Comparison of Antibacterial Activity Against Food-Borne Bacteria of *Alpinia galanga, Curcuma longa,* and *Zingiber cassumunar*

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ABSTRACT

The aim of this study was to compare the antibacterial action of Alpinia galanga Linn., Curcuma longa Linn. and Zingiber cassumunar Roxb. against food-borne bacteria and to search for the most effective fraction from these plants. The crude extracts and the essential oils of the plant rhizomes were used as test fractions. Several strains of food-borne bacteria were used as test microorganisms. The crude ethanolic extract of A. galanga inhibited Corynebacterium sp., Staphylococcus aureus and four strains of Escherichia coli. C. longa and Z. cassumunar inhibited only Corynebacterium sp. The essential oils of the two plants exhibited dramatically stronger antibacterial activity than their crude extracts. The antibacterial activity of only 40 µg of A. galanga essential oil was as effective as 15,000 µg of its crude extract in inhibiting E. coli. The essential oil of Z. cassumunar inhibited E.coli and Pasteurella multocida, whereas its crude extract did not. The essential oils of A. galanga and Z. cassumunar offered the highest potential for further investigating their minimum inhibitory and bactericidal concentrations. The antibacterial action of the oils appears to be a bactericidal effect. Of the plants studied, A. galanga was the most effective at inhibiting food-borne bacteria. The antibacterial activity of the essential oil of A. galanga was two times higher than that of Z. cassumunar.

Keywords: *Alpinia galanga, Curcuma longa, Zingiber cassumunar,* Food-borne bacteria, Essential oil, Extract

INTRODUCTION

Food safety is a highly important issue for both consumers and the food industry, particularly with the rising number of cases of food-associated infections. The food manufacturing industry is continually working to control the pathogen level in food products. The most effective way to minimize food-contaminated microorganisms is to add an effective antimicrobial agent into food products. While both chemical and natural agents are used today, consumers increasingly demand natural agents as additives as they are considered healthier, less toxic, and more natural tasting.

Plants in the family Zingiberaceae are widely grown in Thailand and other tropical areas. *A. galanga* has been used as a medicine for curing stomach aches in China and Thailand (Yang and Eilerman, 1999). Its fresh rhizome has a characteristic fragrance as well as pungency, hence its rhizome is used as an essential component in Thai curry paste and other Asian foods. It has been reported that the crude extracts of *A. galanga* have antioxidant and antimicrobial activities (Habsah et al., 2000, Mayachiew and Devahastin, 2008). Janssen and Scheffer (1985) reported that the monoterpenes in the essential oil from fresh rhizomes of *A. galanga* exhibit an antimicrobial activity against *Trichophyton mentagrophytes*.

Curcuma longa, or tumeric in English, is a yellowish rhizome which has been used by traditional medicine practitioners in Southeast Asia and West Africa to treat cough, fever, liver and urinary diseases, inflammation, vascular disorders, and hypertension (Bakhru, 1997). Phytochemical screenings have shown that the main constituents of *C. longa* are curcumin, zingiberine, phellandreen, sabenene, cineol, borneaol, sesquiterpenes, curcuminoids, and essential oils (Jayaprakasha et al., 2005). In addition, it also contains fats, fibres, vitamins, proteins, minerals, and carbohydrates (Bakhru, 1997). *Curcuma longa* has been extensively studied for its biological activities, including: anti-microbial (Lutomski et al., 1974), anti-platelet (Srivastava et al., 1995), hepatoprotective (Miyakoshi et al., 2004), hypoglycemic (Sharma et al., 2006), anti-ulcer (Kositchaiwat et al., 1993), wound healing (Sidhu et al., 1998), neuroprotective (Rajakrishnan et al., 1999), anti-hemolytic (Mathuria and Verma, 2007), anti-arthritic (Funk et al., 2006), and anti-oxidant (Toda et al., 1985; Adaramoye et al., 2002).

