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Garlic as a Functional Material:
—Antibacterial Activity of Garlic Peel against *Colletotrichum acutatum*—

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Aomori Prefecture produces garlic on a large scale. The yield is about 80% of the national output. In proportion to the amount of garlic, large amount of peel is also produced. However, peel is disposed of as an industrial wastes, due to lack of effective utilization. We studied garlic peel to develop its uses. We hypothesized that it would not only have physical but also physiological protective activity toward the bulb which is important for proliferation of garlic. To prove our hypothesis, we explored antibacterial compounds from garlic peel. A highly active antibacterial fraction (minimum inhibitory concentration: 6 μ g/ml) against *Colletotrichum acutatum* was obtained from ethyl acetate extracts of the peel. Thus, the results suggest an effective and important use of the garlic peel.

Key words: Garlic Peel, Hypothesis, Antibacterial Activity, *Colletotrichum acutatum*, Effective utilization

1. INTRODUCTION

Aomori Prefecture is famous as an apple-producing district. It also produces garlic on a large scale (*Allium sativum*). The garlic yield is about 80% of the national output. Therefore, we considered garlic suitable for studying natural product chemistry and applying it to functional materials.

There have been many reports about the pharmacologic effects of garlic and its related compounds (Fig. 1). Alliin and allicin are known as active antibacterial compounds ; DAS has an immunity control effect; and Z-ajoene is a potent inhibitor of platelet aggregation [1].

However, chemical study of the bioactivity of garlic peel is

rare. Recently, phenol compounds from garlic peel have been reported as antioxidants [2].

In proportion to the amount of garlic produced in Aomori Prefecture, lot of peel is produced. However, peel is disposed of as an industrial waste, due to the lack of effective utilization.

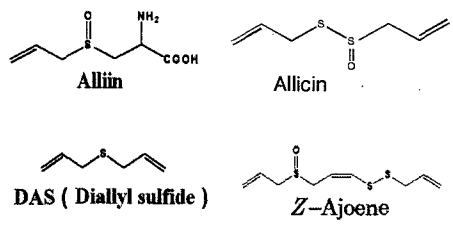


Figure 1. Typical bioactive compounds from garlic.

We studied garlic peel to develop ways to effectively utilize it. First, we considered what kind of physiological role peel played in garlic. We hypothesized that, not only physically but also physiologically, the garlic peel would protect the bulb which was important for proliferation of garlic.

This may lead to development of ways for effective utilization of garlic peel.

2. MATERIALS AND METHODS

Material

Garlic peel was provided by the Field Corps and Horticulture Experiment Station, Aomori Prefecture Agriculture and Forestry Research Center (Rokunohe, Aomori 033-0071, Japan), and JA-Takkomachi (Takko, Aomori, 039-0201, Japan).

Extraction

Powdered garlic peel (50 g) was stirred with ethyl acetate (800 ml) at room temperature for 24 hours in a nitrogen-rich atmosphere. The extract obtained was filtered and the residue extracted with ethyl acetate (600 ml) under the same conditions. This procedure was performed three times (50g × 3) and the combined ethyl acetate extracts were evaporated and dried *in vacuo*. The weight of ethyl acetate extracts was 819.5 mg.

Separation

The extract (819.5 mg) was subjected to low-pressure liquid chromatography (LPLC) on a glass column (450 × 34 mm) with silica gel 60N (180 g, Kanto, catalogue number 37561-79) and eluted gradually with n-hexane and ethyl acetate (2:1, 600 ml ; 1:1, 200 ml ; 1:2, 200 ml). Fractions (250 ml each) were collected and concentrated to give four fractions ; the third fraction (Fr. III, 18.4 mg) showed activity. Fr. III was applied twice to prepatative thin layer chromatography (TLC) on silica gel (20 × 20 cm, Merck silica gel 60 F_{254}, catalogue number 1.05715.0009) in dichloromethane : diethyl ether : n-hexane (10:1:7) to yield Fr. III-1(Rf: 0.44, 1.1 mg) as an active fraction.

