Therapeutic Effects on Murine Oral Candidiasis by Oral Administration of Cassia (*Cinnamomum cassia*) Preparation

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We examined the effects of spices and herbs on *Candida albicans* growth using *in vitro* assay and therapeutic activity of some selected herbal preparations against murine oral candidiasis. All tested samples: lemongrass (*Cymbopogon citratus*), lemon balm (*Melissa officinalis*), thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), roselle (*Hibiscus sabdariffa*), green tea (*Camellia sinensis*), and cassia (*Cinnamomum cassia*) inhibited *Candida* mycelial growth *in vitro*. The results of this assay showed that the anti-*Candida* activity of lemongrass, green tea, and cassia is stronger than that of the other tested herbs. Oral administration of lemongrass or green tea did not result in significant improvement in the murine oral candidiasis, while the administration of cassia improved the symptoms and reduced the number of viable *Candida* cells in the oral cavity. The results of *in vitro* *Candida* growth assay including GC/MS analysis suggested that cinnamaldehyde in the cassia preparation was the principal component responsible for the inhibitory activity of *Candida* mycelial growth. These findings suggest that oral intake of a cassia preparation is a clinical candidate for a prophylactic or therapeutic tool against oral *Candida* infection.

**Key words**: *Candida albicans*, murine oral candidiasis, lemongrass, cassia

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**Introduction**

Oral candidiasis occurs opportunistically and frequently by the excessive growth of *Candida albicans* which is one of the members of the oral microbial flora in a healthy human.¹² The pathogenic symptoms such as severe oral inflammation induced by oral candidiasis aggravate the quality of life of immunosuppressed individuals and elderly people.³⁴ Since spices and herbs have been widely used as anti-infection tools in daily life, we investigated their anti-*Candida* activity in our search to develop new therapeutics of oral candidiasis.⁵ Previously, we reported the therapeutic effect of clove (*Syzygium aromaticum*) in a murine oral candidiasis model, however, its stimulatory and palate-numbing taste prevented its clinical application as food.⁶ To develop other applications, we have attempted to find another spice or herbal food candidate with therapeutic efficacy greater than or equal to that of clove⁷. In this study, we examined the inhibitory activity on *Candida* mycelial growth by *in vitro* assay and evaluated the therapeutic effect by a murine oral candidiasis model of a hot water extract of herbal foods and spice which have been used for tea or a beverage.⁸⁹ We found that cassia had the strongest anti-*Candida* activity among the tested samples and that a cassia preparation, which was relatively easy to intake, has therapeutic activity against murine oral candidiasis⁵⁷.

**Materials and Methods**

*C. albicans* strain

The *C. albicans* strains of TIMM 1768 and TIMM 2640 were isolated clinically and maintained at the Research Institute of Medical Mycology, Teikyo University. The strain of TIMM 2640, which was shown to induce oral candidiasis in a murine model, has been used for...
animal experiments, and TIMM 1768, the mycelia of which strongly adhere to plastic plates, has been used in the crystal violet staining assay. Cultures were stored at −80°C in Sabouraud dextrose broth (Becton Dickinson, MD, USA) containing 0.5% yeast extract (Becton Dickinson), and 10% glycerol (v/v, final concentration) in our laboratory until use. Strain TIMM 1768 was cultured on a Sabouraud dextrose agar plate for 18h at 37°C and strain TIMM 2640 was cultured on a GS agar plate (Eiken Chemical Co., Ltd. Tokyo, Japan) for 20h at the same temperature. The cells of both strains were harvested with a microspatula, and suspended in RPMI 1640 medium containing 2.5% fetal calf serum (RP medium). The C. albicans strain TIMM 1768 was used for in vitro experiments and TIMM 2640 was used for in vivo Candida oral inoculation.

