Kasetsart J. (Nat. Sci.) 40 : 159 - 165 (2006)

Antimicrobial Activity of Spice Extracts against Pathogenic and Spoilage Microorganisms

Ekkarin Pattaratanawadee[1], Chitsiri Rachtanapun[1]*, Penkhae Wanchaitanawong[2] and Warapa Mahakarnchanakul[1]

ABSTRACT

The ginger, galangal, turmeric, and fingerroot extracts were determined for their antimicrobial activities against foodborne pathogenic bacteria, spoilage bacteria and fungi by using agar dilution assay. *Salmonella enterica* serotype Typhimurium and *Eschericia coli* O157:H7 were resistant to ginger, galangal, turmeric, and fingerroot extracts. Minimal inhibitory concentrations (MICs) of ginger, galangal, turmeric and fingerroot extracts against those gram-negative bacteria were 8–10% (v/v). Fingerroot extract showed stronger inhibitory activity against *Listeria monocytogenes*, *Bacillus cereus*, and *Staphylococcus aureus* than ginger, turmeric, and galangal extracts. MICs of fingerroot extract was 0.2–0.4% (v/v). For the spoilage bacteria *Lactobacillus plantarum* and *L. cellobiosus*, galangal extract gave the most efficiency of with MIC at 4% (v/v). The results showed that fingerroot and ginger extracts had antifungal activity ranging from 8 to 10 and ≥10% (v/v) against *Aspergillus flavus*, *A. niger*, *A. parasiticus* and *Fusarium oxysporum*, respectively. Moreover, inhibition over time of *E. coli* O157:H7 was studied in TSB added with spice extracts at concentrations ranging from 8 to 10% (v/v). The 8% galangal and 10% fingerroot extracts showed bactericidal effect at 36 hours and 9 hours, respectively. While 8% turmeric extract showed bacteriostatic effect. In conclusion, rhizomatous spice extracts had antimicrobial effect against some spoilage and pathogenic microorganisms, thus it has potential to be used as natural preservative agents.

Key words: antimicrobial activity, spice, spice extracts, pathogenic microorganisms, spoilage microorganisms

INTRODUCTION

Nowaday, consumers increasingly concern about food safety. The foods must not contaminate with either pathogenic microorganisms or hazard chemical agents. Moreover, consumers prefer fresh foods, that have long shelf–life. Therefore, it has led to a search for novel antimicrobial compound from natural sources. Spices are used widely in the food industry as flavors and fragrances. Besides, they also exhibit useful antimicrobial properties (Pruthi 1976; Roller, 2003) and were used in food industry for shelf-life extension and wholesomeness.

Some rhizomatous members of the Zingiberaceae family such as ginger (*Zingiber*

[1] Department of Food Science and Technology, Faculty of Agro – Industry, Kasetsart University, Bangkok 10900, Thailand.
[2] Department of Biotechnology, Faculty of Agro – Industry, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: fagicrt@ku.ac.th

officinale Rosc), galangal *(Alpinia galanga* Stuntz), turmeric (*Curcuma longa* Linn.), and fingerroot (*Boesenbergia pandurata* Schltr) have been used extensively as condiment for flavoring and local medicines for stomachache, carminative, and antiflatulent. They are known to contain various antimicrobial agents (Hirasa and Takemasa, 1998; Guzman and Siemonsma, 1999; Oonmetta – aree *et al.*, 2006). For example, protein from ginger rhizome was able to inhibit various fungi including *Botrytis cinerea, Fusarium oxysporum, Mycosphaerella arachidicola* and *Physalospora piricola* (Wang and Ng, 2005). Furthermore, the ethanol extract of the galangal had inhibitory effect against *Staphylococcus aureus* (Oonmetta – aree *et al*., 2006). The antimicrobial effects of essential oil from turmeric could inhibit *Bacillus cereus* growth (Burt, 2004). In addition, both maceration and high–intensity ultrasound of the isopropanol extract of the fingerroot had antimicrobial efficacy against *Salmonella* Typhimurium DT104 (Thongson *et al*., 2004). However, those previous studies about antimicrobial of Zingiberaceae extracts, were obtained from using different solvents, and various extraction methods. That caused incomparable antimicrobial activity.

