The individual antibacterial activity of Thai red curry paste's dry ingredients against *Listeria monocytogenes* using different ethanolic extraction

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Abstract

Thai red curry paste is a main ingredient of Thai Red curry. The paste contains herbs which have the potential to be natural antibiotics. Six herbs contained in Thai red curry paste were tested for their individual antibacterial activity against Listeria monocytogenes 10403S. Six herbs tested were Chilli (Capsicum annuum), Kaffir lime (Citrus hystrixShallot (Allium ascalonicum L.), Garlic (Allium sativum), Lemongrass (Cymbopogon citratus), and Galangal (Alpinia galanga). The agar disk diffusion method was used for in-vitro screening antibacterial activity of each crude extract against L. monocytogenes 10403S on Beef Heart Infusion media (BHI) with five different ethanol concentrations; 0, 25, 50, 75, and 95%. The 95 % ethanol crude extracts gave the highest antibacterial activity against L. monocytogenes 10403S in all herbs. The dry galangal 95% crude ethanolic extract showed the highest antibacterial activity; 10.50 ± 1.16 mm. The minimum inhibitory concentrations (MICs), using a broth dilution method, were found to be between 4-128 µl/ml. The minimum bactericidal concentrations (MBCs), using a broth dilution method, were greater than 32-128 µl/ml. The results showed that the six dry herbs have significant antibacterial activity against the food-borne pathogen, L. monocytogenes 10403S.

***Keywords*:** *Listeria monocytogenes* 10403S, antibacterial activity, Thai red curry paste, ingredients.

Introduction

Nowadays, there are many diseases that bacteria are one of the main causes. Bacteria are the most difficult to control and easy to spread rapidly. Some evidence as H5N1, diarrhea by *Vibrio, and Listeria monocytogenes* in food which is a potential danger.

Listeria is the name of a bacteria 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 live in food processing plants and contaminate a variety of processed meats. *Listeria* is unlike many other germs because it 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 which leads 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)

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 consisted 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 against *Listeria monocytogenes* 10403S

growth. Therefore, the objective of this experiment was to investigate the individual antibacterial activity of each herb in Thai Red Curry Paste dry form under different ethanolic extraction conditions against *L. monocytogenes* 10403S.

Materials & Methods

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.

Garlic and shallot were peeled and chopped into small pieces. Galangal was peeled and sliced. Lemongrass was sliced only the stem part. All of them were air dried in oven (Memmert, UM500) at 45^0C for overnight. Then, dried herbs were blended in food blender to reduce the size. For chili, dry chili was purchased from a local market in Bangkok and ground into powder. Herb powder was stored in refrigerator at 6^0C until use.

B. Extraction

Weight 20g herb powder on top- loaded balance, 1 decimal (ZEPPER model ES- 300). Add 180 ml of ethanol (Rung- Sap Co.,Ltd.) by vary the percentage of ethanol as following 0%, 25%, 50%, 75%, 95%. Then soak for 48 hours at room temperature and stirred every 12 hours. After 48 hours, the liquid part was separated by filter pass through thin cloth. Then crude extract was centrifuged (Chermle model Z230A) at 5000 rpm for 5 min. Supernatant was collected and concentrated in water bath (Schutzart DIN40050- IP20) at 45^0C until become very concentrate slurry. Crude extract was kept in freezer at -20^0C until use. Crude extract was diluted 100 mg/ml by Ethanol 95% as final concentration. 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 *Listerial monocytogens* into 10-ml fresh Brain heart infusion broth (BHI) 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 by Culture tube Rotator SCI (Stuart Scientific), until OD_{600} reach 0.1 (SPECTRONIC, model GENESYS 5) which is their early log (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, sterile cotton bud was also sterilized at 121°C for 15 minutes. Area on each agar (BHI agar) plate was divided into four parts. Each plate was swabbed 100 µl of culture on the agar by using sterile cotton bud under aseptic technique. After agar plate dried, each area was placed by a paper disc; first part and second part for 15µl of herb extract, third part for 15µl of control (ethanol in different concentration), and last part for 15µl of 100 mg/ml Penicillin-G (Fluka BioChemika). All plates were incubated at 37°C for 24 hours. Clear zone result was 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 extracted were add to the 1ml fresh broth in different concentration as following 128, 64, 32, 16, 8, 4, 2, 1 and 0.25µl/ml. 100µl of culture 0.1 OD_{600} was incubated at 37^0C for 24 hours. The MIC test negative result tubes were chosen for MBC Test 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 as clear zone (mm)

Herb and Spice	% Ethanol (clear zone in mm.)		
	95	75	50
Lemongrass	8.95 ± 0.86	7.72 ± 0.47	8.25 ± 0.33
Chili	9.17 ± 1.37	7.58 ± 0.29	7.18 ± 0.37
Galangal	10.5 ± 1.16	9.42 ± 3.70	7.17 ± 0.35
Garlic	8.00± 0.6	7.55 ± 0.16	7.04 ± 0.14
Shallot	7.875 ± 0.6	7.72 ± 0.47	7.80 ± 0.35
Kaffir lime	10.17 ± 1.96	7.71 ± 0.26	7.17 ± 0.35
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.

As can be seen in table 1, it showed that using different percentage of extraction solvents effect antibacterial activity. Using higher percentage of ethanol gives better antibacterial activity. The highest activity measured by using agar disc diffusion are 95% ethanolic extraction of galangal and kaffir lime peel. From the work of Farnworth and Bunyapraphatsara (1992), the essential oils from both fresh and dried rhizomes of galangal shown to have antimicrobial activities against bacteria, fungi, yeast and parasite. In this case correlate to this study galangal shows 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*. From table 1, chili, lemongrass, garlic and shallot showed antibacterial activity but slightly different in antibacterial activity.

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

Herbs	MIC (µl/ml)	MBC (µl/ml)
Chili	32	32
Shallot	128	>128
Kaffir lime	8	32
Garlic	128	>128
Lemongrass	32	32
Galangal	4	32

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*. Kaffir lime and Galangal shows least MIC value which correlate to results obtained from agar disc diffustion in table 1. While the minimum MBC value of extraction for chili, kaffir lime, lemongrass and galangal is 32 µl, the minimum MBC value for shallot and garlic is 128 µl.

From the works of Ikigai *et al* (1993) and Otake *et al* (1991), they suggest 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 also rich in phenolic compounds and besides exerting antimicrobial effect they may 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 microorganism and spoilage microorganism. Thus that from the result in this research found out that 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*) using ethanol extractions showed promising antimicrobial activity sources to exploit.

Conclusion

In conclusion, the dry galangal 95% crude ethanolic extract showed the highest antibacterial activity; 10.50 ± 1.16 mm. The minimum inhibitory concentrations (MICs), using a broth dilution method, were found to be between 4-128 µl/ml. The minimum bactericidal concentrations (MBCs), using a broth dilution method, were greater than 32-128 µl/ml. The results showed that the six dry herbs have significant antibacterial activity against the food-borne pathogen, *L. monocytogenes* 10403S. Thus that ethanol 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*) showed promising antimicrobial activity sources.

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