Preparation of spices, herbs, cinnamaldehyde, coumarin, and cinnamaldehyde-coumarin mixture

Lemongrass (Cymbopogon citratus), lemon balm (Melissa officinalis), thyme (Thymus vulgaris), rosemary (Rosmarinus officinalis), and roselle (Hibiscus sabdariffa) were harvested and manufactured, and clove (Syzygium aromaticum), and cassia (Cinnamomum cassia) were collected and imported by S&B Inc. (Tokyo). Green tea (Camellia sinensis) was purchased from ITO EN, LTD. (Tokyo). All of the samples were milled and weighed in plastic tubes. Each preparation was suspended by hot water at 90°C and immersed in a water bath of the same temperature for 5 min. The suspensions were collected and designated as a 100% original preparation. The original cassia preparation was centrifuged (3,000 rpm, 5 min) and separated into the upper layer, the supernatant, and the lower layer, the precipitate. An original cassia preparation was also filtered through filter paper (Whatman, Maidstone, England) or a disk filter (Millipore, MA, USA). The filtrate was collected in a plastic tube. These preparations were used in the experiments. Cinnamaldehyde and coumarin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and dissolved in dimethylsulfoxide (DMSO) at 10% w/w prior to dilution with RP medium for in vitro assay.

Evaluation of inhibitory activities of spice and herbal preparations against Candida growth in vitro

Evaluation of inhibitory activities against the mycelial growth of Candida was made by the methods described previously. After the Candida cells (TIMM 1768) were cultured on a Sabouraud dextrose agar plate for 18h at 37°C, the cultures were harvested and suspended with RP medium. The suspension was centrifuged (3000 rpm, 5 min) and separated into supernatant and precipitate. The precipitate was washed twice with RP medium and the cell suspension was prepared in the same medium at 5 × 10^6 cells/ml.

A crystal violet (CV) staining assay was performed to evaluate the extent of mycelial growth of C. albicans as described previously. Cassia preparations, cinnamaldehyde, coumarin, the mixture of cinnamaldehyde and coumarin (8:7:1.4) in DMSO, and DMSO (control) were diluted with RP medium, respectively. A 96-well flat bottom microplate received a mixture of 100 μl of Candida cell suspension and 100 μl of each sample. The microplate was incubated for 18h at 37°C in a 5% CO2 atmosphere, following which the medium in the wells was discarded by inverting the microplate. Candida mycelia, which adhered to the bottom of the wells, were sterilized by treatment with 70% ethanol and stained with 0.01% CV. After the microplate was washed with water and dried, 150 μl of isopropanol containing 0.04 N HCl and 50 μl of 0.25% sodium dodecyl sulfate were added to the wells and mixed. The absorbance at 620 nm of quadruplicate samples was measured spectrophotometrically.

Animals

All animal experiments were performed in accordance with the guidelines for the care and use of animals approved by Teikyo University. The experimental procedure of the murine oral candidiasis model was described previously. Six-week-old female ICR mice (Charles River Japan, Inc., Yokohama, Kanagawa) were used for all animal experiments. The mice were randomized, kept in cages housing 4 to 7 individuals, and were given food and water ad libitum. During the experiments, the photoperiods were adjusted to 12 h of light and 12 h of darkness daily, and the environmental temperature was maintained at 21°C all day. To induce an orally immunsuppressed condition, 100 mg/kg of prednisolone (Mitsuka Pharmaceutical Co., Japan) was injected subcutaneously to mice 20 to 24 hours before oral infection. Following prednisolone injection, 0.08% of tetracycline hydrochloride (Takeda Shering Purau Animal Health Co., Japan) was administered with drinking water. On the day of infection, animals were anesthetized by intramuscular injection with 100 μl of 0.2% chlorpromazine chloride (Wako Pure Chemical Industries, Ltd.) in the femur, and were orally infected with about 2.0 × 10^6 cells/ml of viable cells of C. albicans TIMM 2640 in RP medium. Oral infection was performed by means of rubbing and rolling a cotton swab (baby cotton buds; Johnson & Johnson Co., Tokyo) inside all parts of the mouth. The number of Candida
cells inoculated in the oral cavity was calculated to be about $1 \times 10^6$ cells/mouse based on the difference in viable cell number adhering to the cotton swabs before and just after oral inoculation.

**Oral administration of lemongrass, green tea, and cassia**

Lemongrass preparations at concentrations of 62.5%, 31.3%, 6.25%, and 1.25%, and green tea preparations at concentrations of 6.25% and 1.25% in drinking water were administered continuously from the day of infection. Since the animals did not take cassia preparation ad libitum via drinking water, fifty μl of the cassia suspension was administered in the oral cavity of the *Candida* infected mice 5 times: at 3 hour, 21 hour, 27 hour, 45 hour, and 51 hour after *C. albicans* inoculation, using a top-rounded needle, and was spread over all parts of the mouth. The weight of dry matter of cassia was measured after drying at 105℃.

