

Antibacterial effects of *Mume Fructus* Water Extract against *Salmonella typhimurium* in Murine Salmonellosis

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ABSTRACT

The present study was undertaken to estimate the antibacterial effect of *Mume Fructus* water extract (MFWE) against murine salmonellosis. At MFWE concentrations ranging from 25 to 100 µg/ml, the antibacterial effect was not showed on *Salmonella typhimurium* (*S. typhimurium*). On the other hand, bacteria without MFWE had a tendency to proliferate up to 8 h after incubation. Oral administration of MFWE at the dose of 40 mg/ml showed a therapeutic effect for *S. typhimurium* infected BALB/c mice. The mortality of MFWE-treated mice was 80% at 12 days, while that of MFWE-untreated mice was 100% at 9 days after a lethal dose of *S. typhimurium* infection. The results of our study strongly indicate that MFWE has potential as an effective of salmonellosis.

Keywords: *Mume Fructus* water extract, antibacterial effect; salmonellosis, *Salmonella typhimurium*

I. Introduction

Mume Fructus is made from the unripe peeled fruit of *Prunus mume* Sieb. et Zucc which have been smoked with dry rice straw.¹⁾ Traditionally, *Mume Fructus* is used as medicinal herb for the treatment of fatigue, diarrhea, and fever.²⁾ Several research studies reported that *Mume Fructus* had antimicrobial activities against pathogenic microorganisms.^{3,4)}

Salmonella causes various disease syndromes, such as self-limiting enteritis, fatal infection in animals, food-borne infection, and typhoid fever in humans.⁵⁾ For the prevention and treatment of salmonellosis, antimicrobial agents are generally used. However, recent studies have been reported that salmonella resistant to antibiotics increased.⁶⁾

According to the increase of antibiotic resistance microbe, many researchers have been forced to

search new antimicrobial substances in medicinal herbs to solve the problem of antibiotic resistance.⁷⁾

In the present study, the therapeutic potentials of *Mume Fructus* water extracts (MFWE) were evaluated for its antibiotic property against *Salmonella typhimurium* (*S. typhimurium*) *in vitro* and *in vivo*.

II. Materials and Methods

1. *Mume Fructus* Crude Extract

Mume Fructus was purchased from the Korea National Animal Bio Resource Bank (Gyeongnam, Korea). Air-dried and crushed *Mume Fructus* (100 g) was extracted with 100 ml distilled water by heating at 121°C, 3 h. The supernatant from the extraction was centrifuged at 5000 rpm for 10 min, and then filtered through filter paper (Whatman No. 2, USA). The filtrates were evaporated to dryness at 54°C. The residue was dissolved in distilled water, adjusted to 40 mg/ml final concentration, and sterilized by passage through a Corning syringe filter (0.20 µm, Japan).⁸⁾

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2. Bacterial Culture and Media

Salmonella enterica serovar Typhimurium (*Salmonella typhimurium*) ATCC 14028 cells were maintained as frozen glycerol stock and cultured in Luria-Bertani (LB) broth or LB broth containing 1.5% agar. Bacteria were grown at 37°C with vigorous shaking to a stationary phase in LB broth.

3. Determination of Antibacterial Activity

Bacteria were diluted with phosphate-buffered saline (PBS) solution, pH 7.4, to 2×10^4 /ml, added to different concentrations (0, 25, 50, 100 µg/ml) of MFWE, and incubated at 37°C for 0, 2, 4, and 8 h. After incubation and proper dilution, 100 µl of each solution was plated onto LB agar to assess bacterial colony forming units (CFUs).

4. MFWE Treatment for Murine Salmonellosis

Specific pathogen free (SPF) female BALB/c mice (Orient Bio, Seoul, Korea) aged 6-8 weeks, weighing 26 ± 4 g each, were used in this study. All mice were kept at $23 \pm 1^\circ\text{C}$ with a 12-h light/dark cycle. They had free access to water and diet and were acclimatized for at least 2 weeks before starting the experiments. Two groups of 10 mice each were used for bacterial infection. Mice were infected intraperitoneally with 2×10^4 CFUs of *S. typhimurium*.⁹⁾ After bacterial infection, two groups of mice were orally treated with 0.1 ml of sterile PBS with/without MFWE (40 mg/ml) every 24 h during 12 days. Infected mice were examined for the viability every 24 h. All procedures described were reviewed and approved by the Animal Ethical Committee of Gyeongsang National University.

