Research Note

Inhibition of Aspergillus niger and Aspergillus flavus by Some Herbs and Spices

MEI-CHIN YIN* AND WEN-SHEN CHENG

Institute of Nutritional Science, Chungshan Medical and Dental College, 110, Sec. 1, Chien-Kou N. Rd., Taichung, Taiwan, Republic of China

ABSTRACT

The inhibitory effect of water-soluble extracts of garlic bulbs, green garlic, green onions, hot peppers, ginger, Chinese parsley, and basil on the growth of Aspergillus niger and Aspergillus flavus was examined. Garlic bulbs, green garlic, and green onions showed an inhibitory effect against these two fungi. The influence of heat, acid, and salt upon the inhibitory effect of these three herbs was further studied. Increasing the temperature from 60 to 100°C resulted in a significant decrease in the inhibitory effect of garlic bulbs against the fungi tested. Green garlic and green onion lost their antifungal activity against A. niger after being treated at 80 and 60°C, respectively. For A. flavus, the inhibitory effect of green garlic declined significantly with an increase in temperature. However, the antifungal activity of green onions against A. flavus was heat stable. For both fungi tested in this study, the antifungal activity of these spice plants was not affected by acid treatments at pH values 2, 4, or 6, or salt by treatments at concentrations of 0.1, 0.2, 0.3, and 0.4 M (P > 0.05).

The presence and growth of fungi in foods may cause food spoilage and result in a reduction in its quality and quantity. Some Aspergillus species are xerophilic fungi and are responsible for many cases of food and feed contamination. A. niger is commonly involved in fruit spoilage, while A. flavus produces aflatoxins in food. Aflatoxins are known to be potent hepatocarcinogens in animals and humans. Because of health and economic considerations, the search for antifungal agents is extensive. Natural plant extracts may provide an alternative way to protect food or feed from fungal contamination.

Garlic bulbs (Allium sativum L.), green garlic (Allium fistulosum L.), green onions (Allium fistulosum L. var. caespitosum, scallion), hot peppers (Capsicum annuum L.), ginger (Zingiber officinale R.), Chinese parsley (Coriandrum sativum L.), and basil (Ocimum basilicum L.) are commonly used as spices, herbs, or seasoning in Chinese food preparation to provide flavor and aroma. In addition to their uses as ingredients in food preparation, these herbs or spices have antibacterial properties that have been widely studied. The essential oil of Chinese parsley has exhibited an inhibitory effect on the growth of Aeromonas hydrophila in cooked pork. The extracts of young ginger and hot peppers have been found to be effective in inhibiting the growth of Micrococcus luteus. Several studies have reported that garlic bulb extract can inhibit the growth of bacteria, fungi, and viruses in culture media and food systems. It has also been shown that the antimicrobial activity of garlic bulbs is due to allicin (diallyl thiosulfinate), ajoene, and other sulfite compounds. Allicin is absent in intact garlic, but is derived from its precursor, alliin, through enzymatic hydrolysis when the garlic tissue is injured. It has been shown that alliinase is responsible for this hydrolysis. Allicin can further generate ajoene, a stronger antifungal component than allicin. Allicin acts as an inhibitor of respiratory SH-group enzymes, and ajoene destroys the integrity of cell walls. The inhibitory effect of garlic bulb extract on the growth of A. niger in culture media has been studied previously. However, little is known about the antifungal activity of other herbs. The first objective of this study was to examine the antifungal activity of some herbs and spices on A. niger and A. flavus. Second, the influence of heat, acid, and salt upon the antifungal activity of these spice plants was studied.

MATERIALS AND METHODS

Herb and spice extract preparation. The antifungal activity of the following herbs and spices was examined: garlic bulbs, green garlic, green onions, ginger, hot peppers, Chinese parsley, and basil. Both old ginger and young ginger were tested. These plant foods were purchased at a local market. A 20-g edible portion of each food sample was chopped and homogenized in 20 ml of sterile distilled water in a Waring blender at high speed for 3 min at room temperature (25°C). The mixture was filtered through Whatman No. 1 filter paper. The filtrate was then sterilized by passage through a 0.22-μm pore-size filter. The filtrate was collected in a sterile vial and stored at 4°C until used.