**Scoring the severity of oral infection**

The procedure of scoring the severity of oral infection was performed as described previously. On the 3rd day after inoculation mice were sacrificed and the severity of the lesion of the tongue was evaluated by scoring the fur on each tongue and the squamous disorder as follows: 0, normal; 1, fur in less than 20%; 2, fur in more than 21% but less than 90%; 3, fur in more than 91% and the squamous layer; 4, thick fur in more than 91% and the squamous layer.

**Evaluation of the number of viable *Candida* cells on tongue of a mouse**

Cheek, tongue, and soft palate of the mice were swabbed using a cotton swab on the 3rd day after inoculation for microbiological evaluation. After swabbing, the cotton end was cut off and placed into 5 ml of sterile saline. *Candida* cells were resuspended by mixing on a vortex mixer and diluted by a series of 10-fold dilutions of sterile saline. Fifty μl of each dilution was incubated on a *Candida* GS plate for 20 h at 37℃. The CFU of *Candida* cells were counted and the total numbers per swab were calculated.

**Statistical analysis**

The data of scores were compared by the non-parametric Mann-Whitney U test. Statistical analysis of the log_{10}CFU of *C. albicans* isolated from each mouse part was compared using a Student’s t-test. *P* values of $<0.05$ were considered statistically significant. All calculations were performed using a statistical software program (Stat View: Abacus Concepts, Berkeley, Calif.). All mean values given in the text include the standard deviation of the mean.

**Histological analysis**

The histological analysis was performed as described previously. Specimens of tongue were taken from sacrificed animals, fixed in 10% formalin solution and O.C.T. compound. Specimens 10-μm thick were sectioned using a cryostat and stained with periodic acid - schiff (PAS) stain for histological observation.

**Gas chromatography and mass spectrometric (GC/MS) analysis**

The contents of cinnamaldehyde and coumarin in the cassia preparation were analyzed by gas chromatography and mass spectrometry (GC/MS) using a Shimadzu model GCMS-QP2010Plus equipped with an Rtx-5MS column (RESTEK, 30 m × 0.25 mm, 0.25 μm film thickness).

**Results**

**Inhibitory activities of herbs and spices against *C. albicans* mycelial growth**

The inhibitory activities of lemongrass, lemon balm, spearmint, thyme, rosemary, roselle, and green tea, all commonly used as ‘herbal tea’ or ‘tea’ against mycelial growth of *C. albicans* were examined as shown in Table 1. The inhibitory activity was compared by IC_{50} or IC_{80} of each preparation. In the tested herbal teas, IC_{50} of lemongrass and green tea preparations were relatively low, that is, less than 6.25%, and their IC_{80} was from 6.25% to 12.5% and less than 6.25%, respectively. These data indicated that the inhibitory activity of lemongrass and green tea was stronger than other herbal tea samples. We also investigated spice, cassia and clove in a hot water extract against *Candida* mycelial growth; the data is shown in Table 2. IC_{50} and IC_{80} of the cassia preparations were 1.0 - 5.0%, and relatively lower than that of clove preparations (5.0 - 25.0%). The inhibitory activity of cassia preparation against *Candida* growth was relatively stronger than that of clove. Lemongrass, green tea, and cassia exercise their anti-*Candida* effect at almost the same dose level as is used in daily life. These data suggested that all three may be candidates as effective agents for the prevention and improvement of oral candidiasis.

**Effects of oral administration of lemongrass and green tea on experimental candidiasis**

The efficacy of administering lemongrass and green tea to counteract oral candidiasis was examined using the oral candidiasis murine model with TIMM 2640. In
In this experiment, considering the results of IC₅₀ and IC₈₀, the most concentrated lemongrass extracts were prepared and administered *ad libitum* to give as much of the preparations as possible; the results are shown in Table 3. Lemongrass and green tea were prepared at a dose of 1.25%, which was almost the same dose level as when they were used as a conventional food, and at a higher dose to check their anti-*Candida* activity. In the group of mice administered 6.25% of lemongrass, the clinical score tended to be relatively lower than that of other groups, but was not statistically significant. When 6.25% of green tea was administered, the number of viable *Candida* cells was increased. The tongues of the mice treated with lemongrass and green tea preparations showed no significant improvement in the symptoms or viable cell numbers of *C. albicans*.