5. Statistical analyses

The data were analyzed by a one-way analysis of variance (ANOVA), followed by Student's *t*-test. The results are expressed as mean \pm SD. A mean difference was significant at the 0.05 level.

III. Results and Discussion

Antibacterial effect of MFWE against *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) is presented in Fig. 1. At 2 h after incubation,

inhibition of *S. typhimurium* growth at the dose of MFWE 100 µg/ml was significantly higher than that of control (no treatment) ($p < 0.05$), but there was no significant difference compared with control (no treatment) at 8 h after incubation. The bacteriocidal effect of MFWE was not showed on *S. typhimurium*. Bae *et al.*¹⁾ reported that the butanol fraction of *Prunus mume*, which is a raw material of *Mume Fructus*, had an antimicrobial effect on *S. typhimurium* at the concentration of 500 µg/g, which is 5 times higher than that of *Mume Fructus* in the present study.

The therapeutic effects of MFWE against *S. typhimurium* were shown in Fig. 2. The mortality of

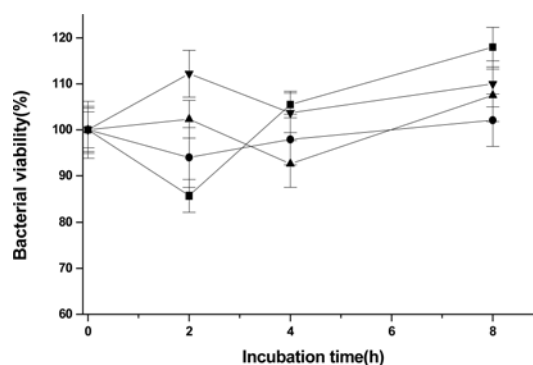


Fig. 1. Antibacterial effect of *Mume Fructus* water extract (MFWE) against *Salmonella typhimurium*. MFWE diluted with PBS were used at the concentration with 0 (▲), 25 (▼), 50 (●), and 100 (■) µg/ml. Bacterial viability was measured based on CFUs on culture plates.

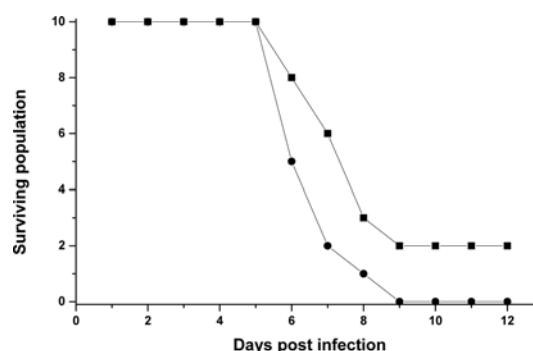


Fig. 2. Mortality rate of mice infected with *S. typhimurium* after treatment of *Mume Fructus* water extract (MFWE). ■, the group treated with 40 mg/ml MFWE (n=10); ●, the group untreated with MFWE (n=10).

mice infected with *S. typhimurium* was counted during the experimental period. The mortality rate in the untreated MFWE was 100% at the 9th day. However, the group treated with 40 mg/ml HCWE survived two mice until the 12th day, and the mortality rate in the treated group was 80%. According to the previous research,⁴⁾ the extract mixture of *Mume Fructus*, *Coptidis Rhizoma*, and *Schizandrae Fructus* (5:3:2) significantly reduced the mortality of chicken infected with *Salmonella gallinarum*. The mortality rate of the extract mixture fed group was 33%, and that of the non-extract mixture fed group was more than 83%. In our study, the mortality rate of the treated MFWE at the dose of 40 mg/ml was lower than that of the research carried out Kwon *et al.*⁴⁾ It assumed that the different result of mortality rate was depended on experimental conditions.

IV. Conclusions

This study was performed to investigate the antibacterial effect of *Mume Fructus* water extract (MFWE) against *Salmonella typhimurium*. The effect was studied using different concentrations of MFWE (25, 50, and 100 µg/ml), but inhibition of the pathogenic bacterial growth due to increasing the concentration of MFWE was not observed.

To identify the mortality rate of BALB/c mice with *S. typhimurium* infection, the dose of MFWE 40 mg/ml was administered for 12 days. The mortality rate of MFWE-treated mice was 80% at 12 days, while that of MFWE-untreated mice was 100% at 9 days after infection of *S. typhimurium*.

In conclusion, our results demonstrate that MFWE at the concentration of 100 µg/ml does not take effect against *S. typhimurium*, while at the dose of 40 mg/ml, MFWE possesses the therapeutic effect for the infection of *S. typhimurium*.

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