Zingiber cassumunar is used as a traditional medicine in Southeast Asia, especially in Thailand and Indonesia. It has also long been used as an embrocation (Phongbunrod, 1965). Phytochemical and pharmacological studies have demonstrated that phenylbutenoids and their dimers are the major active compounds. These compounds from *Z. cassumunar* have been found to have antioxidant (Jitoe et al., 1992), anti-inflammatory (Panthong et al., 1990), antifungal (Bin et al., 2003), and anticancer activities (Lee et al., 2007). They can also be used as insecticidal constituents (Nugroho et al., 1996).

Bacterial contamination of food can lead to certain food-borne diseases. Although the antimicrobial actions of these three Zingiberaceus plants – *A. galanga*, *C. longa*, and *Z. cassumunar* – have been widely reported, an understanding of their activity against food-borne bacteria is very limited. The purpose of this study was to compare the antibacterial action of these three plants against food-borne bacteria and to search for the most effective fraction from these plants.

MATERIALS AND METHODS

Plant materials

Rhizomes of *A. galanga*, *C. longa* and *Z. cassumunar,* aged 6-12 months and cultured in the northern part of Thailand, were collected. Voucher specimens were deposited at the Herbarium of the Faculty of Pharmacy, Chiang Mai University, Thailand.

Essential oil extraction

The fresh rhizomes were chopped and subjected to hydro-distillation for 6 h using a Clevenger apparatus to obtain the essential oil fraction. The essential oil obtained was dried using anhydrous sodium sulphate and then stored in an airtight dark bottle at 4°C until use.

Preparation of the crude extracts

Dried rhizome powder of the plants was prepared by slicing the fresh rhizomes into small pieces and drying at 60°C for 48 h. The dried rhizome was ground into fine powder to prepare for solvent extraction. Dried powdered sample of *A. galanga, C. longa,* and *Z. cassumunar* were separately weighed and macerated in a suitable solvent, e.g., ethanol, hexane, or ethyl acetate for four cycles at room temperature. Each cycle lasted 7 days with 1 h mechanical stirring everyday. The volume ratio of the powdered sample to the solvent used was approximately 1:3. The filtrates of the same plant from each macerated solvent and cycle were pooled. The solvent was removed under reduced pressure at 45°C by using a rotary evaporator. The resulting extracts were stored in dark bottles at 4°C until use.

Microbial strains

The food borne microorganisms used in this study were composed of *Escherichia coli* ATTCC 25922, *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 14028, field strains of Pasteurella multocida, *Corynebacterium* sp., and three field strains of *E. coli*. Tryptic soy broth (TSB) or tryptic soy agar (TSA) from Merck, Darmstadt, Germany was used for culturing the bacteria. All strains were stored at -20°C in glycerol and regenerated twice before testing.

Screening for antimicrobial activity

Comparative antimicrobial activity of the essential oils and the crude extracts was studied by using the agar diffusion method (NCCLS, 1999). A single colony of the test bacteria was transferred into TSB and incubated overnight. Three ml of each culture were mixed with 100 ml of melted TSA at about 45°C and poured onto the surface of an agar plate containing 2% agar. Wells 8 mm in diameter were made after the agar was solidified. Each test oil or crude extract was diluted to the appropriate concentration and filled into the well. Distilled water and pure solvent were used as negative controls. The plates then were incubated at 37°C for 18-24 h. The experiment was done in triplicate. The inhibition zone (including the diameter of the well) was recorded.

Minimum inhibitory and bactericidal concentrations

Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined with the essential oil by means of a broth dilution method introduced by Yu et al. (2004). Tween 20 was used to solubilize the extracts. All tests were performed in TSB. Serial doubling dilutions of the oil were prepared. The final concentration of each bacterial test strain was adjusted

to 4 x 10^4 cfu/ml. Plates were incubated at 37°C for 24 h. The MIC is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. The microorganism growth was indicated by the turbidity. To determine MBC, broth was taken from each well and further incubated in TSA at 37°C for 24 h. The MBC was defined as the lowest concentration of the essential oil at which incubated microorganism was completely killed. Each test was performed in three replicates.

