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Antimicrobial Effect of Phytochemicals to *Listeria monocytogenes* Isolated from Slaughterhouses

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ABSTRACT - The objective of this study was to investigate the inhibitory effect of natural antimicrobials on *Listeria monocytogenes* isolated from Korean slaughterhouses. A mixture of 15 strains of *L. monocytogenes* at low (3 Log CFU/mL) or high (7 Log CFU/mL) concentration was exposed to various extracts (grapefruit seed extract, citrus fruit extract, ginger extract, pear extract, Japanese apricot concentrate, balloon flower extract, jujube extract, and omija extract) at 0.001-4.0 μg/mL. Ginger extract, pear extract, Japanese apricot concentrate, balloon flower extract, jujube extract, or omija extract showed no antimicrobial effects on high-concentration of *L. monocytogenes* (7 Log CFU/mL). However, grapefruit seed extract and citrus fruit extract showed antibacterial effects against *L. monocytogenes* at 3 and 7 Log CFU/mL with MBCs of 0.001 and 0.002 μg/mL, respectively. These results indicate that grapefruit seed extract and citrus fruits extract can be used to control *L. monocytogenes* as natural antimicrobials.

Key words : *Listeria monocytogenes*, Antimicrobial effect, Grapefruit seed extract, Citrus fruits extract, Slaughterhouses

Listeria monocytogenes is a Gram-positive non-spore forming, facultative anaerobic, and short rod foodborne pathogen[1]. The pathogen can cause a listeriosis which can be severe to pregnant women, newborns, and immunocompromised people[2] with 20-30% of fatality rate[3]. *L. monocytogenes* is ubiquitous in the natural environment, and has usually been isolated from foods of animal origin including meat, sausages, cheeses, and milk products[3].

L. monocytogenes has frequently been isolated from slaughterhouses and meat processing plants. In Korea, a total of 290 samples including carcasses (n = 140), feces (n = 136), and the environment (n = 14) samples were collected from nine slaughterhouses, and fifteen (5.2%) of *L. monocytogenes* were isolated[4]. In 373 samples from carcasses (n = 50), pluck sets (n = 250), and the slaughterhouse environment (n = 73) of ten slaughterhouses in Finland, *L. monocytogenes* was detected in six slaughterhouses and 9% of the samples[5]. *Listeria* spp. was detected in seven sites (3.2%) of three slaughterhouses (27.3%) in eleven slaughterhouses in Italy, and *L. monocytogenes* was isolated from 7.1% of hand-wash basins, and 13.3% of the cold room floor in the slaughterhouses[6]. *L. monocytogenes* in the slaughterhouse environment can easily migrate to raw meat processed at the slaughterhouses and can cause food poisoning[7,8], and they can cross-contaminate processed-meat products. Thus, antimicrobials have been applied to control *L. monocytogenes* in the processed meat, but consumers prefer to use natural antimicrobials than synthesized antimicrobials[9].

Therefore, the objective of this study was to evaluate activities of natural antimicrobials to control the growth of *L. monocytogenes* isolated from domestic slaughterhouses environment.

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Materials and Methods

Preparation of natural antimicrobials

Grapefruit seed extract (Quinabra, Eldorado, Brazil), citrus fruit extract (Quinabra), ginger extract (Serim Food, Bucheon, Gyeonggi, Korea), pear extract (Serim Food), jujube extract (Sanjung Food, Eumseong, Chung-cheong bukdo, Korea), Japanese apricot concentrate (Sanjung Food), omija extract (Sanjung Food), and balloon flower extract (Sanjung Food) were purchased from online stores. Each antimicrobials was diluted with distilled water to final concentration of 8 μg/mL.