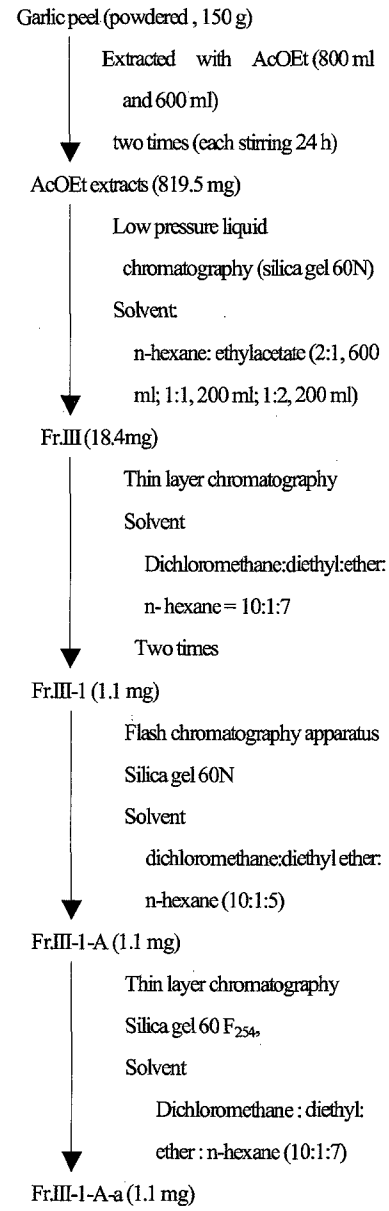


Figure 2. Extraction of garlic peel and purification.

Fr. III-1 was further separated by flash chromatography apparatus (Eyela Ceramic Pomp VSP-2050, Eyela RI-UV Monitor RI20UV, solvent rate 3ml/min) on a glass column (220 × 15 mm) with silica gel 60N (Merck silica gel 0.040-0.063 mm, catalogue number 1.09385.1000) using the solvent system of dichloromethane: diethyl ether: n-hexane (10:1:5). Fractions (5 ml) were collected and concentrated to yield four fractions; the first fraction (Fr. III-1-A, 1.1 mg) showed high activity. Finally, Fr. III-1-A was separated by preparative TLC on silica gel (20 × 20cm, Merck silica gel 60 F_{254}, catalogue number 1.05715.0009) in dichloromethane: diethyl ether: n-hexane (10:1:7) to yield Fr. III-1-A-a (Rf: 0.44, 1.1 mg) as a very high active fraction (minimum inhibitory concentration (MIC): 6 μ g/ml).

Test for Biological Activity

Fractions of garlic extract were dissolved in 5% dimethyl sulfoxide (DMSO) at 500 ug/ml and assayed for anti-fungal activity to *Colletotrichum acutatum* (isolated from Bush clover (*Lespedeza bicolor*), infectious to apple) using the paper disk method. MICs were determined by diluting the samples serially 2-, 4-, 8-, and 10-fold with 5% DMSO. The test to determine MICs was performed in a 9 -cm Petri dish containing 20 ml potato dextrose agar (Difco, Daigo 9.75 g in 250 ml distilled water). The plate was evenly coated with 500 ul of *Colletotrichum* spore suspension at a concentration of 3×10^6 to 3.7×10^6 spores in 1 ml distilled water, air-dried briefly, and then placed by five sterilized paper disks (1.0 cm in diameter). Serially diluted garlic extract of 50 ul was spotted onto the disk and incubated at 20 °C for 48 h in the dark. The same volume of 5% DMSO was used for negative control. The lowest concentration to produce inhibition zone was defined as the MIC. The tests were all triplicated. When different MIC values were obtained between the triplicates, the higher value was adopted.

