Natural Antimicrobial Activity of Thai Red Curry's Herbs on *Salmonella* Typhimurium DT104b

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Abstract

The outbreaks of Salmonella *spp. have been reported as the major cause of food-borne illnesses worldwide. Thai red curry paste is an important ingredient of cultural foods and consists of herbs that have potential natural antibacterial activity. The objective of this experimental study was to measure the individual antibacterial activity of Thai red curry herbs on* Salmonella *under three different extraction methods (Kaeng Kathi - oil using fresh coconut milk, Kaeng Kathi - oil using UHT coconut milk, and Kaeng Pa - water). During the conducted experiments, the individual activities of the extracted herbs on* Salmonella enterica *serovar Typhimurium U302 (DT104b) were investigated by agar diffusion method using SS agar followed by MIC and MBC assay using broth dilution method. Among the three extraction methods, the extraction using fresh coconut milk gave the highest antibacterial activity in all herbs, followed by UHT coconut milk and water. Fresh coconut milk extracted cumin* (Cuminum cyminum) *and lemongrass* (Citronella citrate) *gave the highest antibacterial activity, 1.6 ± 0.22 cm and 1.4 ± 0.24 cm, respectively. The MIC assay showed 125 µl/ml and 100 µl/ml, while the MBC showed 125 µl/ml and >150 µl/ml, respectively. Thai red curry paste ingredients possess a significantly promising antibacterial activity against the food-borne pathogen* S. enterica *Typhimurium U302 (DT104b).*

***Keywords**: Kaeng Kathi, Kaeng Pa,* Salmonella enterica *Typhimurium.*

1. Introduction

In recent years, food safety concerns have been focused on pathogens, such as *Salmonella*, which are recognized as a primary cause of food poisoning worldwide and massive outbreaks have occurred in several parts of the world (Van Immweseel *et al*. 2005).

It is estimated that globally around 86% of salmonellosis cases are food-borne (Majowicz *et al*. 2010). *Salmonella* Typhimurium is one type of *Salmonella* serovars that has a remarkable ability to survive and becomes the major cause of salmonellosis food-borne illness. This is reflected by the fact that 6.6% of food-borne outbreaks were attributable to this serotype in an international study (Greig and Ravel 2009).

One type of food ingredients with the potential to be the natural source of antibiotics is Thai red curry paste. Thai red curry paste is a very important ingredient for different kinds of Thai curries: Kaeng Pa (water base) and Kaeng Kathi (coconut-milk base), and contains various kinds of herbs and spices that are rich sources of biologically active antimicrobial compounds. The inhibitory effect of these herbs and spices on a variety of microorganisms was described by Arora and Kaur (1999).

As these herbs and spices are contained in Thai red curry paste, it became worthwhile to investigate their potential as a natural antibacterial compound in order to fight against *Salmonella* Typhimurium DT104b. Therefore, the objective of this experimental study is to

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investigate the individual antibacterial activities of each herb/spice contained in Thai red curry paste under three extraction methods (Kaeng Kathi - oil using fresh coconut milk, Kaeng Kathi - oil using UHT coconut milk, and Kaeng Pa using water) against *S.* Typhimurium DT104b.

2. Materials and Methods

2.1 Preparation of Plant Samples

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

Every herb was cleaned properly. The dry chili was cut into small pieces and soaked in water for 20 min before use. The kaffir lime peel was cut off from the fruit. The dry cumin was cleaned before use by passing it through running water. Also, the shallot was peeled and chopped into fine pieces, the garlic was peeled and chopped, the lemongrass stem was sliced, and the galangal was peeled and sliced. Then 50 grams of each herb were weighed out and ground up in an automatic mortar for 15 min.

2.2 Extraction Conditions

The three extraction models, based on Thai homemade authentic cooking, consisted of Kaeng Kathi (fresh coconut milk extraction model), Kaeng Kathi (UHT coconut milk extraction model), and Kaeng Pa (water extraction model).