Inoculum preparation

Fifteen *L. monocytogenes* strains SMFM-SI-1, SMFM-SI-2, SMFM-SI-3, SMFM-SI-4, SMFM-SI-5, SMFM-SI-6, SMFM-SI-7, SMFM-SI-8, SMFM-SI-9, SMFM-SI-10, SMFM-SI-11, SMFM-SI-12, SMFM-SI-13, SMFM-SI-14, and SMFM-SI-15 which were isolated from slaughterhouses in Korea[4] were used in this study. Each *L. monocytogenes* strain was cultured in 10 mL of tryptic soy broth with 0.6% yeast extract (TSBYE; Becton, Dickinson and Company, Sparks, MD, USA) at 30ºC for 24 h. One hundred microliter of each culture was inoculated to 10 mL of TSBYE, and incubated again at 30ºC for 24 h. The cultures were centrifuged at 1,912 × g and 4ºC for 15 min, and the cell pellets were washed twice with phosphate buffered saline (PBS, pH 7.4; 8.0 g of NaCl, 0.2 g of KH_2PO_4, 1.5 g of Na_2HPO_4, and 0.2 g of KCl in 1 L of distilled water). These suspensions

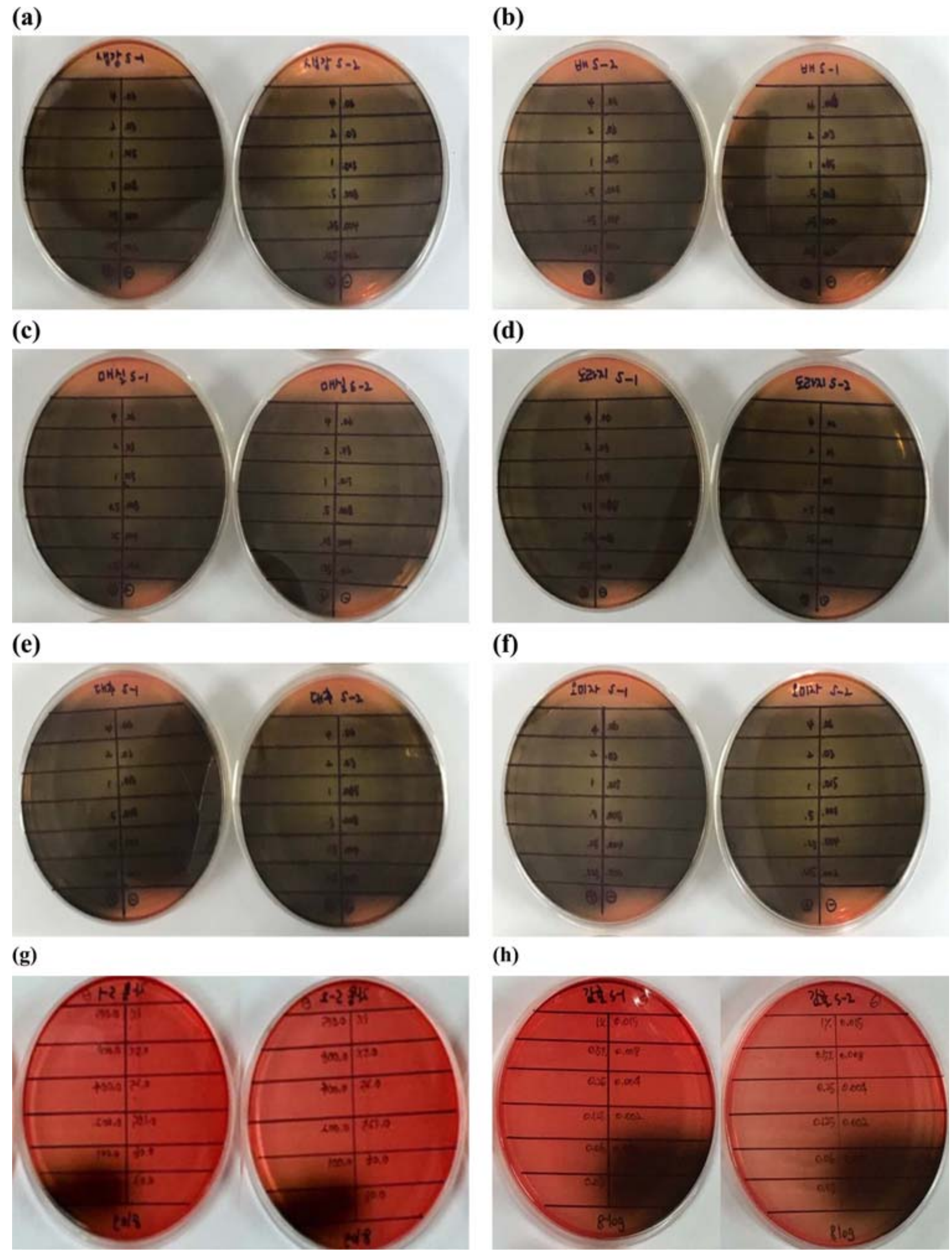

Fig. 1. Identification of minimum bactericidal concentrations of ginger extract, pear extract, Japanese apricot concentrate, balloon flower extract, jujube extract, omija extract, grapefruit seed extract, and citrus fruit extract against *Listeria monocytogenes* at 7 Log CFU/mL. (a) ginger extract; (b) pear extract; (c) Japanese apricot concentrate; (d) balloon flower extract; (e) jujube extract; (f) omija extract; (g) grapefruit seed extract; (h) citrus fruit extract.

(a) **(b)**

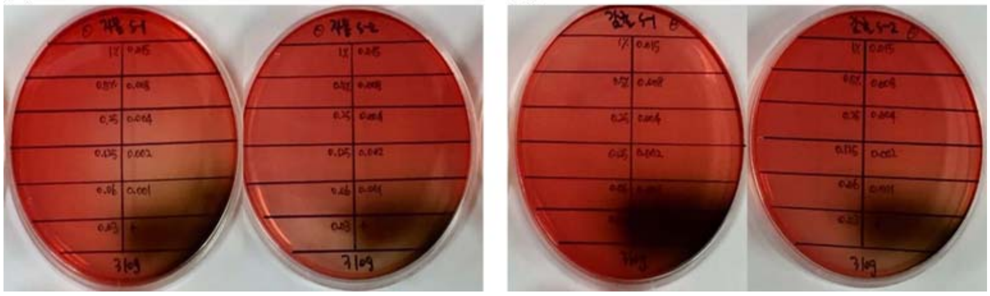

Fig. 2. Identification of minimum bactericidal concentrations of grapefruit seed extract and citrus fruit extract against *Listeria monocytogenes* at 3 Log CFU/mL. (a) grapefruit seed extract; (b) citrus fruit extract.

