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In-vitro Individual Antibacterial Activity Of Thai Red Curry Paste Ingredients Against Salmonella Enterica Enteritidis (Human) And Listeria Monocytogenes 10403s

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ABSTRACT

Thai red curry paste consists of herbs which have potential to be natural antibacterial agents. Therefore 7 herbs of Thai curry paste ingredients; Chili (*Capsicum annuum*), Kaffir lime (*Citrus hystrix*), Cumin (*Cuminum cyminum* L.), Shallot (*Allium ascalonicum* L.),Garlic (*Allium sativum*), Lemongrass (*Cymbopogon citrates*), and Galangal (*Alpinia galangal*), were investigated for their individual antibacterial activity againt *Salmonella enterica* Enteritidis (human) and *Listeria monocytogenes* 10403S by agar diffusion method Agar under 3 extraction3; water, UHT coconut milk and fresh coconut milk. *In-vitro* antibacterial screening results, fresh coconut milk extraction showed the highest potential in inhibiting *S. enterica* Enteritidis (human), in all herbs and the highest antibacterial activity was found in garlic; 01.00 ± 0.18 cm, using water extraction. For *L. monocytogenes* 10403S, the highest antibacterial activity was found in lemon grass using fresh coconut milk extraction; 0.90 ± 0.12 cm. The minimum inhibitory concentration (MICs), using broth dilution method, of all herbs were between 80 to >160µl/ml. The minimum bactericidal concentrations (MBCs) of all herbs under three extractions, using broth dilution method, showed >160µl/ml in both bacteria. The herbs in Thai red curry paste ingredient showed the significantly promising antibacterial activity against food-borne pathogen, *S. enterica* Enteritidis (human) and *L. monocytogenes*10403S.

Key words: Thai red curry paste, Antibacterial, *Salmonella enterica* Enteritidis (human), *Listeria monocytogenes* 10403S, Herb.

INTRODUCTION

In recent years, food safety concerns have been focused. There are a lot of outbreaks all over the world in varieties of foods. *Salmonella* sp. and *Listeria monocytogenes* are recognized as a primary cause of food poisoning worldwide and massive outbreaks have been occurred. *Salmonella* sp. is a rod-shaped, motile bacterium, non spore forming and Gram-negative[1]. There is a widespread occurrence in animals, especially in poultry and swine[1]. Environmental sources of the organism include water, soil, insects, factory surfaces, kitchen surfaces, animal feces[1]. In the last 20 years or so, *S.* Enteritidis has become the single most common cause of food poisoning in the United States. *S*. Enteritidis causes a disease almost identical to the very closely related *S.* Typhimurium[1].

L. monocytogenes is a Gram-positive bacterium, motile by flagella. Some studies suggest that 1-10% of humans may be intestinal carriers *of L. monocytogenes*[2]. It can be isolated from soil and other environmental sources. *L. monocytogenes* is quite hardy and resists the deleterious effects of freezing, drying, and heat[2]. About 2500 cases of listeriosis occur each year in the United States[2]. Most cases of listeriosis and most deaths occur in adults with weakened immune systems, the elderly, pregnant women, and newborns[2]. Outbreaks of

listeriosis have been linked to a variety of foods especially processed meats and dairy products made from unpasteurized milk[2].

Thai red curry paste is very important ingredient for Thai red curry, and contains 7 kinds of herbs and spices. These herbs and spices are rich sources of biologically active antimicrobial compound [3,4,5,6,7,8,9,10,11]. These scientific reports have been described the inhibitory effect of these herbs and spices on a variety of microorganisms [3,4,5,6,7,8,10,11]. Thai red curry paste is main ingredient in many Thai red curry menus. Thai red curry paste with water is called Kang-Pa and with coconut milk is called Kang-Kati. Thai red curry paste consists of herbs and spices, which have potential to be natural antibacterial agents; Chili (*Capsicum annuum*), Kaffir lime (*Citrus hystrix*), Cumin (*Cuminum cyminum* L.), Shallot (*Allium ascalonicum* L.), Garlic (*Allium sativum*), Lemongrass (*Cymbopogon citrates*), and Galangal (*Alpinia galangal*).

Since these 7 herbs and spices contained in Thai red curry paste may have the potential to possess antibacterial properties, it becames worthwhile to investigate their potential as a natural antibiotics in order to against *S. enterica* Enteritidis (human) and *L. monocytogenes*10403S growth. Therefore, the objective of this experiment is to investigate the individual antimicrobial activities of each herbs and spices contained in Thai red curry paste under three different extraction conditions; water (as in Kang-Pa), UHT coconut milk (as in Kang-Kati), and fresh coconut milk (as in Kang-Kati).