**Therapeutic effects on murine oral candidiasis of oral administration of cassia preparation**

The inhibitory effect of the administration of cassia preparation on murine oral candidiasis was also examined. This preparation was administered by force-feeding using a top-rounded needle since this suspension contained numerous small particles which precipitated rapidly. By using force-feeding, it was possible for the volume of the sample (50 μl/mouse) to be reduced so that the concentration was increased. The results are shown in Table 4 and details are as follows. In control mice two days after infection, the symptoms of oral candidiasis appeared on the lingual mucosa of the *C. albicans* infected animals. There were expanded atrophic areas and patchy areas of smooth mucosa over the dorsal of the tongue of the infected mice which had had no treatment. In the group of mice treated with a high dose of cassia preparation, the clinical score was significantly reduced compared with the control group. A typical tongue of the control group and the cassia administered group is shown in Fig. 1A and 1B, respectively. The tongue treated with a high dose of cassia preparation appeared normal and partially healthy. By histological analysis, PAS-positive fungi were observed on the severe lesions near the oral

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**Table 1. Inhibitory effects of hot water extracts of various herbs for *C. albicans* mycelial growth**

<table>
<thead>
<tr>
<th>Agent</th>
<th>IC₅₀ (mycelial growth) ¹⁾ (concentration: %)</th>
<th>IC₈₀ (mycelial growth) ¹⁾ (concentration: %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemongrass</td>
<td>&lt; 6.25</td>
<td>6.25-12.5</td>
</tr>
<tr>
<td>Lemon balm</td>
<td>6.25-12.5</td>
<td>50.0 &lt;</td>
</tr>
<tr>
<td>Spearmint</td>
<td>12.5-25.0</td>
<td>25.0-50.0</td>
</tr>
<tr>
<td>Thyme</td>
<td>6.25-12.5</td>
<td>50.0 &lt;</td>
</tr>
<tr>
<td>Rosemary</td>
<td>25.0-50.0</td>
<td>50.0 &lt;</td>
</tr>
<tr>
<td>Roselle</td>
<td>&lt; 15.6</td>
<td>15.6-31.3</td>
</tr>
<tr>
<td>Green Tea</td>
<td>&lt; 6.25</td>
<td>&lt; 6.25</td>
</tr>
</tbody>
</table>

Inhibitory effects were evaluated by the CV staining assay as described in materials and methods. Each sample (4 g) was extracted by hot water (20 ml), and the extracts (100%) were diluted by the medium and their anti-*Candida* activity tested.

¹⁾ The concentrations of the extracts providing 50% or 80% inhibition (IC₅₀, IC₈₀) of *Candida* mycelial growth are indicated.

**Table 2. Inhibitory effects of hot water extracts of spice for *C. albicans* mycelial growth**

<table>
<thead>
<tr>
<th>Agent</th>
<th>IC₅₀ (mycelial growth) ¹⁾ (concentration: %)</th>
<th>IC₈₀ (mycelial growth) ¹⁾ (concentration: %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassia</td>
<td>1.0-5.0</td>
<td>1.0-5.0</td>
</tr>
<tr>
<td>Clove</td>
<td>5.0-25.0</td>
<td>5.0-25.0</td>
</tr>
</tbody>
</table>

Inhibitory effects were evaluated by the CV staining assay as described in materials and methods. Each sample (4 g) was extracted by hot water (20 ml), and the extracts (100%) were diluted by the medium and their anti-*Candida* activity tested.

¹⁾ The concentrations of the extracts providing 50% or 80% inhibition (IC₅₀, IC₈₀) of *Candida* mycelial growth are indicated.
Table 3. The effects of herbal tea preparations against oral candidiasis mice

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of mice</th>
<th>Viable Candida cells (log10 CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16</td>
<td>5.61 ± 0.44</td>
</tr>
<tr>
<td>Lemongrass 62.5%</td>
<td>10</td>
<td>5.28 ± 0.75</td>
</tr>
<tr>
<td>Lemongrass 31.3%</td>
<td>5</td>
<td>5.80 ± 0.27</td>
</tr>
<tr>
<td>Lemongrass 6.25%</td>
<td>5</td>
<td>5.79 ± 0.31</td>
</tr>
</tbody>
</table>