were mixed, and its optical density was measured at 600 nm with UV-spectrophotometer (OPTIZEN 2120UV, Mecasys Co., Ltd., Daejeon, Korea). The OD_{600} was adjusted to 0.1 (8 log CFU/mL) to be an inoculum.

Determination of minimum bactericidal concentration (MBC)

MBCs were determined by the conventional two-fold dilution method[10,11] with some modifications. Three hundred sixty microliters of each antimicrobial extract solution was added to the 96-well cell culture plate (SPL Life Sciences Co., Ltd., Pocheon, Gyeonggi, Korea). One hundred eighty microliter aliquots of TSBYE were placed to the other wells, and two-fold dilution of the antimicrobial extracts was performed to have 4.0, 2.0, 1.0, 0.5, 0.25, 0.125, 0.06, 0.03, 0.015, 0.008, 0.004, 0.002, and 0.001 μg/mL of concentrations. Twenty microliter aliquots of *L. monocytogenes* inoculum were then inoculated into each well of the 96-well plates to have 7 Log CFU/mL of *L. monocytogenes*. To have 3 Log CFU/mL of *L. monocytogenes*, the inoculum was diluted with PBS and inoculated. The wells containing TSBYE and *L. monocytogenes* were positive controls, and the wells containing TSBYE and antimicrobials were negative controls. The plate was incubated at 30°C for 24 h. A loopful of the culture in the plate was then streaked onto the Palcam agar (Becton, Dickinson and Company), and the Palcam agar plates were incubated at 30°C for 48 h. After incubation, colony formation on the plates was confirmed.