MATERIALS AND METHODS

Plant sample preparation

Plant samples; Chilli (*C. annuum*), Kaffir lime (*C. hystrix*), Cumin (*C. cyminum* L.), Shallot (*A. ascalonicum* L.), Garlic (*A. sativum*), Lemongrass (*C. citrates*), and Galangal (*A. galangal*), which were bought from local market in Bangkok, Thailand. Every herb was cleaned by tap water. Dry chili was cut into a small pieces and soaked in water 20 minute before use. Kaffir lime was used only the peel. Shallot and garlic were peeled and chopped into small pieces. Lemongrass was sliced only the stem part. Galangal was peeled and sliced.

Extraction

The 50 g of each herb was grounded by auto mortar for 15 min. The water, UHT coconut milk, and fresh coconut milk were used as extractant. For water extraction, 200 ml water was added into 50 g of each grounded herb and boiled for 1 hr by using the hot plate (VELP SCIETIFICA, model Are2) and stirred every 5 minutes. This method is the same as authentic cooking Thai red curry using water or Kang-Pa.

For UHT coconut milk extraction, 80 ml UHT coconut milk was boiled for 5 minutes by using the hot plate (VELP SCIETIFICA, model Are2), and added 50 g grounded herb and stirred for 5 minutes. Then, coconut milk solution (1 UHT coconut milk: 2 water) was added, continue boiled until 1 hr and stirred every 5 minutes. This method is the same as authentic cooking Thai red curry using UHT coconut milk or Kang-Kati.

The fresh coconut milk was prepared by using ratio 1 coconut: 1 water; mixed them together, soaked for 5 minutes and separated coconut part out of coconut milk by squeezing. For fresh coconut milk extraction, 80 ml of fresh coconut milk was boiled for 5 minutes by using the hot plate (VELP SCIETIFICA, model Are2), added 50 g of grounded herb and stirred for 5 minutes. Then, coconut milk solution (1 coconut milk: 2 water) was added, continue boiled until 1 hr and stirred every 5 minutes. This method is the same as authentic cooking Thai red curry using fresh coconut milk or Kang-Kati. The cooking temperature was controlled in the range of 98-100 °C.

Preparation of the culture

The stock culture was prepared by inoculating one loopful of *S. enterica* Enteritidis (human) into 10 ml fresh Nutrient Broth (NB) and *L. monocytogenes* 10403S into 10 ml fresh BHI medium and shaken on Culture tube Rotator SCI (Stuart Scientific) at 37°C for overnight. Then 1 % v/v overnight culture was inoculated into 10 ml of fresh NB at 37 °C overnight by Culture tube Rotator SCI (Stuart Scientific), until OD600 reach 0.1 (SPECTRONIC, model GENESYS 5) as the early log phase of both bacteria.

Antibacterial Assay

BSAC disc diffusion method for antimicrobial Susceptibility Testing version 8 [12]was used for antibacterial activity assay. Each plate was swabbed 100 µl of culture on the agar by using sterile cotton bud under aseptic technique. The 30µl herb extract was added into sterile disc. The extractants; water, UHT coconut milk, and fresh coconut milk were used as negative control. The 100 mg/ml Penicillin-G (Fluka BioChemika) was used as positive control. All plates were incubated at 37°C for 24 hours. The clear zone was used as criterion of the antibacterial activity. All experiment was performed in duplicate and repeated four times. The data were collected and calculated for mean and standard deviation using Microsoft Excel 2007.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determination

The MIC (Minimum Inhibitory Concentration) method was modified from BSAC disc diffusion method for Antimicrobial Susceptibility Testing version 8 [12]. Only herb, which showed positive antibacterial activity, was tested for MIC. For MIC test, the herb extracted as following concentration; 0, 10, 20, 40, 80, and 160 µl /ml, was added into 1 ml fresh NB. Then 100 µl/ml of culture with OD600 reach 0.1 (early log phase) was inoculated, and incubated at 37°C for 24 hours.

The negative MIC result tubes were chosen for MBC determination by taking 1 loop of negative MIC was streaked on NA then incubated at 37°C 24 hours. The growth of culture in each plate was observed. All experiment was performed in duplicate and repeated three times.

RESULTS AND DISCUSSION

Antibacterial Activity

The individual natural antibacterial activity of seven herbs as the ingredients in Thai red curry paste against *S. enterica* Enteritidis (human) were shown in table 1.