The objective of this study was to determine the antimicrobial activity of maceration ethanol extracts obtained from ginger, galangal, turmeric, and fingerroot by determining the minimum inhibitory concentrations (MICs) against pathogenic bacteria, spoilage bacteria and fungi.

MATERIALS AND METHODS

Spices preparation

Rhizomes of ginger (*Zingiber officinale* Rosc), galangal *(Alpinia galanga* Stuntz), turmeric (*Curcuma longa* Linn.) and fingerroot (*Boesenbergia pandurata* Schltr) were purchased from a local market in Bangkok, Thailand. The fresh spices were washed by 50 ppm chlorine solution, sliced and dried in a tray – dryer oven at 50°C for 24 hours. Dried spices were kept frozen in a plastic zip bag until use.

Spice extracts preparation

Ten grams of the dried spices were minced by the blender and extracted by 100 ml of 50% (v/v) ethanol in 250 ml erlenmeyer flask, then shaken at 250 rpm in a reciprocal shaker at room temperature for 24 hours. After maceration, two flasks were collected and mixed together. The spice extracts were separated from plant materials by filter paper (Whatman no.4). The solvent was evaporated by rotary evaporator until few milliliters left and adjusted volume of the extracts by adding 50% (v/v) ethanol to get 25 ml in volumetric flask. Solid particle of the extracts was separated by centrifugation at 3,000 rpm for 15 min. Supernatant was filtered through 0.45 mm membrane. The filtrate was kept in the sterile vial at 0°C until use.

Microorganisms preparation

Tested pathogenic bacteria comprised of *Salmonella enterica* serotype Typhimurium DT104 which are multi-antibiotic resistant strains (2380, 2486, 2576 2582) and typical strain (13311), *Escherichia coli* O157:H7, *Listeria monocytogenes* (strain 101, 108, 310, Scott A, and V7), *Bacillus cereus*, and *Staphylococcus aureus*. *S.* Typhimurium, *E. coli* O157:H7 and *S. aureus* were grown in tryptic soy broth (TSB) at 37°C, except *B. cereus* was cultured at 30°C, for 18 hours. *Listeria* sp. was grown in TSB containing 0.6% yeast extract (TSBYE) at 37°C for 18 hours. Spoilage bacteria namely *Lactobacillus plantarum* (strain PD26 and PD110) and *Lactobacillus cellobiosus* (strain RE33, PD32, PD40, PD55 and PD112) were grown in DE MAN, ROGOSA and SHARPE (MRS) broth at 37°C for 18 hours. Fungi namely *Aspergillus flavus, Aspergillus niger*, *Aspergillus parasiticus* and *Fusarium oxysporum*

were grown in malt extract agar (MEA) at 25°C for 4 days.

Determination of minimum inhibitory concentrations

An agar dilution assay was modified from the National Committee for Clinical Laboratory Standards (NCCLS, 2002) and used, for determination of the MIC. Tube 13 ml molten agar were mixed with 2 ml spice extracts to obtain final concentrations ranging from 0.1 to 10% (v/v) and then poured into plate and allowed the agar to solidify. Bacterial strains were cultured and serially diluted in 0.1% (w/v) peptone water to *ca.* 7 log CFU/ml. The test strains were then added to the plate in 1 µl spots to maintain *ca*. 4 log CFU/spot and incubated at 37°C, except *B. cereus* was cultured at 30°C for 24 and 48 hours. Mycelium of the fungi was cultured on MEA for 4 days and cut with a sterile cork borer (5 mm diameter) and laid well the agar plug on MEA contained tested spice extracts. The plates were incubated at 25°C for 7 days before determination. The MICs of bacteria or fungi were defined as the lowest concentration at which no growth was observed after incubation for 24 hours or 7 days, respectively.

