), while those of *Salmonella Typhimurium* were killed within 4 h at the MIC (16 µg/ml). The dose-killing activity of xanthorrhizol against other foodborne bacteria was reached after treatment with 2 × MIC of xanthorrhizol in 4 h for *C. perfringens* and *L. monocytogenes,* 8 h for *S. aureus,* and 12 h for *V. parahaemolyticus* with a 5.0- to 6.0-log reduction in counts. Overall, at its super-MICs, xanthorrhizol showed significant bactericidal effect in dose- and growth time-dependent manners.

**pH and thermal stability.** Xanthorrhizol was tested at pH 3.0, 7.0, and 11.0 in combination with high temperature (121°C, 15 min) for measuring pH and thermal stability. Table 2 shows that MICs and MBCs of xanthorrhizol against *L. monocytogenes* and *V. parahaemolyticus* were not changed after autoclaving at all pH ranges. The results indicate that xanthorrhizol can maintain its antibacterial activity under various food-processing conditions.

**DISCUSSION**

One method for inhibiting foodborne pathogenic bacteria to improve food safety is to use antimicrobial food preservatives. Recently, interest in natural antimicrobial compounds with broader spectra has been increasing. Xanthorrhizol was effective against all gram-positive bacteria (*B. cereus*, *C. perfringens*, *L. monocytogenes*, and *S. aureus*) tested at low concentrations (MICs of 8 to 16 µg/ml). In contrast, among six gram-negative foodborne bacteria tested, only *Salmonella Typhimurium* and *V. parahaemolyticus* were found to be sensitive to xanthorrhizol, while *C. jejuni*, *E. coli O157:H7*, *S. sonnei*, and *Y. enterocolitica* were not affected by xanthorrhizol. It was previously reported that gram-positive bacteria were more sensitive to plant essential oils than the gram-negative bacteria due to the bacterial membrane structure (23, 27).

Studies of the antimicrobial activities of key constituents from plant origin could provide information on their potential application as natural preservatives against foodborne pathogens. The MICs of carvacrol, thymol, cinnamic acid, and other phenolic compounds from herbs and spices against some foodborne bacteria in vitro have been reported to be ~1 mM (2). Carvacrol at its MICs (0.5 to 1.0 mM) accomplished growth inhibition of some foodborne bacteria such as *B. cereus*, *Bacillus circulans*, and *E. coli O157:H7* in apple juice (16). Monocaprylin at 5 mM was effective for killing *E. coli O157:H7* in apple juice (22). Another compound, vanillin, at 40 mM completely inactivated *L. monocytogenes* and *E. coli O157:H7* over a broad pH range in an apple juice medium model (21).

Previous studies have shown that phenolic compounds may act on microbial cell walls or membranes. They inhibit microbial growth by changing microbial cell permeability, which leads to the loss of intracellular molecules such as protein, DNA, RNA, and ATP (6). Phenolic compounds could also affect cellular wall, membrane integrity, and microbial physiological responses (14). Carvacrol and thymol, the principal components of oregano and thyme oils, killed bacteria pathogens mostly by damaging their cytoplasmic membrane integrity (17). Cinnamic aldehyde, a major component of cinnamon oil, showed antimicrobial mechanism by disrupting bacterial cell membrane or inhibiting glucose uptake (10). However, the mode of antimicrobial action of xanthorrhizol has not been elucidated.

Since xanthorrhizol imparts strong antibacterial activity to a broad spectrum of gram-positive and gram-negative foodborne bacteria after heat and various pH treatments, it can be used as an effective natural antibacterial agent. Further studies are also necessary to investigate the molecular mechanisms of antibacterial activity brought about by xanthorrhizol.

**ACKNOWLEDGMENT**

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**REFERENCES**

3. Clinical and Laboratory Standards Institute. 2003. Reference meth-

**TABLE 2. MICs and MBCs of xanthorrhizol treated at 121°C for 15 min under various pH conditions**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>pH 3.0</th>
<th>pH 7.0</th>
<th>pH 11.0</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>32</td>
<td>32</td>
<td>16</td>
</tr>
<tr>
<td><em>Vibrio parahaemolyticus</em></td>
<td>32</td>
<td>32</td>
<td>16</td>
</tr>
</tbody>
</table>

* Values are in micrograms per milliliter.

**FIGURE 2.** Time-kill curves of xanthorrhizol against foodborne pathogens at 0 × MIC or control (filled squares), 0.25 × MIC (filled triangles), 0.5 × MIC (filled circle), 1 × MIC (open squares), 2 × MIC (open triangles), and 4 × MIC (open circle) after endpoint (24 h). Values (in parentheses) for 0 ×, 0.25 ×, 0.5 ×, 1 ×, 2 ×, and 4 × MIC, respectively, by species are (A) Bacillus cereus ATCC 271772 (0, 2, 4, 8, 16, and 32 µg/ml); (B) Clostridium perfringens ATCC 3624 (0, 4, 8, 16, 32, and 64 µg/ml); (C) Listeria monocytogenes ATCC 15323 (0, 2, 4, 8, 16, and 32 µg/ml); (D) Salmonella Typhimurium KCCM 11862 (0, 4, 8, 16, 32, and 64 µg/ml); (E) Staphylococcus aureus KCCM 11764 (0, 2, 4, 8, 16, and 32 µg/ml); (F) Vibrio parahaemolyticus ATCC 17802 (0, 2, 4, 8, 16, and 32 µg/ml). All data are presented as means ± standard deviations (SD) of two determinations. Asterisks indicate a significant difference compared with the control group (P < 0.01).