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In vitro Antifungal Activity of Thai Herb and Spice Extracts against Food Spoilage Fungi

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ABSTRACT

The screening of Thai herbs and spices was carried out to investigate their *in vitro* antifungal activity against *Aspergillus niger, A. oryzae* and *Penicillium* sp. by using agar well diffusion method. Of thirteen plants tested, crude ethanol extracts of three, namely *Piper betel, Boesenbergia pandurata, Andrographis paniculata* exhibited antifungal activity against all test microorganisms. *Penicillium* sp. was more resistant to the extracts than *A. niger* and *A. oryzae*. The antifungal index of the selected plant extracts with various concentrations against test fungi was measured by agar dilution assay. With the increase of the concentration, the antifungal activity also increased. A complete fungal inhibition was observed when *Piper betel* extract concentration exceeded 1.50% (v/v). The antifungal index from the other plants ranged from 30 to 60%.

Key words: antifungal, herb extract, spice extract, *Piper betel*, antifungal index

INTRODUCTION

Contamination of foodborne pathogens and spoilage microorganisms is of great concern in food industries. Fungi, especially *Penicillium* species and *Aspergillus* species, are among the major causes of food spoilage, especially bakery products, intermediate-moisture food products, cheese, preserved fruit, and grain. Contamination of wheat bread was mainly *Penicillium* species (90-100%) and also *Aspergillus* species (Legan and Voysey, 1991). Besides the repelling sight of visible growth, fungi may cause off-flavor formation and the production of mycotoxins.

The use of chemical preservatives as antimicrobial agents to extend shelf life of food products and to ensure food safety has been widely practiced. These preservatives have been considered as food additives and are mostly synthetic substances, such as sorbic acid, benzoic acid, propionic acid and their salts. The use of these additives is regulated and limited by law, and their use must be stated on the product's label. However, today consumers demand less use of synthetic additives (Membre *et al.*, 2001).

In recent years, many attempts have put emphasis on the search of natural antimicrobial compounds that can properly serve the needs of food manufacturers and consumers. Various herbs and spices have been recognized by their medicinal value, in particular antimicrobial activity, and used throughout the past as an alternative approach to preserve foods. They contain antimicrobial compounds that may find useful as natural preservatives. Several studies have revealed the results on the preservative action of spices or their

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essential oils (Zaika, 1988). Methanolic extracts of *Solanum xanthocarpun* and *Datura metel* inhibited the growth of *Aspergillus fumigatus, A. flavus and A. niger* and their *in vitro* MICs were found to be 1.25-2.50 mg/ml by microbroth dilution (Dabur *et al.*, 2004). Neilsen and Rios (2000) had found that the mustard essential oil, which primarily contains allyl isothiocyanate, had shown a very significant antifungal effect against all test fungi for more than two weeks.

In Thailand, there are many herbs and spices that exhibit antimicrobial activity and may be used for food preservation. Few studies focused on the potential of herbs and spices as sources of antimicrobial compounds that could inhibit food spoilage fungi. Therefore, the objective of this study was to investigate antifungal properties of ethanol extract of Thai herbs and spices against fungi generally encountered in food spoilage. The antifungal index of the extracts was also determined.

MATERIALS AND METHODS

Plant materials

Thirteen Thai herbs and spices listed in Table 1 were purchased from markets in Bangkok, Thailand. About 500 g of each plant was cut into small pieces and dried at 50°C, for 24 hours. Then the materials were ground by using a grinder (Model HL-22) and kept at room temperature, in sealed plastic bags.

Test microorganisms

The test microorganisms used for antimicrobial activity screening were *A. niger*, *A. oryzae* and *Penicillium* sp. These microorganisms were obtained from the culture collection at the Department of Biotechnology, Kasetsart University, Bangkok, Thailand.

All fungi were cultured on potato dextrose agar (PDA) as the growth medium for all test fungi on petri dishes and incubated at room temperature for 5 days. The mycelium of the fungi was cut with a sterile cork borer (0.8 mm) and used for testing antifungal activity.

Preparation of crude extracts

Each dried, ground plant (10 g) was extracted with 100 ml of 95% ethanol at room temperature for 24 hours. The extract solution was filtered through a Whatman No. 4 filter paper. The

Table 1 List of herbs and spices used in the experiment.

Scientific name	Family name	Common name
Phyllanthus niruri Linn.	Euphorbiaceae	Egg women
Amomum krevanh Pierre	Lauraceae	Bay leave
Terminalia chebula Retz.	Combretaceae	Myrobalan
Piper betel Linn.	Piperaceae	Betel pepper
Curcuma longa	*Zingiberaceae*	*Turmeric*
Mentha cordifoli Opiz.	Labiatae	Kitchen mint
Eugenia caryophyllus Thunb.	Myrtaceae	Clove
Citrus hystrix DC.	Rutaceae	Leech lime, Kaffir
Ocimum sanctum Linn.	Labiatae	Holy basil
Piper sarmentosum Roxb.	Piperceae	
Cinnamomum iners Blume.	Lauraceae	Ceylon cinnamon
Boesenbergia pandurata (Roxb.) Sohltr.	Zingiberaceae	Fingerroot
Andrographis paniculata (Burm. F.) Nees.	Acanthaceae	Creat

solvent was removed from the sample by using a rotary vacuum evaporator (Eyela, Tokyo Rikakikai Co., Ltd., Japan). The sample was rotary evaporated at 50°C until it reached $^1/_4$ its volume. Fifty milliliter of distilled water was then added to the sample and the content was continuously rotary evaporated until it reached about 5 ml volume (Jaturapornchai, 2003). The residue obtained was diluted to the final volume of 15 ml using distilled water.