For Kaeng Kathi (fresh coconut milk extraction model), fresh coconut milk was prepared by weighing 1 coconut : 1 water, mixing it together and soaking it for 5 min. The coconut milk was separated from the grated coconut by squeezing. Then 80-ml fresh coconut milk was boiled for 5 min, 50-g herb substance was added and stirred for 5 min, and lastly coconut milk solution (1 coconut milk : 2 water) was added and stirred.

For Kaeng Kathi (UHT coconut milk extraction model), 80 ml of ready-to-use pasteurized coconut milk was boiled for 5 min, then 50-g herb substance was added and stirred for 5 min, and lastly coconut milk solution (1 coconut milk : 2 water) was added and stirred.

For Kaeng Pa (water extraction model), 50-g herb substance was added to 200 ml of water, stirred and boiled.

For all three extraction models, the boiling on a hot plate (VELP Scientifica, model Are 2) continued for 1 hr and stirring was performed every 5 min. The cooking temperature was controlled in the range of 98-100°C.

2.3 Preparation of the Culture

The stock culture was prepared by inoculating a loopful of *S.* Typhimurium DT104b into 10-ml fresh nutrient broth (NB) and shaked on culture tube rotator SCI (Stuart Scientific) at 37°C for one night. 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 reached 0.1 (SPECTRONIC, model GENESYS 5) for an early log phase (Pitinidhipat and Yasurin 2012).

2.4 Antibacterial Assay

Modified disc diffusion method (Pitinidhipat and Yasurin 2012) was used to test the antibacterial activity of herbs in this experimental study. Sterile discs were made from 2-layered Whatman filter paper number 41 and sterilized at 121°C for 15 min. Additionally, sterile cotton buds were also sterilized at 121°C for 15 min. The area on *Salmonella - Shigella* (SS) agar plates was divided into four parts. Each plate was swabbed 100 µl of culture on the agar by using the sterile cotton buds under aseptic technique. After the agar plates dried up, in each part was placed a paper disc so that the first part and the second part had 30 µl of herb extract, the third part had 30 µl of control, and the last part had 30 µl of 100 mg/ml penicillin-G (Fluka BioChemika). All plates were incubated at 37°C for 24 hrs. Clear zone result was measured as inhibitory effect. The obtained data were collected and calculated for mean and standard deviation using Microsoft Excel 2007. All experiments were performed in duplicate and repeated three times.

2.5 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration Determination

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods (Pitinidhipat and Yasurin 2012) were used. For MIC tests, the herb extracts of the following concentrations were added into 1 ml fresh NB: 0, 25, 50, 75, 100, 125 and 150 µl/ml. Then 100 µl/ml of *S.* Typhimurium DT104b, when OD600 reached 0.1 (early log phase), was inoculated and incubated at 37°C for 24 hrs. The tubes which showed negative results in MIC tests were used in MBC tests. The 1 loop of negative MIC was streaked on nutrient agar (NA). All plates were incubated at 37°C for 24 hrs. The growth of microbes in each plate was observed. All experiments were performed in duplicate and repeated three times.

3. Results and Discussion

3.1 Antibacterial Activity

The seven herbs in Thai red curry paste showed natural antibacterial activities against *S.* Typhimurium DT104b in all three extraction conditions: Kaeng Kathi (fresh coconut milk extraction model), Kaeng Kathi (UHT coconut milk extraction model), and Kaeng Pa (aqueous extraction model). The results are shown in Tables 1-3, and the comparison is shown in Fig. 1.

The results in Tables 1-3 show that each one of the herb extracts contained in Thai red curry paste has potential antibacterial activity. Among the three extraction conditions, the herbs showed varying antibacterial activity results against *S.* Typhimurium DT104b. For example, kaffir lime showed low antibacterial activity in Kaeng Kathi using fresh coconut milk extraction and in Kaeng Pa. However, kaffir lime showed high antibacterial activity in Kaeng Kathi using UHT coconut milk extraction.

In Kaeng Kathi using fresh coconut milk extraction, cumin and lemongrass showed the highest antimicrobial activity with diameters of clear zone of 1.58 ± 0.41 and 1.32 ± 0.59 cm, respectively. The lowest antibacterial activities were found in Kaeng Pa extracted kaffir lime and Kaeng Kathi (UHT coconut milk) extracted cumin, with diameters of clear zone of 0.25 ± 0.37 and 0.27 ± 0.41 cm, respectively.