3. RESULTS AND DISCUSSION

Keeping effective utilization of garlic peel in mind, we proposed a hypothesis about the physiological role of garlic peel. Since the bulb is important for proliferation, we supposed that peel would protect the bulb not only physically but also physiologically. We deduced that peel would prevent the bulb from bacteria causing infection and thus have an antibacterial compound.

Proving our hypothesis, we tried to find the antibacterial compound against *Colletotrichum acutatum* from the garlic peel. *C.acutatum* is known to cause anthracnose in many plants, *e.g.*, strawberry, cucumber, peach, and apple [3]. It has spread worldwide under conditions of high temperature and humidity. In particular, concern has increased that apples produced in Aomori Prefecture would be infected with it owing to global warming. Benomyl and diethofencarb have been used as

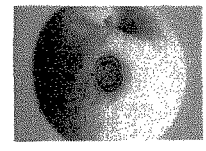
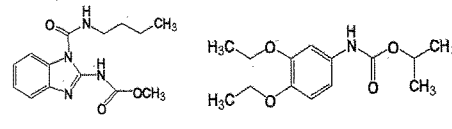


Figure 3. Anthracnose lesion on apple fruit inoculated with *C. acutatum*.

chemical synthetic pesticides against *C.acutatum*. Benomyl was very effective for *C. acutatum*. But benomyl-resistant



Benomyl Diethofencarb

Figure 4. Chemical Synyhetic Pesticides against *C.acutatum*

strains of *C. acutatum* were found in strawberry [4] and cucurbit [5]. On the other hand, diethofencarb had an effect on benomyl-resistant strains of *C. acutatum* [5], but was not effective for susceptible strains of *C. acutatum*. Though the combination of these pesticides was recently used, outbreaks of strains resistant to both these pesticides were reported [6]. Because of this, a new and safe antibacterial compound against *C. acutatum* is required.

First, we extracted garlic peel with ethyl acetate and tested the extracts for antibacterial activity against *C. acutatum*. As the extracts possessed antibacterial activity as predicted, we separated the extracts with various kinds of chromatography and tested respective fractions for antibacterial activity.

Repeated separation followed by activity tests gave Fr. III-1-A-a as the most active fraction. Therefor, we tried to measure its MIC using a new technique that is supported by the literature [7]. The MIC was 6 μg/ml at high value.

As the MIC of 6 μg/ml is very high, it was compared

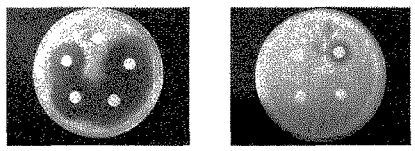


Figure 4. MIC test of Fr. III-1-A-a (60 μg/ml). It was diluted to the value with 5% DMSO aq. solution. Left: control (top, 5% DMSO aq. solution), 1/1 (upper right, 60 μg/ml), 1/2 (lower right, 30 μg/ml), 1/4 (lower left, 15 μg/ml), 1/8 (upper left, 7.5 μg/ml). Right: 1/10 (upper right, 6 μg/ml), 1/20 (lower right, 3 μg/ml), 1/30 (lower left, 2 μg/ml), 1/40 (upper left, 1.5 μg/ml).

To benomyl and diethofencarb. The results are shown in Table 1. The activity of Fr. III-1-A-a was 100-200 times greater than these compounds [8].

Table 1. Comparison of Fr. III-1-A-a with pesticides

Substrate	MIC (μg/ml)
Ethyl acetate extract	250
Fr. III-1-A-a	6
Benomyl	1,250
Diethofencarb	625

Nuclear magnetic resonance measurement of Fr. III-1-A-a had small amount of contaminants. It showed that main element was an organic compound that was different from the pesticides used for treatment of garlic at the Field Corps and Horticulture Experiment Station and JA-Takkomati. In addition, we could not detect Fr. III-1-A-a in extracts from the garlic bulb by TLC. These results established our hypothesis that, garlic peel physiologically protects the bulb, and we can effectively utilize the garlic peel in future.

4. ACKNOWLEDGMENT

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