Inhibition over time

E. coli O157:H7 was grown in TSB at 37°C for 18 hours and transfered 5 ml into 250 ml erlenmeyer flask containing 40 ml TSB with 8%, 9% and 10% of spice extracts. Samples were taken at 0, 1, 3, 6, 9, 12, 24 and 36 hours and viable cells were counted on tryptic soy agar (TSA) after incubation at 37°C for 24 hours.

RESULTS AND DISCUSSION

Antimicrobial activity of ethanolic spice extracts from Zingerberaceae was tested with pathogenic bacteria, spoilage bacteria and fungi by using agar dilution method. In Table 1 MICs of ginger, galangal and fingerroot extracts against *E. coli* O157:H7 were 9-10% (v/v). Similarly, MICs of five of strains *S.* Typhimurium were

Table 1 Minimum inhibitory concentrations (%, v/v) of the ethanolic spice extracts against pathogenic bacteria.

Tested microorganisms	MICs of the spice extracts			
	Ginger	Galangal	Turmeric	Fingerroot
Salmonella Typhimurium				
DT104 strain 2380	10	10	10	10
DT104 strain 2486	9	10	8	8
DT104 strain 2576	8	9	8	9
DT104 strain 2582	9	10	10	10
typical strain 13311	9	10	9	10
Escherichia coli O157:H7	9	10	10	10
Listeria monocytogenes				
strain 101	1	6	0.9	0.3
108	1	5	0.9	0.2
310	2	5	2	0.3
Scott A	2	4	2	0.3
V7	2	5	2	0.4
Bacillus cereus	0.4	6	1	0.2
Staphylococcus aureus	2	7	3	0.3

8-10% (v/v). There were no difference of MICs between the spice extracts of multi-resistant strains (DT104) and typical strain (13311). Fingerroot extract had the highest antimicrobial activity against gram-positive pathogenic bacteria that comprised of *Listeria* sp., *B. cereus* and *S. aureus*. Its MICs against gram-positive pathogenic bacteria ranging from 0.2 to 0.4% (v/v).

Lactic acid bacteria namely *L. plantarum* and *L. cellobiosus* which were isolated from fermented rice noodle process (Sribuathong, 2005), were resistant to ginger, turmeric and fingerroot. However, they were susceptible to the galangal extract. MICs of galangal extract was 4% (v/v). (Table 2)

The fungi in this study were classified as spoilage fungi (*A. niger* and *F. oxysporum*) and aflatoxin producing fungi (*A. flavus* and *A. parasiticus*). Galangal and turmeric extracts could not inhibit the fungal growth at the concentration of 10% (v/v). Ginger and fingerroot had inhibitory efficiency against all tested fungi. MICs of ginger was 10% (v/v). Among spices tested, fingerroot showed the strongest antifungal activity. The MICs of fingerroot for *A. niger* was 8% (v/v).

E. coli O157:H7 is gram negative bacteria which has important to food safety in food production, processing and packaging environment (Burgula *et al*., 2006) so it was chosen as model bacteria to study death kinetic study in TSB. The concentrations of the spice extracts tested were 8- 10% (v/v) which referred to their MICs. Death kinetic of *E. coli* O157:H7 in combination of TSB and spice extracts revealed that ginger could not eliminate the culture, but increasely extended lag phase. Galangal and fingerroot showed bactericidal effect at 8% and 10% (v/v) at 36 and 9 hours, respectively, while 8% turmeric extract showed bacteriostatic effect.

The spice extracts of this research

Table 2 Minimum inhibitory concentrations (%, v/v) of the ethanolic spice extracts against spoilage bacteria.

Tested microorganisms	MICs of the spice extracts			
	Ginger	Galangal	Turmeric	Fingerroot
Lactobacillus plantarum				
Strain PD26	>10	4	10	10
PD110	10	4	10	10
Lactobacillus cellobiosus				
Strain RE33	<6	4	10	10
PD32	10	4	8	6
PD40	10	4	10	10
PD55	>10	4	10	10
PD110	10	4	10	10

Table 3 Minimum inhibitory concentrations (%, v/v) of the ethanolic spice extracts against fungi.