RESULTS AND DISCUSSION

Yields of extraction

Hydrodistillation of the fresh *A. galanga, C. longa,* and *Z. cassumunar* rhizomes yielded essential oils of differing amounts as shown in Figure 1(A). *Z. cassumunar* rhizome gave the highest yield of 0.30% v/w. Aengwanich (2002) reported that *Z. cassumunar* rhizome grown in Khon Kaen contained 3.49% volatile oil. This result showed that the environment plays an important role in the production of compounds in many plants, thus the content of essential oil in the plant may change in response to the environment. *Alpinia galanga* and *C. longa* rhizomes showed the same yield of 0.1% v/w. For solvent extraction of dried rhizome powder, ethanol was first used as an extracting solvent to obtain the crude ethanolic extracts. *C. longa* rhizome produced the highest yield of 4.1% w/w, followed by the rhizome of *Z. cassumunar*, which yielded 1.9% w/w. *A. galanga* rhizome produced the lowest yield of about 1.6% w/w as shown in Figure 1(B).

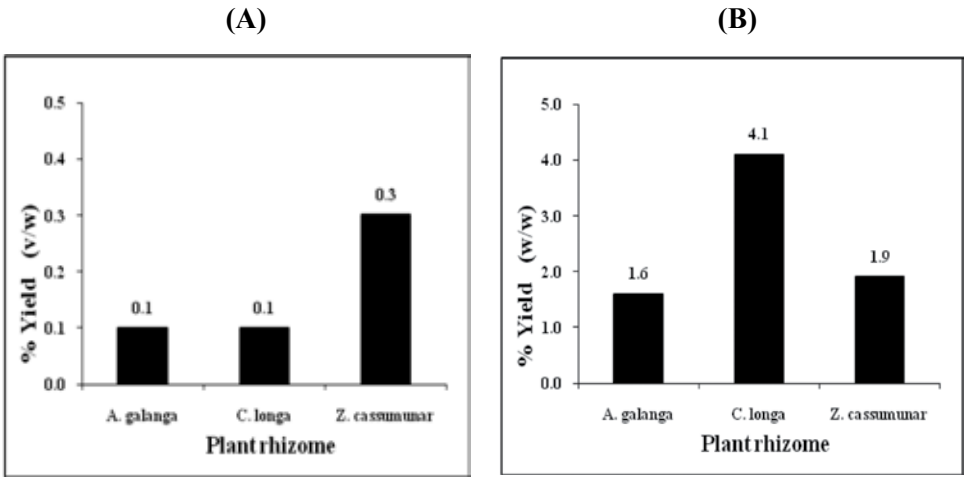

Figure 1. The yield of essential oils (A) and crude ethanolic extracts (B) of the three plants.

Antibacterial activity of the extracts

The comparative study of the antibacterial activity of the three plant crude ethanolic extracts was first done using the standard strains of food-borne bacteria, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *S. typhimurium* ATCC 14028. The amount of each test extract filled in each well was 15 mg/well. The antibacterial potency of the samples expressed as the growth inhibition clear zones is shown in Table 1. Only the extract of *A. galanga* inhibited the test bacteria. It has been reported previously that acetone extracts of *A. galanga* could inhibit the growth of many multi-drug resistant bacteria (Latha et al., 2009). The results from the present study supported the antibacterial activity of this plant. The ethanolic crude extracts of *C. longa* and *Z. cassumunar* did not inhibit the test bacteria. The field strains of bacteria – three stains of *E. coli*, one strain of *P. multocida*, and one strain of Corynebacterium sp. – were investigated. The results are shown in Table 2. *A. galanga* inhibited all test strains, except *P. multocida*, whereas the crude extracts of *C. longa* and *Z. cassumunar* inhibited only one field strain of *Corynebacterium spp*.