The results in table 1 showed that among three extractions, fresh coconut milk extraction showed the highest potential in inhibiting *S. enterica* Enteritidis (human) in among three extractions. The highest antibacterial activity was found in lemon grass extract using fresh coconut milk extraction; 0.93 ± 0.10 cm. While in UHT coconut milk extraction, kaffir lime extract showed the highest antibacterial activity; 0.90 ± 0.12cm. The water extraction showed the lowest antibacterial activity in all herbs.

The individual natural antibacterial activity of seven herbs as the ingredients in Thai red curry

Table 1. Antibacterial activity as clear zone (cm) of seven herb extracts against *S. enterica* Enteritidis (human) under three different extraction conditions

Herb Extract	Water Extraction	UHT coconut milk Extraction	Fresh coconut milk Extraction
Galangal	0.00 ± 0.00	0.00 ± 0.00	0.80 ± 0.00
Shallot	0.89 ± 0.11	0.88 ± 0.11	0.90 ± 0.14
Kaffir lime	0.00 ± 0.00	0.90 ± 0.12	0.83 ± 0.08
Cumin	0.80 ± 0.00	0.00 ± 0.00	0.80 ± 0.00
Dry chili	0.80 ± 0.00	0.89 ± 0.16	0.88 ± 0.11
Lemon grass	0.80 ± 0.00	0.00 ± 0.00	0.93 ± 0.10
Garlic	1.00 ± 0.18	0.00 ± 0.00	0.80 ± 0.00
Negative control*	0.00 ± 0.00	0.00 ± 0.00	0.80 ± 0.00
Penicillin-G		3.70 ± 0.37	

*Water was used as negative control in water extraction.
UHT coconut milk was used as negative control in UHT coconut milk extraction.
Fresh coconut milk was used as negative control in fresh coconut milk extraction.

paste against *L. monocytogenes* 10403S were shown in table 2.

The results in table 2 showed that among three extractions, fresh coconut milk extraction showed the highest potential in inhibiting *L. monocytogenes* 10403S in among three extractions. The highest antibacterial activity was found in lemon grass using fresh coconut milk extraction; 0.90 ± 0.12 cm.

The fresh coconut milk itself also showed the antimicrobial activity. This was due to a half the medium-chain fatty acids in coconut milk compose of lauric acid, which is anti-viral, anti-bacterial, anti-microbial and anti-fungal[13]. The results showed that the fresh coconut milk extraction gave the higher antibacterial activity among three extractions. This phenomenal might be explain that there are both polar and nonpolar active phytochemical compounds in herb extract by using fresh coconut milk more than other extractions due to both polar and non-polar phase in fresh coconut milk. While UHT coconut milk was emulsified during process, which polar and nonpolar phase aligned together, so there was less polar and nonpolar phases. However, the active phytochemical compounds profile of herb extract in each extraction is needed for further investigation by using GC-MS.

The water extracts of galangal (*A. galangal*) showed antimicrobial activity against food spoilage bacteria, food-borne pathogens (*Escherichia coli*, *S. enteriditis*, *Clostridium perfringens, Staphylococcus aureus, Campylobacter jejuni* and *Bacillus cereus* and four different species of fungi (*Saccharomyces cerevisiae, Hansenula anomala, Mucor mucedo* and *Candida albicans*)[19]. While in this experiment, only galangal extract using fresh coconut milk showed antibacterial activity again both *S. enterica* Enteritidis (human) and *L. monocytogenes* 10403S. However, the active compound that contains in the galangal was proof that it gave the highest antimicrobial activity with the non-polar extraction by the non-polar extractant[14]. So this might explain the result that only galangal extract using fresh coconut milk showed antibacterial activity. Siripongvutikorn et al., 2005 studied the antimicrobial and antioxidation effects of Thai seasoning, Tom-Yum; galangal in Thai seasoning Tom-Yum cannot inhibit *L. monocytogenes*[5]. The major active compound of galangal's essential oils are â-farnesene, myrcene and 1, 8 cineole, â-bisabolene, â-caryophyllene and â-selinene [15], which may be responsible for antibacterial activities.