Screening of herbs and spices for their antifungal activity

The agar well diffusion method (Rauha *et al.*, 2000) was modified to test antifungal activity of the herb and spice extracts. Sample wells of PDA agar plates were prepared by using a sterile cork borer (0.88 mm in diameter). Each plate contained 4 wells, evenly distributed around the inoculum of test fungi that was placed at the center. The crude extract of 100 µl was poured into each well. The plates were incubated at room temperature for 5 days. The clear zone surrounding each well indicated its inhibition activity.

Determination of antifungal index (%)

Antifungal assays were performed based on the method described by Wang *et al*. (2005) for testing the selected plant extracts with high inhibition activity. Four amounts (0.15, 0.30, 0.45, 0.6 ml) of each crude extract were individually added into sterile PDA (20 ml) to obtain the final concentration of 0.75, 1.50, 2.25, 3.00% (v/v). PDA without crude extract served as a control. The inocula of 5 days test fungi (0.88 mm) were placed on PDA as shown in Figure 1. The plates were incubated at room temperature for 5 days. The antifungal index (%) was calculated as follows:

$$\text{Antifungal index}\,(\%) = \frac{D_{Control} - D_{Extract}}{D_{Control}} \times 100,$$

where $D_{Control}$ = the diameter of growth in the control plate and $D_{Extract}$ = diameter of mycelial growth in the test plate. Each experiment was done in duplication.

RESULTS AND DISCUSSION

Screening of thirteen herbs and spices for antifungal activity

Thirteen herbs and spices were screened for their antifungal activity by using agar diffusion assay. The results are shown in Table 2. In general, most of the extracts evaluated for antifungal activity were more active against *Aspergillus* spp. compared with *Penicillium* sp. Among the plants showing antifungal activity, *Piper betel*, *Boesenbergia pandurata* and *Andrographis paniculata* showed activity against all test fungi while *Terminalia chebula* and *Ocimum sanctum* did not inhibit the growth of the test fungi. Interestingly, *A. orezae* was the most sensitive fungus to the herb and spice extracts, followed by *A. niger* and *Penicilium* sp.. From this result, the active extracts from *Phyllanthus niruri*, *Amomum krevanh*, *Piper betel*,

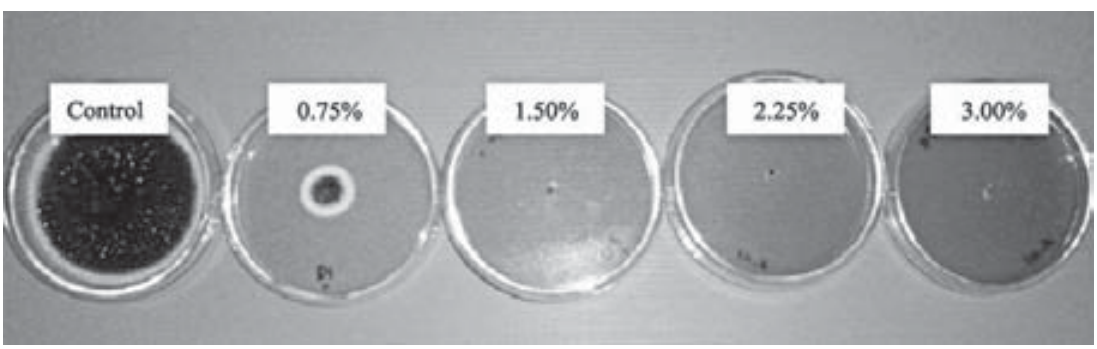

Figure 1 Antifungal effect of ethanol extract from *Piper betel* at various concentrations against *A. niger*.

Table 2 Antifungal activity of herb and spice extracts against test fungi.

Plants	Microorganisms		
	A. niger	*A. oryzae*	*Penicilium sp.*
Phyllanthus niruri	+	+	-
Amomum krevanh	-	+	+
Terminalia chebula	-	-	-
Piper betel	+	+	+
Curcuma mangga	+	+	-
Mentha cordifoli	+	+	-
Eugenia caryophyllus	-	+	-
Citrus hystrix	-	+	-
Ocimum sanctum	-	-	-
Piper sarmentosum	+	-	-
Cinnamomum bejolghota	+	+	-
Boesenbergia pandurata	+	+	+
Andrographis paniculata	+	+	+

- = no inhibition; + = inhibition. The amount of the extract used was 100 µl per well.

