The fresh Thai local herbs *in vitro* antibacterial activity against *Listeria monocytogenes* 10403S

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Abstract

Thai local herbs are one important ingredient of Thai food. Six fresh herbs extracted by five different ethanol concentrations (0%, 25%, 50%, 75%, and 95%) were investigated for their individual antibacterial activity; Chilli (Capsicum annuum), kaffir lime (Citrus hystrix), cumin (Cuminum cyminum L.), shallot (Allium ascalonicum L.), garlic (Allium sativum), lemongrass (Cymbopogon citratus), and galangal (Alpinia galanga) against Listeria monocytogenes 10403S by using the agar disk diffusion method on Brain Heart Infusion media (BHI), five different ethanol concentrations (0%, 25%, 50%, 75%, and 95%). The results showed that 95 % ethanolic crude extracts gave the highest antibacterial activity against L. monocytogenes 10403S. Of all herbs, lemongrass extracted by 95% ethanol showed the highest antibacterial activity; 9.79 ± 1.68 mm. The minimum inhibitory concentrations (MICs), using a broth dilution method, were found to be between 16-128 µl/ml. The minimum bactericidal concentrations (MBCs), using a broth dilution method, were greater than 32-128 µl/ml. This indicated that all Thai local herbs showed the promising antibacterial activity against L. monocytogenes 10403S.

Keywords**:** *Listeria monocytogenes* 10403S, antibacterial activity, herbs

Introduction

Herbs have been used since ancient time for flavoring foods and beverages, and for medicinal purposes with varying success to cure and prevent diseases. Herbs contain innumerable constituents and are valuable sources of new and biological active molecules possessing antimicrobial properties (Negi, 2012). The extracts from plants either as standardized extracts or as a source of pure compounds provide unlimited opportunities for control of microbial growth owing to their chemical diversity (Negi, 2012). Many of them possess antimicrobial activity against a range of bacteria, yeasts and molds. However, the variation in quality and quantity of their bioactive constituents is the major detriments in their food usage (Negi, 2012).

Listeria is bacterium found in soil and water and some animals, including poultry and cattle. It can be present in raw milk and foods made from raw milk. It can also be found in food processing plants and contaminate a variety of processed meats. Unlike many other germs, *Listeria* can grow even in the cold temperature of the refrigerator. The severity of listeriosis in which during severe infections, the bacteria disseminate via the blood and cross the blood–brain barrier resulting in infections of the meninges and the brain. Furthermore, it can cross the fetoplacental barrier in pregnant women, leading to infection of the fetus. It can invade different non-phagocytic cells and is resistant to intracellular killing by macrophages after phagocytosis (Barbuddhe and Chakraborty, 2009; Hamon et al., 2006)

Food is the ideal medium for the spread of harmful agents due to the ability of food to mask the harmful agents by strong flavors,

strong odors, various textures or intense colors. Food and food ingredients are easily in distribution over great distances; thus there is increased potential for widespread impact from food and food ingredients (Sobel and Watson, 2009). Thai red curry paste contains various kinds of herbs and spices. These herbs and spices are rich sources of biologically active antimicrobial compound. Several scientific reports have described the inhibitory effect of these herbs and spices on a variety of microorganisms (Arora and Kaur, 1999). The main ingredient of Thai red curry paste is consists of herbs which have potential to be natural antibacterial agents; chili (*Capsicum annuum*), kaffir lime (*Citrus hystrix*), shallot (*Allium ascalonicum* L.), garlic (*Allium sativum*), lemongrass (*Cymbopogon citrates*), and galangal (*Alpinia galangal*) were used in this study to investigate their potential as a natural antibiotics in order to inhibit *Listeria monocytogenes* 10403S growth.

Therefore, the objective of this experiment is to investigate the individual antibacterial activity of each herb in Thai red curry paste fresh form under different ethanolic extraction conditions against *L. monocytogenes* 10403S.

Methodology

A. Preparation of Plant Samples

Plant samples include the following herbs and spices: chilli (*Capsicum annuum*), kaffir lime (*Citrus hystrix*), shallot (*Allium ascalonicum*), garlic (*Allium sativum*), lemongrass (*Cymbopogon citratus*), and galangal (*Alpinia galangal*) that were bought from local markets in Bangkok, Thailand.

Dry chili was bought from local market in Bangkok, Thailand. It was cut into small pieces and soaked in water 20 minute before use. Remove excess water and grind with blender. Fresh shallot, galangal and garlic were peeled and chopped into small pieces. Fresh lemongrasses were cut into small pieces. Then each of these herbs was grind using blender.