The shallot extract (*A. ascalonicum* L.) in all three extractions showed the antibacterial

Table 2. Antibacterial activity as clear zone (cm) of seven herb extracts against *L. monocytogenes* 10403S under three different extraction conditions

Herb Extract	Water Extraction	UHT coconut milk Extraction	Fresh coconut milk Extraction
Galangal	0.00 ± 0.00	0.00 ± 0.00	0.85 ± 0.10
Shallot	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Kaffir lime	0.80 ± 0.00	0.87 ± 0.12	0.87 ± 0.11
Cumin	0.80 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Dry chili	0.00 ± 0.00	0.00 ± 0.00	0.80 ± 0.00
Lemon grass	0.00 ± 0.00	0.80 ± 0.00	0.90 ± 0.12
Garlic	0.80 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Negative control*	0.00 ± 0.00	0.00 ± 0.00	0.85 ± 0.10
Penicillin-G		2.47 ± 0.21	

*Water was used as negative control in water extraction.
UHT coconut milk was used as negative control in UHT coconut milk extraction.
Fresh coconut milk was used as negative control in fresh coconut milk extraction.

activity against only *S. enterica* Enteritidis (human). Shallot in Thai seasoning, Tom-Yum cannot inhibit *L. monocytogenes,* it confirm the result in this experiment that shallot cannot inhibit *L. monocytogenes* in all three extraction condition[5]. The shallot oil showed that shallot oil has bacteriocidal action on *L. monocytogens*[6] that is conflict with the result in this experiment. But their result on *S.* enteric, it support the result of this experiment that shallot have antibacterial activity as the bacteriostatic[6]. The shallot Extract only was soluble in dimethyl sulphoxide, dimethyl formamide and water which means that the compound present in the shallot is polar compounds[16]. However, the previous study also indicated that the oil extraction method provide a better antibacterial activity than the fresh extraction by manual extractor and squeezing [17].

The results in table 1 and 2 showed that kaffir lime (*C. hystrix*) extract showed antibacterial activity against both both *S. enterica* Enteritidis (human) and *L. monocytogenes* 10403S. It has been reported that the essential oils of kaffir lime peel against all 20 serotypes of *Salmonella* sp. [7]. The crude ethanolic extracts and essential oils showed 10 and 15 mm respectively on *S.* Enteritidis [7], it confirm the result of this experiment that kaffir lime peel in all three extraction condition can inhibit *S. enteriditi*s and *L. monocytogenes*[7]. The use of pressurized hot water extraction on kaffir lime fruit peel and found out that when increase temperature in extraction the phenolic compound content increasing[18] . The kaffir lime peel essential oil chemical composition, was analysed by GC-MS, consisted of several components (limonene 40.65%, terpinene-4-ol 13.71%, a-terpineol 13.20%), and the most active component was a-terpineol, followed by terpinene-4-ol, and limonene [20].

The cumin (*C. cyminum* L.) extract using water extraction showed antibacterial activity against both *S. enterica* Enteritidis (human) and *L. monocytogenes* 10403S. The methanolic, hydroalcoholic and aqueous cumin extracts showed weak activity and the antimicrobial activities decrease in the order [21]. The cumin essential oil showed antibacterial activity against *E. coli, S. aureus,* and *S. faecalis* but *Pseudomonas aeruginosa* and *Klebsiella pneumonia* resisted to cumin essential oil[22]. The GC-MS results showed that Cumin oil contained á-pinene (29.1%), limonene (21.5%), 1,8-cineole (17.9%), and linalool (10.4%) as the major compounds[22]. It was found out that cumin methanolic extract caused damage to bacteria cell membranes and cell leakage[23].

The dry chili in Thai seasoning Tom-Yum cannot inhibit *L. monocytogenes* [5]. It showed that by adding 1% w/v of dried chili in BHI can slightly inhibited the growth of *L. monocytogenes* [24]. The results in table 2showed that only the dry chili extract using fresh coconut milk extraction can inhibit *L. monocytogenes* 10403S. The dry chili extract gave high antibacterial activity in three extractions against *S. enterica* Enteritidis (human). The use of mixed diet with C. annuum at percent 1% and 2%, were effective against *S. typhimurium* infection[25]. The plain and heated extracts were found to exhibit varying degrees of inhibition against *B. cereus, B. subtilis, C. sporogenes, C. tetani*, and *S. pyogenes*[8]. The capsaicin is the major antimicrobial compound of chili that might be antibacterial agent of chili in this experiment but this point need further investigated[8].

The lemon grass (*C. citrates*) antibacterial activity of crude ethanolic extracts showed 10mm on *S.* Enteritidis[7], it confirm the result of this experiment that lemon grass gave high antibacterial activity on *S. enteriditi*s and lemon grass in Thai seasoning Tom-Yum did not inhibit *L. monocytogenes*[5]. This conflict with the result in this experiment that lemon grass extract show antibacterial activity against both *S. enterica* Enteritidis (human) and *L. monocytogenes* 10403S. The aqueous extract was effective against *S. aureus , B. subtilis* while it was inactive on *E. coli, S. typhimurium, P. vulgaris, P. aeruginosa, A. niger* [26]. However, the lemon grass extract using water extraction showed antibacterial activity against *S. enterica* Enteritidis (human) but not *L. monocytogenes* 10403S. The main chemical of lemon grass oil is citral-b and citral-a [9] that might be antibacterial agent of lemon against both *S. enterica* Enteritidis (human) and *L. monocytogenes* 10403S.