Boesenbergia pandurata and *Andrographis paniculata* were selected for further study.

Antifungal index (%) of herbs and spices

Figure 2 shows the effect of various extract concentrations of selected plant extracts on the inhibition of *A. niger*, *A. oryzae* and *Penicillium* sp., respectively. With the increase of concentration (0.75-3.00 %, v/v), antifungal index value of the extracts increased. *Piper betel* extract was found to be very strong antifungal agent, compared with the other plants against all test fungi. *Piper betel* extract at the concentration of 2.25, 1.5 and 1.5 % (v/v) caused complete inhibition, 100% antifungal index, of the growth of *A. niger*, *A. oryzae* and *Penicillium* sp. respectively, while the other extracts showed antifungal index ranging from 30 to 60%.

Crude ethanol extract of *Piper betel* was a mixture of nonvolatile and volatile components. The principal active antimicrobial component of *Piper betel* was eugenol (Kim *et al*., 1995). Several reports have shown the antifungal effect of eugenol against *Aspergillus* spp. and *Penicillium* spp. in various foods (Bullerman *et al*., 1977; Vazquez *et al*., 2001).

In Figure 2, *Piper betel* extracts was the most effective antifungal agent, followed by *Boesenbergia pandurata* and *Andrographis paniculata*. The extract of *Phyllanthus niruri*, showed specific effect against *Aspergillus* spp.

CONCLUSION

This study showed that crude ethanol extracts of 11 Thai herbs and spices could inhibit certain fungi, commonly cause food spoilage. Extracts from *Piper betel*, *Boesenbergia pandurata* and *Andrographis paniculata* inhibited all test fungi. Moreover, *Piper betel* extract at the concentration of 2.25, 1.5, and 1.5 % (v/v) caused complete inhibition, 100% antifungal index, of the growth of *A. niger*, *A. oryzae* and *Penicillium* sp., respectively, while the other extracts showed antifungal index ranging from 30 to 60%. The results revealed that the extract from Thai herbs and spices could be used as source of natural antifungal agents which may be added directly into food or incorporated in packaging materials. More researches are required to explore the antifungal activity of Thai herbs and spices.

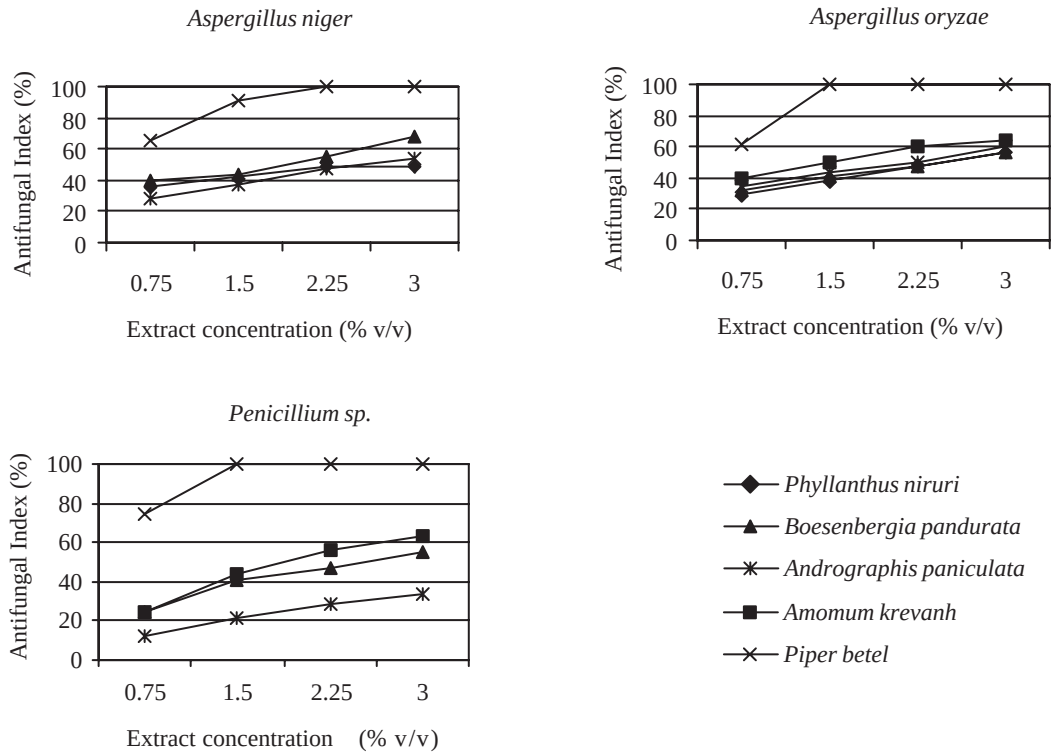

Figure 2 Effect of various concentration of selected herb and spice extracts on the inhibition of *A. niger*, *A. oryzae* and *Penicillium* sp.

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