Table 1. Inhibition zone (mm) of the extracts against the standard strains.

Bacteria	Plant rhizome		
	A. galanga	*C. longa*	*Z. cassumunar*
E.coli ATCC25922	11.0	NZ	NZ
S.typhimurium ATCC14028	7.8	NZ	NZ
S.aureus ATCC25923	11.8	NZ	NZ

Note: NZ = no inhibition zone.

Table 2. Inhibition zone (mm) of the extracts against the field strains.

Bacteria	Plant rhizome		
	A. galanga	*C. longa*	*Z. cassumunar*
E.coli (strain 1)	12.9	NZ	NZ
E.coli (strain 2)	11.7	NZ	NZ
E.coli (strain 3)	11.5	NZ	NZ
P. multocida	NZ	NZ	NZ
Corynebacterium spp.	21.3	17.4	13.5

Note: NZ = no inhibition zone.

The results from this experiment indicated that among the crude ethanolic extracts of the three plants, *A. galanga* produced the most potent extract for use against both Gram-positive and Gram-negative food-borne bacteria. Given the positive test results for *A. galanga*, the dried powder of *A. galanga* rhizome was investigated further by separately macerating in two more solvents: hexane and ethyl acetate. The yield of the hexane extract of *A. galanga* was the highest at 3.4% w/w, whereas those of ethyl acetate and ethanolic extracts were similar at approximately 1.6% w/w (Figure 2). The antibacterial activity of these extracts

was investigated and the results are shown in Table 3. Both ethanolic and ethyl acetate extracts of *A. galanga* demonstrated high antibacterial effect against all test bacterial strains. In contrast, the hexane extract exhibited antibacterial action against only one strain of *S. aureus.*

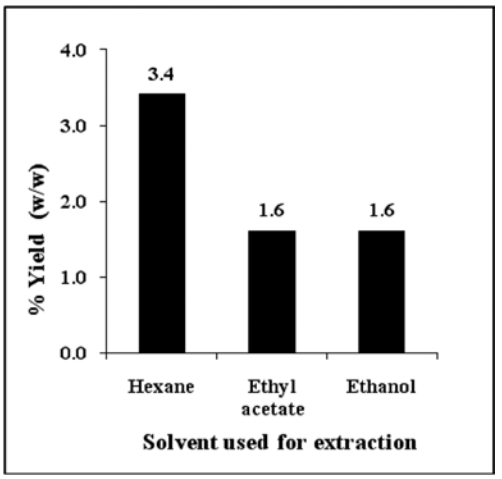

Figure 2. The yield of *A. galanga* extracts obtained from different solvents.

Table 3. Inhibition zone (mm) of *A. galanga* extracts obtained from different solvents.

Bacteria	Extracting solvent		
	Hexane	Ethyl acetate	Ethanol
E.coli ATCC25922	NZ	9.0	11.0
S.typhimurium ATCC14028	NZ	7.8	7.8
S.aureus ATCC25923	21.7	16.5	11.8

Note: NZ = no inhibition zone.

Antibacterial activity against food-borne bacteria of the essential oils was screened for, first with the same standard strains and performance as the crude extracts. The amount of oil used was approximately 40 µg/well. The results are shown in Table 4. Only the essential oils of *A. galanga* and *Z. cassumunar* demonstrated antibacterial activity to the test standard strains. The field strains were further investigated and the results are shown in Table 5. The essential oil of *A. galanga* inhibited all test field strains of *E. coli,* whereas the oil of *Z. cassumunar* inhibited only two field strains of *E. coli*. *Curcuma longa* inhibited only one test field strain of *Corynebacterium* sp.

Table 4. Inhibition zone (mm) of the essential oils against the standard strains.

Bacteria	Plant rhizome		
	A. galanga	*C. longa*	*Z. cassumunar*
E.coli ATCC25922	10.2	NZ	28.2
S.typhimurium ATCC14028	9.5	NZ	35.7
S.aureus ATCC25923	10.0	NZ	17.5

Note: NZ = no inhibition zone.