The antibacterial activity of garlic (*A. sativum*) crude ethanolic extracts and aquous extracts cannot inhibit *Salmonella* sp. but essential oils can inhibit *S.* Enteritidis [7,10]. However, garlic water extraction show antibacterial activity against both *S. enterica* Enteritidis (human) and *L. monocytogenes* 10403S. There were reports about its antibacterial and antifungal properties which come from the substance called allicin (allyl 2-propene thiosulphinate)[27,28,29,30]. Alicin inhibits various thiol-dependent enzymatic systems of bacteria [31]. Garlic was found to be effective against *L. monocytogenes* [32,33]. There was the study of the effect of raising the temperature on the effectiveness of garlic, it was that found that the activity of garlic increased with increase in temperature up to 80 C, beyond which the activity remained constant or decreased [34,35]. So it can explain the result in table1 and 2 that garlic extract using water extraction, that temperature was 98-100 °C, showed antibacterial activity against both *S. enterica* Enteritidis (human) and *L. monocytogenes* 10403S.

Table 3. The minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MCB) of seven herb extracts against *S. Enterica* enteritidis (human) under three different extraction conditions

Extraction	Herbs	MIC (µl/ ml)	MBC (µl/ ml)
Water Extraction	Shallot	80	>160
	Cumin	80	>160
	Dry chili	80	>160
	Lemon grass	80	>160
	Garlic	160	>160
UHT coconut milk Extraction	Shallot	80	>160
	Kaffir lime	80	>160
	Dry chili	80	>160
Fresh coconut milk Extraction	Galangal	160	>160
	Shallot	160	>160
	Kaffir lime	160	>160
	Cumin	80	>160
	Dry chili	80	>160
	Lemon grass	160	>160
	Garlic	80	>160

The minimum inhibitory concentrations (MICs) and The Minimum Bactericidal Concentrations (MBCs)

The MIC, using broth dilution method, is the concentration of herbs extract needed to inhibit the growth of *S. enterica* Enteritidis (human), while the MBC is the concentration of herbs extract needed to completely kill *S. enterica* Enteritidis (human) as the results were shown in table 3. The MIC of shallot, cumin, dry chili, and lemon grass showed 80µl/ml while garlic showed 160µl/ml using water extraction. For UHT coconut milk extraction, MIC of shallot, kaffir lime, and dry chili showed 80µl/ml. For fresh coconut milk extraction, MIC of galangal, shallot, kaffir lime, and lemon grass showed 160µl/ml while cumin, dry chili, and garlic showed 80 µl/ml. The MBC of all herbs under three conditions, using broth dilution method, showed >160 µl/ml.

The MIC, using broth dilution method, is the concentration of herbs extract needed to inhibit the growth of *L. monocytogenes* 10403S, while the MBC is the concentration of herbs extract needed to completely kill *L. monocytogenes* 10403S as the results were shown in table 4. The MIC of kaffir lime and cumin showed 80µl/ml while garlic showed 160µl/ml using water extraction. For UHT coconut milk extraction, MIC of kaffir lime, and lemon grass showed 80 and 160 µl/ml respectively. For fresh coconut milk extraction, MIC of galangal and kaffir

Table 4. The minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MCB) of seven herb extracts against *L. monocytogenes* 10403S under three different extraction conditions

Extraction mode	Herbs	MIC (µl/ ml)	MBC (µl/ ml)
Water Extraction	Kaffir lime	80	>160
	Cumin	80	>160
	Garlic	160	>160
UHT coconut milk Extraction	Kaffir lime	80	>160
	Lemon grass	160	>160
Fresh coconut milk Extraction	Galangal	160	>160
	Kaffir lime	160	>160
	Dry chili	80	>160
	Lemon grass	80	>160

lime showed 160 µl/ml while dry chili and lemon grass was showed 80 µl/ml. The MBC of all herbs under three conditions, using broth dilution method, showed >160 µl/ml.

CONCLUSION

The results showed that all herbs in Thai red curry paste had anitbacteial activity against *S. enterica* Enteritidis (human). While all Thai red curry paste ingredient herbs had antibacterial activity against *L. monocytogenes* 10403S expect shallot. However, all herbs in Thai red curry paste showed the significantly promising antibacterial activity against both food-borne pathogens; *S. enterica* Enteritidis (human) and *L. monocytogenes* 10403S in all extraction conditions. It can benefit to food industry as food safety issue.

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