B. Extraction

20 grams of each herb was weighted on top- loaded balance, 1 decimal (ZEPPER model ES- 300) and then 180 ml ethanol (Rung- Sap Co.,Ltd.) with different concentration (0%, 25%, 50%, 75% and 95%) was added and soak for 48 hours at room temperature and stirred every 12 hours. After 48 hours, the liquid part was separated by filtering through thin cloth. Then, crude extract was centrifuged (Chermle model Z230A) at 5,000 rpm for 5 min. The supernatant was collected and concentrated in water bath (Schutzart DIN40050- IP20) at 45^0C until it become very concentrate slurry. The crude extract was kept in freezer at -20^0C until use. Crude extract was diluted to 100 mg/ml by different percentage of ethanol according to its extraction condition. Diluted crude extracted were 0.2 µm CE filter sterile (Minisart®). Then keep in freezer at -20^0C.

C. Preparation of the Culture

The stock culture was prepared by inoculating a loopful of *L. monocytogens* into 10-ml fresh Brain heart infusion broth (BHI) and incubated at 37^0C for 24 hours (Jouan incubator, model EB280). 1% v/v overnight culture was inoculated into 10 ml of BHI broth and incubated at 37^0C in the Culture tube Rotator SCI (Stuart Scientific), until OD_{600} reached 0.1 (SPECTRONIC, model GENESYS 5) which its early log phase (Pitinidhipat and Yasurin, 2012).

D. Antibacterial Assay

Modified disc diffusion method (Pitinidhipat and Yasurin, 2012) was used to test antibacterial activity of herbs in this experiment. Sterile disc was made from 2-layered of Whatman filter paper number41 and sterilized at 121°C for 15 minutes.

Additionally, a sterile cotton bud was also sterilized at 121°C for 15 minutes. Area on BHI agar plate was divided into four parts. The 100 µl of culture was swabbed on the agar by using sterile cotton. After agar plate dried, placed sterile paper disc into each part; first part and second part for 15µl of herb extract paper disc, third part for 15µl of control paper disc (ethanol in different concentration), and last part for 15µl of 100 mg/ml Penicillin-G (Fluka BioChemika) paper disc. All plates were incubated at 37°C for 24 hours. The inhibition zone diameters were measured by ruler (local manufacturer, contained mm unit). The data were collected and calculated for mean and standard deviation using Microsoft Excel 2007.

E. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination

MIC and MBC methods were modified from Pitinidhipat and Yasurin (2012). For MIC test, crude extracts were added to the 1ml fresh broth in different concentration as following 128, 64, 32, 16, 8, 4, 2, 1 and 0.25µl/ml. The 100µl of culture with 0.1 OD_{600} was incubated at 37^0C for 24 hours. The MIC test negative result tubes were chosen for MBC test and then incubated at 37^0C for 24 hours. All experiments were performed in triplicate and repeated three times.

Results and Discussion

The effect of different ethanolic extractions of six herbs which composed of chilli (*Capsicum annuum*), kaffir lime (*Citrus hystrix*), shallot (*Allium ascalonicum* L.), garlic (*Allium sativum*), lemongrass (*Cymbopogon citratus*), and galangal (*Alpinia galanga*) were tested against *L. monocytogenes*. The inhibition effects of the extractions were measured using agar disc diffusion assay and shown in table 1.

Table 1. Antibacterial activity of Thai local herbs against *Listeria monocytogenes* by disc diffusion methods

Herb and Spice	% Ethanol (clear zone in mm.)		
	95	75	50
Lemongrass	9.79 ± 1.68	7.54 ± 0.14	7.20 ± 0.40
Chili	9.78± 2.53	7.79 ± 0.26	7.50 ± 0.89
Galangal	9.58± 0.76	7.63 ± 0.22	8.35 ± 1.31
Garlic	8.00 ± 0.70	7.22 ± 0.26	7.00 ± 0.00
Kaffir lime peel	8.50 ± 0.88	8.50 ± 0.88	7.50 ± 0.72
shallot	8.77 ± 0.81	8.17 ± 1.28	8.90 ± 2.98
Control (Penicillin-G)	36.9 ± 10.3	44.0 ± 1.66	42.7 ± 1.80
Control (Ethanol)	8.14± 0.52	9.55 ± 2.44	7.79 ± 1.29

Note: For 25% and 0% ethanol extraction, the result shows no inhibition.