Tested microorganisms	MICs of the spice extracts			
	Ginger	Galangal	Turmeric	Fingerroot
Aspergillus flavus	10	>10	>10	>10
Aspergillus niger	10	>10	>10	8
Aspergillus parasiticus	>10	>10	>10	10
Fusarium oxysporum	10	>10	>10	< 8

exhibited inhibitory activity against pathogenic and spoilage bacteria. This is in agreement with many previous studies (Thongson *et al*., 2004, 2005; Oonmetta – aree *et al*., 2006) but there are many methods used for determining antimicrobial effectiveness. This situation led to several difficulties such as comparing results from different laboratories, determining antimicrobial effectiveness, establishing minimal inhibitory concentrations, and evaluating antimicrobial spectrum (Davidson *et al*., 1993).

The ethanolic spice extracts from Zingiberaceae (ginger, galangal, turmeric, and fingerroot) contained many chemical components in their extracts including phenolic compounds and its derivative compounds, the esters of weak acid, fatty acid, terpenes and others (Burt, 2004; Oonmetta – aree *et al.*, 2006). Since the large number of different chemical compounds presented in these crude extracts, therefore, chemical components can affect multiple target sites against the bacterial cells. The chemical

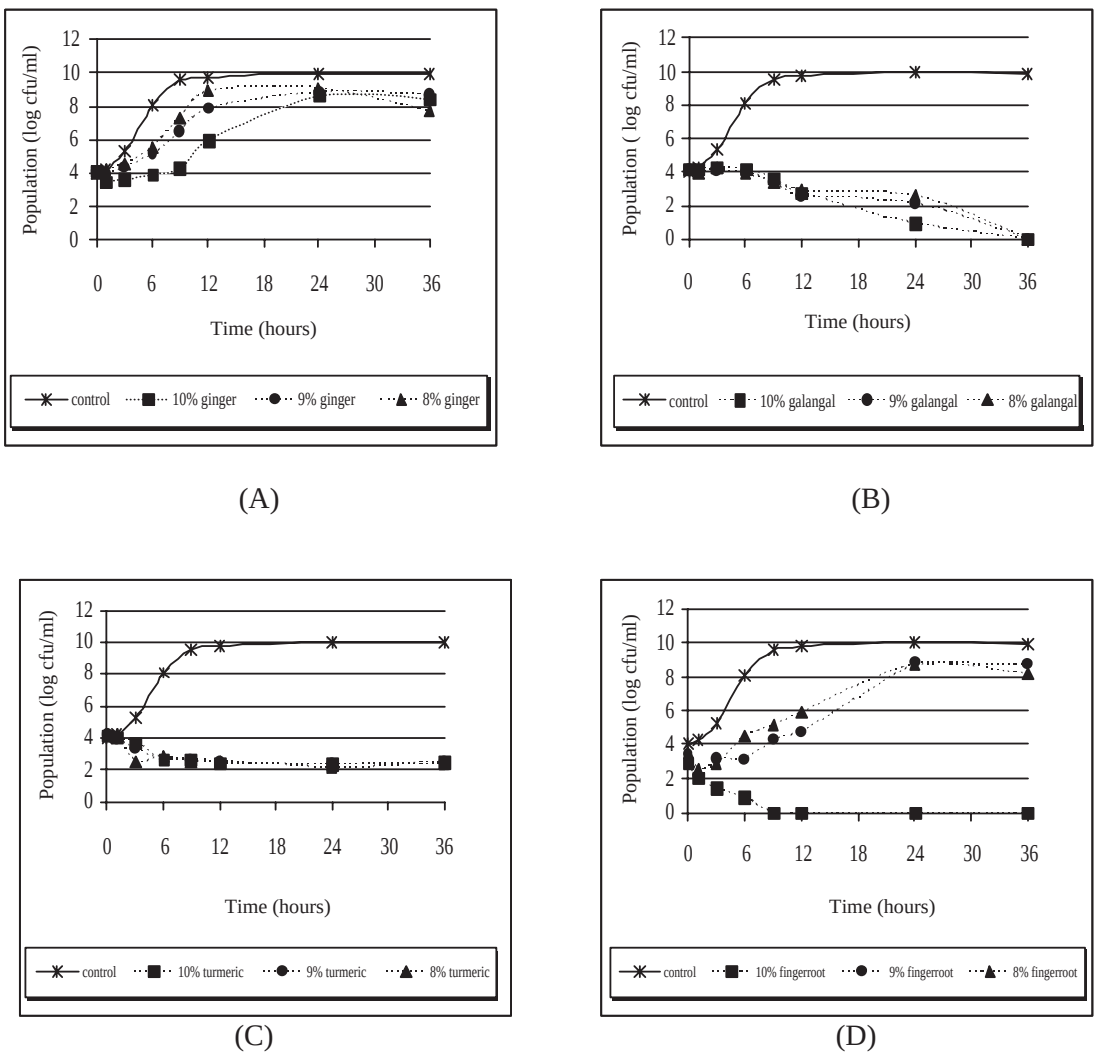

Figure 1 Total viable count of *E. coli O157:H7* in TSB added with spice extracts at various concentrations of ethanolic ginger(A), galangal(B), turmeric(C) and fingerroot(D)

compounds in spice extracts act at many targets such as degradation of the cell wall, damage to cytoplasmic membrane, damage to membrane proteins, leakage of cell contents, coagulation of cytoplasm and depletion of the proton motive force (Burt, 2004). Results of this study demonstrated that the gram–positive bacteria were more susceptible to the ethanol spice extracts than gram–negative bacteria such as *S.