Fungal strains and media. The reference strains, A. niger CCRC 31512 and A. flavus CCRC 31358, purchased from Institute of Food Industrial Development (Shin-chu, Taiwan), were used as test microorganisms in all antifungal assays. The microorganisms were grown by incubating for 72 h on a Sabouraud dextrose agar plate...

* Author for correspondence. Tel: +886-4-3896190, ext. 50915; Fax: +886-4-3890964.
(Difco, Detroit, MI) plate at 37°C. Ten milliliters of 1% Tween were added for spore collection. Spores of fungi were harvested by centrifugation at 1,000 x g for 25 min and washed with 10 ml of sterile distilled water. This step was repeated 3 times. The spore suspension was stored in sterile distilled water at 4°C until used.

**Antifungal assay (disk diffusion assay).** Filter paper disks (5 mm diameter) containing 6 μl of a particular plant extract were applied to the surface of Sabouraud dextrose agar plates previously seeded with a test microorganism. The plates were incubated for 24 h at 25°C, and the zones of inhibition (mm) were determined. The results are expressed as the net zone of inhibition, which represents the subtraction of the diameter (5 mm) of the paper disk from the measured zone.

**Heat treatment.** The extracts of the spices and herbs which showed antifungal activity at room temperature were used to study the influence of heat upon their antifungal activity. The food samples were prepared as described in the procedure for herb and spice extract preparation. Before the sample was prepared for homogenization, 20 g of each food sample were wrapped in aluminum foil and placed into ovens at certain temperatures for 15 min. The temperatures used were 25, 40, 60, 80, and 100°C. Then the food sample in aluminum foil was mixed with 10 ml of sterile distilled water in a Waring blender. The aluminum foil was removed by filtering the mixture through Whatman No. 1 filter paper. The filter paper and filtrate were further mixed with another 10 ml of sterile distilled water in a Waring blender at high speed for 3 min.

**Acid and salt treatments.** The food samples were prepared as described in the procedure for herb and spice extract preparation with the difference that a 20-g edible portion of each food sample was soaked in 20 ml of sterile distilled water at different pH values or a different NaCl concentration for 15 min. The pH values used were 2, 4, 6, and 7. The pH value was adjusted with concentrated HCl. The NaCl concentrations used were 0, 0.1, 0.2, 0.3, and 0.4 M.

**Statistical analysis.** The inhibition zones were calculated as means ± SD (n = 5). The data on temperature, acid, and salt interactions were evaluated by two-way analysis of variance. The difference in significance was determined by the least significant difference test.

**RESULTS**

The growth of both *A. niger* and *A. flavus* was significantly inhibited (P < 0.05) by the extracts of garlic bulbs, green garlic, and green onions. The extracts of the other food samples examined in this study did not show any antifungal activity against the growth of these two fungi. The inhibitory effect of these three food samples against both *A. niger* and *A. flavus* growth at 25°C differed significantly; it was greatest for garlic bulb extract, less for extract of green garlic, and least for green onion extract. The influence of heat treatments upon the inhibitory effect of these three food samples against the growth of *A. niger* and *A. flavus* is presented in Tables 1 and 2. The 25°C groups were used as a control. The inhibitory effect of garlic bulb extract on the growth of both *A. niger* and *A. flavus* significantly decreased (P < 0.05) with increasing temperatures from 60 to 100°C. The antifungal activity of green garlic on *A. niger* extract was not observed after these samples were treated at 80°C. Also, green onion extract lost its inhibitory effect on the growth of *A. niger* after being treated at 60°C. As shown in Table 2, the inhibitory effect on *A. flavus* growth was still detectable for all samples after they were treated at 100°C.