From table 1, using different percentage of extraction solvents effect antibacterial activity. Using higher percentage of ethanol gave better antibacterial activity. The activities measured by using agar disc diffusion at 95% ethanolic extraction showed highest activities. According to table 1, lemongrass showed highest activity then followed by chilli, galangal, shallot, kaffir lime peel and garlic. Lis-Balchin and Deans (1997) studied 93 commercial essential oils against 20 *L. monocytogenes* strains. Lemongrass was among the oils that exhibited antibacterial activity against all the *Listeria* strains tested. The results from Lis-Balchin and Deans (1997) correlate to this research and found out that not only the oil form of lemongrass that showed activity against *L. monocytogenes* but also the ethanolic extraction form. Red chilli is in *Capsicum* spp. and it contains capsaicin which is reported as antimicrobial agents (Cichewicz *et al*, 1996; Molina-Torres *et al*, 1999). Also from the work of Leuschner and Lelsch (2003) showed that adding 1% w/v of dried chilli in BHI can slightly inhibited the growth of *L. monocytogenes* correlate to the result from this study which ethanolic extraction of chilli showed activity against *L. monocytogenes*. From the work of Farnworth and

Bunyapraphatsara (1992), the essential oils from both fresh and dried rhizomes of galangal were shown to have antimicrobial activities against bacteria, fungi, yeast and parasite. In this case, theses finding correlate to the result from this study which galangal show to have inhibition against *L. monocytogenes*. From the work of Nanasombat and Lohasupthawee (2005) showed that kaffir lime peel crude ethanolic extract showed antibacterial activity against 20 serotypes of *Salmonella* and 5 species of other enterobacteria using disk diffusion method. Thus, not only kaffir lime peel has activity against *Sallmonella* but also against *L. monocytogenes*.

Table 2: MICs and MBCs of crude extracted derived from herbs sample (µl/ml) using 95% ethanol extraction, *L. monocytogens* was test on BHI broth

Herbs	MIC (µl)	MBC (µl/ml)
Chili	16	32
Shallot	128	>128
Kaffir lime	32	32
Garlic	64	128
Lemongrass	>128	NT
Galangal	128	>128

Note: NT = Not tested.

The result from table 2 showed MIC and MBC results of six herbs which composed of chilli (*Capsicum annuum*), kaffir lime (*Citrus hystrix*), shallot (*Allium ascalonicum* L.), garlic (*Allium sativum*), lemongrass (*Cymbopogon citratus*), and galangal (*Alpinia galanga*) were tested against *L. monocytogenes*. The minimum inhibitory concentrations (MICs), using a broth dilution method, were found in the range of 16-128 µl/ml. The minimum bactericidal concentrations (MBCs), using a broth dilution method, were greater than 32-128 µl/ml.

From the works of Ikigai *et al*. (1993) and Otake *et al.* (1991), they suggested that the antimicrobial activity of plant in form of extract is most likely due to the combined effects of adsorption of polyphenols to bacterial membranes with membrane disruption and subsequent leakage of cellular contents. Herbs and spices are also rich in phenolic compounds and besides exerting antimicrobial effect they may be used to preserve the foods by reducing lipid oxidation as they are reported to have significant antioxidant activity (Scwarz *et al*, 2001; Shahidi *et al*, 1997; Shan *et al*, 2009; Tanabe *et al*, 2002; Yanishlieva *et al*, 2006). From above mentioned properties, the major targets for those antimicrobials could be food poisoning microorganisms and spoilage microorganisms. Thus that from the result in this research found out that six herbs Chilli (*Capsicum annuum*), Kaffir lime (*Citrus hystrix*), Shallot (*Allium ascalonicum* L.), Garlic (*Allium sativum*), Lemongrass (*Cymbopogon citratus*), and Galangal (*Alpinia galanga*) using ethanol extractions showed promising antimicrobial activity sources to exploit.

Conclusion

All crude extracts using 95% ethanol as extraction solvent had the highest antibacterial activity against *L. monocytogenes* 10403S. The fresh lemongrass 95% crude ethanolic extract showed the highest antibacterial activity; 9.79 ± 1.68 mm. The minimum inhibitory concentrations (MICs), using a broth dilution method, were found in the range of 16-128 µl/ml. The minimum bactericidal concentrations (MBCs), using a broth dilution method, were greater than 32-128 µl/ml. This indicated that all Thai local herbs showed the promising antibacterial activity against *L. monocytogenes* 10403S.

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