* Typhimurium DT 104 strain 2380 exhibited more resistant than *L. monocytogenes* strain 101 when they were tested with ginger extract. The reason would be that lipopolysaccharide (LPS) layer of gram–negative bacteria in outer membrane having high hydrophobicity and acts as a strong barrier against hydrophobic molecules (Smith-Palmer *et al*., 1998). It can pass through cell wall of gram–positive bacteria easier than the gram–negative bacteria because cell wall of the gram–positive contained peptidoglycan and lack of outer membrane (Lambert *et al*., 2001).

Mode of action of the spice extracts which can inhibit the fungal growth showed alterations in the morphology of the hyphae, which appeared severely collapsed. Plasma membrane disruption, mitochondrial destruction, lack of cytoplasm, folding of the nuclear membrane, thickened cell wall caused by chemical components of spice extract (Rasooli *et al.*, 2005). As explained so far, chemical compounds with hydroxyl group (-OH) or an aldehyde group (-CHO) tend to exhibit strong antimicrobial activity. It is well known that a hydroxyl group can form hydrogen bonds with the active site of an enzyme, resulting in its deactivation. The growth inhibition by the aldehyde group is considered due partially to their reactions with sulhydryl groups involved in microbial growth (Hirasa and Takemasa, 1998)

Agar dilution assay is method to determine the approximate concentration, time–kill curve of bacteria is a descriptive test that evaluated the effect of the spice extracts on growth, such as inhibitory effect or bactericidal effect. In addition, it was found that *E. coli O157:H7* in broth medium was more sensitive than the one on agar medium due to, a better immersed cell exposed to antimicrobial agent incorporated to broth medium.