Table 5. Inhibition zone (mm) of the essential oils against the field strains.

Bacteria	Plant rhizome		
	A. galanga	*C. longa*	*Z. cassumunar*
E.coli (strain 1)	10.5	NZ	NZ
E.coli (strain 2)	10.4	NZ	11.4
E.coli (strain 3)	9.3	NZ	11.7
P. multocida	NZ	NZ	NZ
Corynebacterium spp.	NZ	17.4	NZ

Note: NZ = no inhibition zone.

The essential oils of the three plants exhibit higher inhibition potential against food-borne bacteria than their crude extracts. The essential oils of *A. galanga* and *Z. cassumunar* were selected for further determination of minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC), since both oils exhibited antibacterial activity against more strains than *C. longa* oil. The standard strains of *S. aureus* and *E.coli* were used as the representative of the Gram-positive and Gram-negative bacteria, respectively. The results are shown in Figure 3. The MIC values of the essential oils of *A. galanga* and *Z. cassumunar* to the test Gram-positive *S. aureus* as shown in Figure 3(A) and Gram-negative *E. coli* as shown in Figure 3(B) were of the same values as their corresponding MBC. This suggested that the inhibitory bacterial action of the essential oils was of a bactericidal effect. The MBC value of *Z. cassumunar* oil against both test strains was two times higher than that of *A. galanga* oil, indicating the bactericidal effect of *A. galanga* oil was two times stronger than that of *Z. cassumunar*.

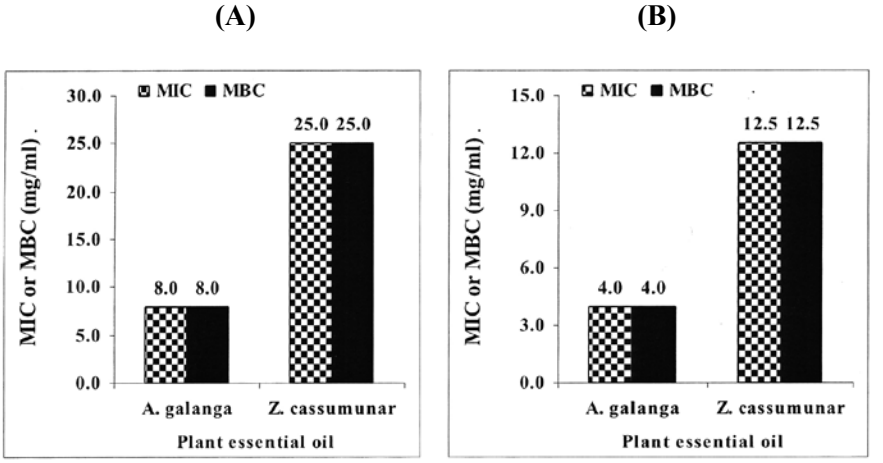

Figure 3. MIC and MBC values of the essential oils against *S. aureus* (A) and *E. coli* (B).

CONCLUSION

The present study has demonstrated the comparative antibacterial activity among three edible rhizomes of *A. galanga, C. longa,* and *Z. cassumunar*. The extract and essential oil of *A. galanga* were the most effective fractions in comparison with the other two extracts and essential oils, respectively. Both extract and essential oil of *A. galanga* inhibited most strains of food-borne bacteria used in this study. The essential oil of *A. galanga* exhibited dramatically stronger antibacterial activity than its crude extract. The essential oil of *Z. cassumunar* inhibited some field strains of *E. coli*, whereas the essential oil of *C. longa* inhibited only one strain of *Corynebacterium* sp. The study on MIC and MBC of the essential oils of *A. galanga* and *Z. cassumunar* indicated that the antibacterial action of both oils was a bactericidal effect. The antibacterial activity of the essential oil of *A. galanga* was approximately two times higher than that of *Z. cassumunar*.

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