Results and Discussion

The MBCs of grapefruit seed extract, citrus fruit extract, balloon flower extract, ginger extract, Japanese apricot concentrate, jujube extract, omija extract, and pear extract for *L. monocytogenes* isolated from slaughterhouses were investigated. *L. monocytogenes* was not destroyed at 7 log CFU/mL by ginger extract, pear extract, Japanese apricot concentrate, balloon flower extract, jujube extract, and omija extract regardless of concentration (Fig. 1). The result for ginger extract was similar to the result of previous studies, which reported that the ginger extract had no antimicrobial effect on *L. monocytogenes* at 10~100%[12], and water and 50% ethanol extracts of ginger at concentration up to 10% also did not show any antimicrobial activity against *L. monocytogenes*[13]. However, the antimicrobial effects against *Escherichia coli* and *Salmonella* Typhi were shown by Ekwenye et al.[14], and the effect was improved as the extract was prepared by ethanol, compared to water. Omija seeds extracts were found to be antibacterial against *Lactobacillus plantarum*, *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli*, and *Salmonella* Typhimurium[15], and Kim et al. also reported that omija extract has strong antibacterial activity against *B. subtilis*, *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa*[16]. However, in our study the extract did not show antilisterial activity. Japanese apricot extract also showed strong antimicrobial activity on *B. subtilis*, *E. coli*, and *P. aeruginosa*, and the jujube extract showed a slightly weaker antimicrobial activity against these kinds of bacteria[16], but these extracts do not have antilisterial activities.

The MBCs of grapefruit seed extract and citrus fruits extract against *L. monocytogenes* at 7 Log CFU/mL were 0.002 μg/mL and MBC to *L. monocytogenes* at 3 Log CFU/mL was 0.001 μg/mL (Fig. 2).

In conclusion, grapefruit seed extract and citrus fruits extract can be used as antimicrobials to destroy *L. monocytogenes* isolated from slaughterhouse environment.

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국문요약

본 연구에서는 도축장에서 분리된 *Listeria monocytogenes*를 저해할 수 있는 식물성 항균 물질을 탐색하고, 각 항균 물질의 최소살균농도(MBC)를 확인하였다. 잠재적인 항균 물질로 자몽종자 추출물, 감귤류 추출물, 생강 추출물, 배 추출물, 매실 농축액, 도라지 추출물, 대추 추출물, 오미자 추출물을 선정하였고, 대상 균주로는 국내 도축장에서 분리한 15 개의 *L. monocytogenes* 균주를 혼합하여 사용하였다. MBC 확인 결과, 최대 4.0 μg/mL 농도의 생강 추출물, 배 추출물, 매실 농축액, 도라지 추출물, 대추 추출물, 오미자 추출물은 고농도의 *L. monocytogenes* (7 Log CFU/mL)에 대해 균 저해 효과가 전혀 나타나지 않았다. 반면, 7 Log CFU/mL의 *L. monocytogenes*에 대한 자몽종자 추출물과 감귤류 추출물의 MBC는 0.002 μg/mL인 것으로 확인되었다. 3 Log CFU/mL의 *L. monocytogenes*에 대한 MBC를 시험해 본 결과, 자몽종자 추출물과 감귤류 추출물의 MBC는 0.001 μg/mL로 나타났다. 이상의 결과로 볼 때 자몽종자 추출물과 감귤류 추출물은 *L. monocytogenes*를 제어하기 위해 적용될 수 있을 것으로 판단된다.

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