CONCLUSION

The ethanol spice extracts from some rhizomatous members of the Zingiberaceae family namely ginger, galangal, turmeric, and fingerroot were used to test for antimicrobial activities by agar dilution assay. The results showed that fingerroot extract was the most effective for growth inhibition of *L. monocytogenes*, *B. cereus* and *S. aureus*. MICs of fingerroot for those gram-positive bacteria were 0.2–0.4% (v/v), while the MICs for gram–negative pathogenic bacteria namely *E. coli O157:H7* and *S.* Typhimurium were 8–10% (v/v). In addition, *L. plantarum* and *L. cellobiosus* showed the most distinctive sensitively to the galangal extract at 4% (v/v). For the fungi, galangal and turmeric extracts could not inhibit mycelium growth of *A. flavus*, *A. niger*, *A. parasiticus* and *F. oxysporum* at concentration 10% (v/v). However, *A. niger* and *F. oxysporum* were the most susceptible to fingerroot extract that the MICs were 8% and less than 8% (v/v), respectively. Combination of TSB and galangal extract to death kinetic study of *E. coli* O157:H7 revealed that at 8% (v/v) had the most effective to inhibit in 36 hours. Concludingly, rhizomatous spice extracts have potential to use as natural preservative agents in food to control or protect against spoilage and pathogenic microorganisms providing shelf-life extension and food safety.

LITERATURE CITED

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods – a review. **Int. J. Food Microbiol**. 94: 223–253.

Burgula, Y., D. Khali, S. Kim, S.S. Krishnan, M.A. Cousin, J.P. Gore, B.L. Reuhs and L.J. Mauer. 2006. Detection of Escherichia coli O157:H7 and Salmonella Typhimurium using filtration followed by Fourier-Transform Infrared Spectroscopy. **J. Food Prot.** 69: 1777-1784

Davidson P.M. and A.L. Branen. 1993. **Antimicrobials in Foods**. 2nd ed. Marcel Dekker, Inc., New York. 647 p.

Guzman C.C. and J.S. Siemonsma, eds. 1999. **Plant Resources of South – East Asia No 13 Spices**, Prosea Bogor Indonesia 1999, Indonesia.

Hirasa K. and M. Takemasa. 1998. **Spice Science and Technology**. Marcel Dekker, Inc., New York.

Lambert, R.J.W., P.N. Skandamis, P.J. Coote and G.J.E. Nychas. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. **J. Appl. Microbiol**. 91 (3): 453-462.

Nevas, M., A.R. Korhonen, M. Lindstorm, P. Turkki and H. Korkeala. 2004. Antibacterial Efficiency of Finnis spice essential oils against pathogenic and spoilage bacteria. **J. Food Prot**. 67: 199-202

NCCLS. 2002. **Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals: Approved Standard-Second Edition**. 2nd ed. Pennsylvania. 80 p.

Oonmetta – aree, J., T. Suzuki, P. Gasaluck and G. Eumkeb. 2006. Antimicrobial properties and action of galangal (*Alpinia galanga* Linn.) on *Staphylococcus aureus*. **LWT.** 39: 1214-1220.

Pruthi J.S. 1976. **Spices and Condiments**. National Book Trust, New Delhi.

Rasooli, I., M.B. Rezaei and A. Allameh. 2006. Growth inhibition and morphological alterations of *Aspergillus niger* by essential oils from *Thymus eriocalyx* and *Thymus x-porlock*. **Food Control** 17: 359 - 364

Roller S. 2003. **Natural Antimicrobials for the Minimal Processing of Foods**. 1st ed. Woodhead Publishing Limited and CRC Press LLC, New York. 306 p.

Smith-Palmer, A.J. Stewart and L. Fyfe. 1998. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. **Lett. Appl. Microbiol**. 26: 118-112.

Sribuathong, S. 2005. **Screening and isolation of lactic acid bacteria from fermented rice noodle (Kanomjeen) starter.** M.S. thesis, Kasetsart University. Bangkok.

Thongson, C., P.M. Davidson, W. Mahakarnchanakul and J. Weiss. 2004. Antimicrobial activity of ultrasound – assisted solvent – extracted spices. **Lett. Appl. Microbiol**. 39: 401-406.

Thongson, C., P.M. Davidson, W. Mahakarnchanakul and P. Vibulsresth. 2005. Antimicrobial effect of Thai spices against Listeria monocytogenes and Salmonella Typhimurium DT104. **J. Food Prot**. 68: 2054-2058.

Wang, H. and T. B. Ng. 2005. An antifungal protein from ginger rhizomes. **Biochem. Biophys. Res. Commun**. 336: 100–104.