Garlic has been proven to inhibit the growth of gram-positive and gram-negative bacteria including strains of *Pseudomonas*, *Proteus*, *Escherichia*, *Staphylococcus*, *Salmonella* (Iwalokun *et al*. 2004). Kaffir lime has been used extensively in traditional Thai medicine (Saralamp *et al*. 1996). Red chili contains capsaicin, a homovanillic acid derivative (8-methyl-N-vanillyl-6-moneamide), which is an irritant and vasoactive component (Tellez *et al*. 1993; McElroy *et al*. 1994). Previous studies with different levels of dietary capsaicin, either natural or synthetic, have demonstrated reductions in *S. enteritidis* organ invasion with no adverse effects on body weight and feed consumption on 11, 16 or 19 day-old broilers and leghorn chickens (Tellez *et al*. 1993; McElroy *et al*. 1994). Galangal, also known as the greater galangal, is indigenous to Southeast Asia. The essential oils and extracts of greater galangal rhizomes have been studied extensively and have been proven to have antifungal, antigiardial, antiamoebic, antimicrobial, and antioxidant activities (Hsu *et al*. 2010). Despite extensive studies on the rhizome of the greater galangal plant, the antimicrobial properties of its flowers have not been investigated (Hsu *et al*. 2010). Cumin is an aromatic plant of the Apiaceae family, and has a broad antibiotic spectrum against both gram-positive and gram-negative bacteria (Sheikh *et al*. 2010). Cumin oil shows a high antifungal activity against various pathogenic fungi. It is also used as a fumigant or additive in the storage of foodstuff (Sheikh *et al*. 2010). Lemongrass, a perennial herb, is widely cultivated throughout the warm tropical climate of the world. The essential oil is used as food flavoring, and an ingredient in cosmetics and perfumes (Shin 2005). The inhibitory activities of lemongrass oil against different microbial species and insects have been documented (Shin 2005).

Table 1. Antibacterial activity as clear zone (cm) of seven herbs extracted in Kaeng Kathi (fresh coconut milk model) on *S.* Typhimurium DT104b.

Herb and Spice	Kaeng Kathi
Galangal	0.98 ± 0.23
Shallot	0.97 ± 0.23
Kaffir lime	0.77 ± 0.11
Cumin	1.58 ± 0.41
Dry Chili	0.92 ± 0.08
Lemon grass	1.32 ± 0.59
Garlic	0.93 ± 0.22
Fresh coconut milk*	0.81 ± 0.38
Penicillin-G*	2.04 ± 0.27

*Fresh coconut milk and 100 mg/ml penicillin-G were used as positive control.

Table 2. Antibacterial activity as clear zone (cm) of seven herbs extracted in Kaeng Kathi (UHT coconut milk model) on *S.* Typhimurium DT104b.

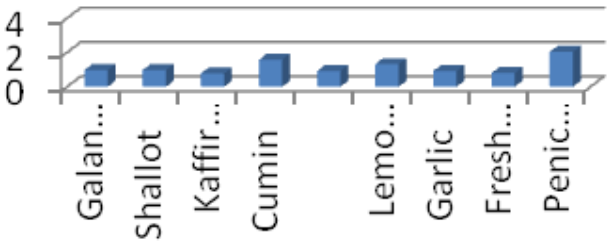
Herb and Spice	Kaeng Kathi
Galangal	0.49 ± 0.37
Shallot	0.88 ± 0.17
Kaffir lime	0.90 ± 0.14
Cumin	0.27 ± 0.41
Dry Chili	0.82 ± 0.10
Lemongrass	0.82 ± 0.10
Garlic	0.80 ± 0.61
UHT coconut milk*	0.62 ± 0.27
Penicillin-G*	1.82 ± 0.24

*UHT coconut milk and 100 mg/ml penicillin-G were used as positive control.