Exp. 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of mice</th>
<th>Viable Candida cells (log10 CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>5.10 ± 0.39</td>
</tr>
<tr>
<td>Lemongrass 6.25%</td>
<td>6</td>
<td>5.56 ± 0.22</td>
</tr>
<tr>
<td>Lemongrass 1.25%</td>
<td>5</td>
<td>5.29 ± 0.17</td>
</tr>
</tbody>
</table>

Exp. 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of mice</th>
<th>Viable Candida cells (log10 CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>5.10 ± 0.39</td>
</tr>
<tr>
<td>Green Tea 6.25%</td>
<td>6</td>
<td>5.67 ± 0.31**</td>
</tr>
<tr>
<td>1.25%</td>
<td>5</td>
<td>5.66 ± 0.29</td>
</tr>
</tbody>
</table>

Exp. 3

4 g of lemongrass or green tea was extracted by hot water (20 ml) and the extracts were designated as 100% original sample.

Scores of the tongue disorder were determined as described in materials and methods.

The data were reported as the combination of three experiments (Exp.1〜Exp.3).

Viable Candida cells in each mouse were evaluated by CFU counted on a Candida GS agar plate.

Statistically significant difference was not observed.

P values of < 0.01 were considered as significant. (**: p values of < 0.01)

Table 4. The effects of cassia preparation by oral administration against oral candidiasis mice

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of mice</th>
<th>Viable Candida cells (log10 CFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>6.18 ± 0.15</td>
</tr>
<tr>
<td>Cassia 100%</td>
<td>7</td>
<td>5.67 ± 0.33**</td>
</tr>
<tr>
<td>Cassia 25%</td>
<td>8</td>
<td>6.04 ± 0.34</td>
</tr>
<tr>
<td>Cassia 5%</td>
<td>8</td>
<td>5.98 ± 0.49</td>
</tr>
</tbody>
</table>

Cassia (4 g) was extracted by hot water (20 ml) and designated as 100% original preparation.

Scores of the tongue disorder were determined as described in materials and methods.

Viable Candida cells in each mouse were evaluated by CFU counted on a Candida GS agar plate.

P values of < 0.01 were considered as significant. (**: p values of < 0.01)

epithelium of tongues of the non-treated mice as depicted in Fig. 2. On the tongues of mice treated with the cassia preparation, there were fewer PAS-positive fungal hyphae and lesional infiltration of inflammatory cells seemed relatively mild (Fig. 2B). Table 4 shows that oral treatment with 100% of cassia preparation improved the oral symptoms of the Candida infected mice, that is, there was a reduction of clinical score of the tongues and a lower number of CFU from the oral cavity. Table 4 also shows that administering 25% or 5% of cassia preparation did not improve the clinical score of tongues and decrease the viable cell numbers of C. albicans. The 100% cassia preparation contained about 200 mg/ml of dry matter.
Characterization of Candida inhibitory agent in the cassia preparation

The original cassia preparation obtained by the hot water extract was muddy, and contained insoluble small materials as described above (approximately 200 mg/ml). Thus, we examined whether the active principal in this cassia preparation for anti-Candida activity was soluble or not. After the hot water-extracted preparation was centrifuged, the sample separated into two layers, the upper layer of the supernatant and the lower layer of the precipitate. The inhibitory activity of each sample for Candida mycelial growth was shown by the number of dilutions providing 50% growth inhibition (Table 5). The number of dilutions for 50% growth inhibition of the original cassia preparation and lower layer was from 256 to 1024. For the upper layer, 20 - 100 times dilution was needed to achieve the same effect. All samples showed 80% inhibition of Candida mycelial growth by the same dilution level giving 50% inhibition. When the original cassia preparation was passed through filter paper (No.
2. Whatman, USA) or a disk filter (0.45 μm Millex filter, Millipore, USA), the inhibitory activity of *Candida* mycelial growth of each sample decreased to 1/100 the level of the original sample (data not shown). These data show that the majority of anti-*Candida* activity in the cassia preparation was associated with insoluble parts of the suspension particles.

