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NOTE

Akinobu Tanaka · Hyo Jung Kim · Shojiro Oda
Kuniyoshi Shimizu · Ryuichiro Kondo

Antibacterial activity of moso bamboo shoot skin (*Phyllostachys pubescens*) against *Staphylococcus aureus*

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Abstract This study examined the antibacterial activity of moso bamboo shoot skin (*Phyllostachys pubescens*). Bamboo shoot skin itself and its dichloromethane extract had antibacterial activity against *Staphylococcus aureus*. Results suggest the possibility of effective utilization of antibacterial materials from bamboo shoot skins that are mainly discarded at present.

Key words Antibacterial activity · Moso bamboo shoot skin · *Phyllostachys pubescens* · *Staphylococcus aureus*

Introduction

Recently, chemical food additives like paraben[1,2] have been reported to have harmful effects. However, harmful compounds like paraben are still used as preservatives in foods, cosmetics, and medical products. Meanwhile, consumer demand for the safety of additive agents is increasing year on year, leading to popular movements to reduce the addition of synthetic chemical compounds to foods and cosmetics. The emergence of materials, preferably natural products, having less harmful effects would be highly desirable if their safety could be guaranteed. *Staphylococcus aureus* is a major food pathogen. These bacteria also live on human skin and infect the body through wounds or food. They produce several toxins such as enterotoxin-A and -D.[3] These toxins cause abdominal pain, vomiting, and diarrhea. It is important to prevent the growth of such bacteria in foods; research into natural products that can achieve this objective is underway in many laboratories.

Bamboo is well known for its usefulness. For example, its roots and leaves have been used as medicinal products. Also, studies on the medicinal properties of bamboo leaves have identified antioxidant, anticancer, and antibiotic properties.[4,5] From these studies, various active compounds have been separated from the leaves, such as flavones, glycosides, phenolic acids, coumarin lactones, anthraquinones, and amino acids.[6–10] Also, 2,6-dimethoxy-*p*-benzoquinone obtained from the skin of bamboo trees and the chitin-binding peptides Pp-AMP1 and Pp-AMP2 separated from bamboo shoots have antibiotic activities.[11,12] Tricin and taxifolin from the bamboo sheath reportedly have antioxidant activity.[13] However, no studies on the antibacterial activity of bamboo shoot skin have been reported.

This study focused on moso bamboo shoot skins (*Phyllostachys pubescens*), which have been traditionally used for packaging food. For example, bamboo shoot skin is used as a preservative container to maintain the taste of tea in China and has been used as packaging materials for rice balls and meat in Japan. Fukuoka prefecture is the major production area of bamboo shoots in Japan. A lot of food goods are produced from bamboo shoots annually. At the present time, a large amount of waste bamboo shoot skin is produced from these industrial food processes. These bamboo shoot skins are often composted; however, it is difficult to use bamboo shoot skins as compost because of their persistence. As a result, most bamboo shoot skins are burned, but this is not an effective use of a potential resource. This study aims to examine the antibacterial activity of moso bamboo shoot skin.

Materials and methods

Materials

Moso bamboo shoot skins were used in this study. These were harvested at Miyako, Fukuoka prefecture, Japan, and were provided by Life Design Co. Ltd (Fukuoka, Japan).

A. Tanaka · H.J. Kim · K. Shimizu (✉) · R. Kondo
Department of Agro-environmental Sciences, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
Tel.+81-92-642-3002; Fax +81-92-642-3002
e-mail: shimizu@agr.kyushu-u.ac.jp

S. Oda
Life Design Co. Ltd., Miyako, Fukuoka 824-0822, Japan

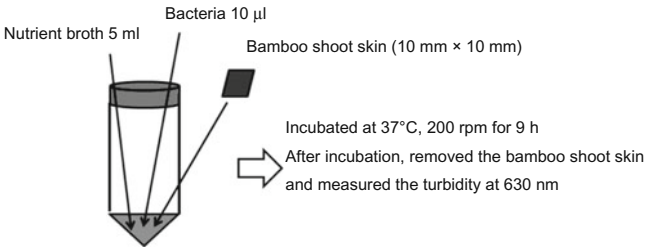

Fig. 1. Scheme of antibacterial assay I: antibacterial activity of bamboo shoot skin

The skins were dried at room temperature and used in the following experiments.

Strains. The test strain was *Staphylococcus aureus* (NBRC 12732).

Antibacterial assay I

In this experiment, we evaluated the antibacterial activity of moso bamboo shoot skin (10 mm × 10 mm plate) itself. Moso bamboo shoot skins were cut into 10 mm × 10 mm pieces, and these pieces were washed in 70% ethanol to kill bacteria. These bamboo shoot skins are referred to as untreated bamboo shoot skins. Other bamboo shoot skins (10 g, 10 mm × 10 mm plates) were sequentially treated by soaking in *n*-hexane (100 ml), dichloromethane (100 ml), methanol (100 ml), and water (100 ml) for 24 h at 180 rpm to prepare extract-free bamboo shoot skins, which are referred to as treated bamboo shoot skins. A single colony of the test strain was taken and added to 20 ml of nutrient broth (NB) medium. This medium was incubated at 37° ± 1°C at 160 rpm for 12 h. After that, NB medium was added to the bacterial suspension to prepare a bacterial concentration at 10^5 CFU/ml. Five milliliters of NB medium, 50 µl of bacteria suspension, and a piece of bamboo shoot skin were incubated at 37°C at 200 rpm for 9 h. Then, the bamboo shoot skin was removed from the medium. Finally, bacteria growth was measured by a microplate reader (630 nm). The significance of the differences between each group was determined by Tukey's test. The scheme of this assay is shown in Fig. 1.