**TABLE 1. The inhibitory effect of heat-treated herb extract against *A. niger***

<table>
<thead>
<tr>
<th>Herb</th>
<th>25°C</th>
<th>40°C</th>
<th>60°C</th>
<th>80°C</th>
<th>100°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic bulbs</td>
<td>43.2 ± 0.6</td>
<td>41.8 ± 0.8</td>
<td>33.7 ± 0.4</td>
<td>27.3 ± 0.5</td>
<td>13.4 ± 1.0</td>
</tr>
<tr>
<td>Green garlic</td>
<td>22.5 ± 0.8</td>
<td>15.3 ± 0.3</td>
<td>7.4 ± 1.0</td>
<td>—b</td>
<td>—</td>
</tr>
<tr>
<td>Green onions</td>
<td>11.3 ± 0.5</td>
<td>5.6 ± 1.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

a The dry weights for garlic bulbs, green garlic, and green onions were 4.3, 3.7, and 3.4 g, respectively.

**DISCUSSION**

Tansey and Appleton (18) reported that garlic bulb extract showed antifungal activity against the growth of *A. niger*. Yoshida et al. (19) reported that the growth of *A. niger* ATCC 16404 was inhibited by garlic bulb extract, in which allicin and ajoene were responsible for the antifungal activity of garlic bulbs. The results of the present study not only support the finding that garlic bulb extract possesses antifungal activity against *A. niger* and *A. flavus*, but also extend the inhibitory effect to green garlic and green onions.
The comparison of the activity of these three food samples against \textit{A. niger} and \textit{A. flavus} growth suggests that the quantity of antifungal components was different in these herbs.

Barone and Tansey (3) reported that garlic bulb extract did not show any antifungal activity following a heat treatment at 121°C for 10 min. Chen et al. (4) also observed that the antibacterial components of ginger, hot pepper, and basil were heat labile and that the antibacterial activity was not detectable after these herbs were heated at 80°C for 10 min. Similar results were observed in this antifungal study. The inhibitory effect of garlic bulb extract against \textit{A. niger} and \textit{A. flavus} declined with increasing treatment temperatures from 60 to 100°C. The antifungal activity of green garlic and green onion against \textit{A. niger} was not observed after they were treated at 80 and 60°C, respectively. However, the heat treatments used in this antifungal study did not influence the inhibitory effect of green onion extract against \textit{A. flavus}. Therefore, it cannot be concluded that all antifungal components of herbs and spices are heat labile because the inhibitory effect of green onion extract on \textit{A. flavus} continued following the heat treatment. The effect of heat on the antifungal activities of herbs and spices may result from either heat decomposition or the inhibition of the action of an enzyme such as allinase, which is required for the generation of allicin and \textit{S}-methyl-\textit{L}-cysteine sulfoxide (SMCSO) in garlic bulb extract (8) on other antifungal components. However, the available data cannot explain the antifungal behavior of green onions under the influence of heat.

As shown in Table 1, the inhibitory effect of green onion on \textit{A. niger} growth was more sensitive to heat when compared with other food samples. However, green onion extract showed a heat-stable inhibitory effect against the growth of \textit{A. flavus} (Table 2). The results for green garlic also supported the supposition that more than one factor determines its antifungal activity, because green garlic lost its antifungal effect on \textit{A. niger} after being treated at 80°C, while its inhibitory effect on \textit{A. flavus} was detectable after it was heated at 100°C. These results might suggest that the antifungal components are different for these two fungi. One other possibility is that, although the antifungal component(s) was (were) similar, other heat-labile factor(s) interfered with the action of the antifungal component(s) in these foods.

There is a possibility that the antifungal component(s) of these three food samples was (were) stable to acid levels of pH $\geq$2. It has been reported that allicin and ajoene are the antifungal components found in garlic bulbs, in which allicin is stable to acid and ajoene is formed from allicin (15, 19). Therefore, allicin and/or ajoene might also be the antifungal component(s) of the other two food samples. Garlic bulbs also contains SMCSO, a potent antimicrobial component (14). These authors reported that SMCSO is heat-labile, pH-dependent, and precipitated at pH 4.0. In this study, acid treatments at pH values of 2 and 4 did not lead to any decrease in antifungal activity of garlic bulb extracts. Thus, this result suggests that SMCSO played only a very mild antifungal role in garlic bulbs, if any. Since acid or salt treatments did not affect the antifungal activity of these herbs, their use in preventing fungal contamination in food may not be affected by the presence of acid or salt.

REFERENCES