Content of cinnamaldehyde and coumarin in the cassia preparation

To assess the active principals in the cassia preparation, the amount of cinnamaldehyde and coumarin in the preparation was examined, since these are known to be major components in cassia essential oil and reported as active substances for its antimicrobial activity. GC/MS analysis showed that original cassia preparation contained 19.5 mg/ml of cinnamaldehyde and 3.09 mg/ml of coumarin (Table 5). The contents in the supernatant were 5.38 mg/ml and 0.99 mg/ml, respectively, which were from 1/3 to 1/4 of the original and the precipitate fraction. This suggests that the precipitate fraction has not only anti-*Candida* activity but also includes the active principal component.

Inhibitory effects of cinnamaldehyde and coumarin against *Candida* mycelial growth

The *Candida* inhibitory activities of cinnamaldehyde and coumarin in the cassia sample were examined, and Table 6 shows their activities on the mycelial growth of *C. albicans in vitro*. IC₅₀ and IC₈₀ of cinnamaldehyde were 8.19–20.5 μg/ml. IC₅₀ of coumarin was 51.2–128 μg/ml and IC₈₀ of coumarin was 128–320 μg/ml; this indicated that the specific activity of cinnamaldehyde was superior to that of coumarin. Cassia preparation contained 19.5 mg/ml of cinnamaldehyde which was about 6–7 times that of coumarin. Therefore, we can assume that cinnamaldehyde in the cassia preparation has a more major role in anti-*Candida* activity than does coumarin. This assumption was tested by further experiments.

**Discussion**

We assessed the inhibitory activity of herbal tea and spice preparation against *Candida* mycelial growth. The results of *in vitro* assay showed that lemongrass, green tea, and cassia have stronger anti-*Candida* activity than the other tested herbs. By several infection experiments, we found that oral administration of cassia preparation inhibited *Candida* growth in the oral cavity and improved lesions on the lingual surface of the mouse tongue in a murine oral candidiasis model. This is the first report, as far as we know, to show that cassia as a conventional food showed a growth inhibitory effect against *Candida* cells and improved the symptoms of oral candidiasis in *Candida*-infected mice.

We also investigated the components in the cassia preparation which exercised a therapeutic effect as shown in Table 5. When the cassia preparation was centrifuged, it separated into an upper layer and lower layer. The *Candida* inhibitory activity of supernatant, of which dry matter content was about 1/10 of the original sample, was nearly 10 times lower than that of original sample. Additionally, when the sample was filtered to remove the particles, the inhibitory activity was reduced to 1/100th. These results indicate that under the conditions of hot water extraction in food processing, most of the active components were not dissolved in the water but were attached to or included in the cassia preparation.
particles.

The major substances playing a leading role in the inhibition of *Candida* mycelial growth in the cassia preparation were investigated. GC/MS analysis showed that cinnamaldehyde and coumarin were included in the cassia suspension. Both compounds are recognized to be dominant components of the essential oil in the cassia preparation. Table 5 shows that the concentration of cinnamaldehyde and coumarin in the supernatant was about 3 to 4 times lower than those of original sample, respectively. It can be assumed that the dose of cinnamaldehyde or coumarin corresponded to the inhibitory effect against *Candida* growth in this assay.

Table 5 also shows that the concentration of cinnamaldehyde was about 5 to 7 times as much as that of coumarin in all tested samples. Additionally, compared with IC₅₀ and IC₈₀ of cinnamaldehyde (8.19 μg/ml–20.5 μg/ml), coumarin (51.2–128 μg/ml, 128–320 μg/ml), and their mixture (8.19 μg/ml–20.5 μg/ml). cinnamaldehyde was assumed to contribute more to inhibition of the *Candida* growth than coumarin in the cassia sample (Table 6). We therefore concluded that cinnamaldehyde was playing the leading role in the *Candida* inhibitory activity in the cassia preparation, but that there might also be influence from other components. This assumption can be supported by the finding that this substance associated with particles in the cassia preparation, because cinnamaldehyde is known to be slightly soluble in water.

In this study, we demonstrated that cassia also may possibly be a candidate for improving or preventing oral candidiasis. Further work will be needed to establish details of a therapeutic usage of cassia preparation for oral candidiasis, such as antifungal effects for other *C. albicans* strains or *Candida* species, conditions of preparation, optimal dosage, period of administration, and interaction between the preparations and *Candida* cells. It is our hope that our studies will facilitate development of the application of spice and herbal foods in dietary use to prevent and improve oral candidiasis in elderly people and immunocompromised patients.

### References


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