Antibacterial assay II

In this experiment, we evaluated the antibacterial activity of extracts from moso bamboo shoot skin. Moso bamboo shoot skins (powder, 100 g) were serially extracted with *n*-hexane (1 l, weight of *n*-hexane extract was 0.17 g), dichloromethane (1 l, 0.18 g), methanol (1 l, 0.93 g), and water (1 l, 2.41 g) for 24 h at 180 rpm. A single colony of test strain was taken and added to 20 ml of NB medium. This medium was incubated at 37°C ± 1°C at 160 rpm for 12 h. After that, NB medium was added to the bacterial suspension to prepare a bacterial concentration at 10^5 CFU/ml. Each extract (*n*-hexane, dichloromethane, methanol, and water) was dissolved in dimethyl sulfoxide (DMSO) at maximum concentration. Subsequently, serial twofold dilutions of the test extracts were prepared in DMSO. Into each well of a 96-well plate, 89 µl of NB medium, 10 µl of bacteria suspension, and 1 µl of DMSO with or without each extract were added. The plate was incubated at 37° ± 1°C at 1160 rpm for 12 h. The minimum inhibitory concentration (MIC) was the lowest concentration of the test extract that demonstrated no visible growth.

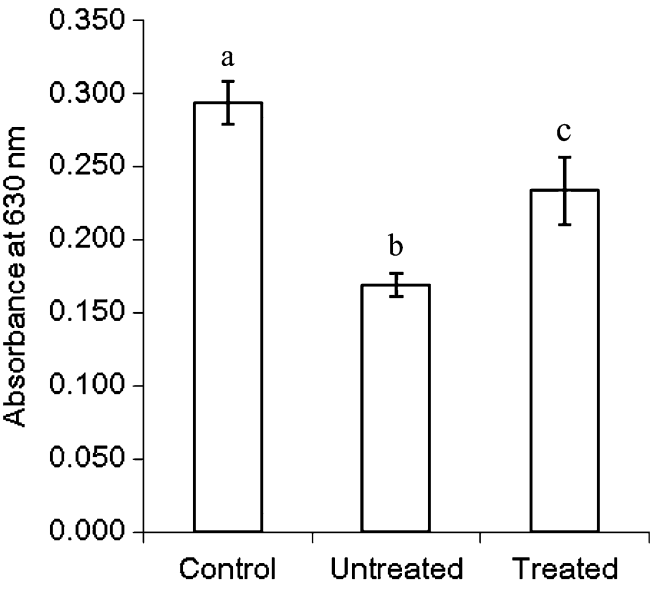

Fig. 2. Antibacterial activity of bamboo shoot skin against *Staphylococcus aureus*. Each value is expressed as the mean ± SD ($n = 3$). *Different letters* indicate significant differences at $P < 0.01$ by Tukey's test. Significant differences were apparent between the control, untreated, and treated bamboo shoot skin in terms of antibacterial activity

Results and discussion

The results of antibacterial assay I are shown in Fig. 2. The absorbance at 630 nm of each sample (control, untreated, and treated) were 0.294 ± 0.0150, 0.169 ± 0.0075, and 0.233 ± 0.0229, respectively. Absorbances of untreated and treated samples were significantly lower than that of the control. From this result, it was determined that bamboo shoot skin itself can inhibit the growth of *S. aureus*. Also, the absorbance of the untreated sample (0.169 ± 0.0075) was lower than that of treated sample (0.233 ± 0.0229). This result indicates that untreated bamboo shoot skin had stronger activity than treated skin. The difference in activities between the untreated and treated bamboo shoot skins indicates that bamboo shoot skin includes some antibacterial compounds that can be extracted by solvents. It seems that antibacterial compounds were eluted from bamboo shoot skin itself into the extraction media. Next, we evaluated the antibacterial activities of extracts prepared from bamboo shoot skin.

The results of antibacterial assay II are shown in Table 1. The MIC of dichloromethane extract was 200 µg/ml. The

Table 1. Antibacterial activity (μg/ml) of extracts from bamboo shoot skin against *Staphylococcus aureus*

Extracts	MIC (μg/ml)
n-Hexane	>200
Dichloromethane	200
Methanol	>800
Water	>400

Minimum inhibitory concentration (MIC) values are means ($n = 3$) of separate experiments

MICs of *n*-hexane, methanol, and water extracts could not be determined under the experimental conditions used. Only the dichloromethane extract showed strong antibacterial activity.

From these experiments, two important facts were elucidated. First, bamboo shoot skin has antibacterial activity against *S. aureus*. Second, the active compounds can be extracted by dichloromethane. Previously, 2,6-dimethoxy-*p*-benzoquinone was reportedly isolated from the skin of bamboo trees as an antibacterial compound.[11] However, 2,6-dimethoxy-*p*-benzoquinone was not detected in extracts of bamboo shoot skin by high-performance liquid chromatography analysis (data not shown). These results indicated that antibacterial compounds other than 2,6-dimethoxy-*p*-benzoquinone are present in bamboo shoot skin. Currently, bamboo shoot skins discarded from food processing activities are mainly incinerated and the rest are composted. This study indicates the possibility of bamboo shoot skins as antibacterial materials. The consumer demand for food safety and safe additives is increasing year on year; bamboo shoot skins might help satisfy these demands as packaging materials to maintain food safety. In addition, there is a problem with environmental persistence when bamboo shoot skins are composted. One of the reasons for this persistence is their antibacterial activity; however, this antibacterial activity can be reduced by sequential extraction. Therefore, it is likely that treated bamboo shoot skins would be more easily decomposed and converted to compost.

This is the first report about the antibacterial activity of bamboo shoot skins. Currently, bamboo shoot skins are either burned or composted. However, this study indicates the potential of bamboo shoot skins as a source of antibacterial materials. This study may make it possible to utilize bamboo shoot skins more effectively.

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