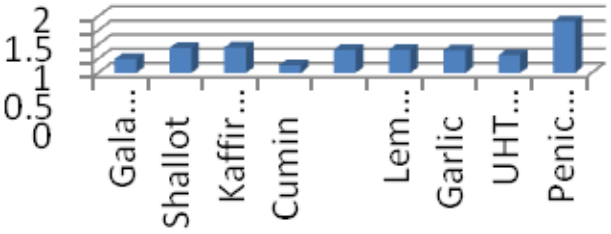
Table 3. Antibacterial activity as clear zone (cm) of seven herbs extracted in Kaeng Pa model on *S.* Typhimurium DT104b.

Herb and Spice	Kaeng Pa
Galangal	0.65 ± 0.31
Shallot	0.74 ± 0.05
Kaffir lime	0.25 ± 0.37
Cumin	0.90 ± 0.14
Dry Chili	0.69 ± 0.23
Lemon grass	0.47 ± 0.35
Garlic	0.90 ± 0.14
Water*	0.62 ± 0.26
Penicillin-G*	1.62 ± 0.50

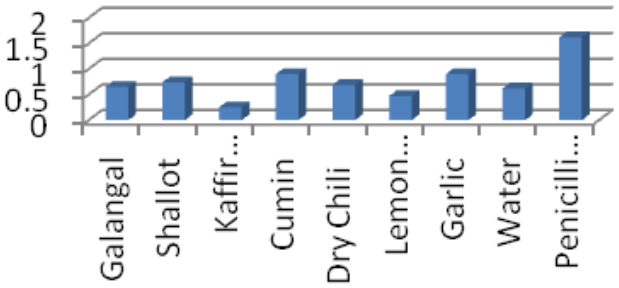
*Water and 100 mg/ml penicillin-G were used as positive control.



(a) Kaeng Kathi (fresh coconut milk) model.



(b) Kaeng Kathi (UHT coconut milk) model.



(c) Kaeng Pa model.

Fig. 1. Antibacterial activity as clear zone (cm) of individual herb crude extracted against *S. Typhimurium* DT104b in different extraction models: (a) Kaeng Kathi (fresh coconut milk) model; (b) Kaeng Kathi (UHT coconut milk) model; and (c) Kaeng Pa model.

Generally, the higher antibacterial activity of coconut milk as an extractant (Kaeng Kathi model) compared to water (Kaeng Pa model) is possibly caused by two reasons.

First, coconut milk contains the bioactive compounds, medium-chain saturated fatty acids such as lauric acid, caproic acid, and caprylic acid, that have powerful antimicrobial properties. For example, lauric acid has been proven to inhibit effectively the growth of *Chlamydia trachomatis*, *Helicobacter pylori*, *Neisseria gonorrhoeae* and *Candida albicans* (Sia *et al*. 2010). Those results (Sia *et al*. 2010) support the observation of the coconut milk's antibacterial activities in the conducted experiments with both fresh and UHT coconut

milk, 0.81 ± 0.38 and 0.62 ± 0.27 cm, respectively. Also, the fresh coconut milk contains a higher amount of fat content, approximately 24% (Pehowich *et al*. 2000) while commercial UHT coconut milk fat content is approximately 17-20%. This explains the results that fresh coconut milk has higher antibacterial activity compared to UHT coconut milk.

Second, higher fat content means that the solvent becomes more non-polar. This polarity property may affect the extraction process by having different extraction rates for distinct herbs and the amount of antimicrobial compound extracted may be different among these herbs.

The water itself showed relatively the same antimicrobial activity against *S. Typhimurium* DT104b compared to both fresh and UHT coconut milk. However, the water used was tap water, which possibly contained chlorine capable of inhibiting the growth of *S. Typhimurium* DT104b.

As shown in Tables 1-3, the herb extracts of Thai red curry paste are very promising for becoming an alternative natural antimicrobial source.

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

The MIC, using broth dilution method, is the concentration of herb extract needed to inhibit the growth of *S. Typhimurium* DT104b, while the MBC is the concentration of herb extract needed to completely inhibit *S. Typhimurium* DT104b (Table 4).

The results from Table 4 indicate that in Kaeng Kathi using fresh coconut milk, galangal showed the highest antibacterial activity (the lowest MIC, 25 µl/ml) against *S. Typhimurium* DT104b, while in Kaeng Kathi using UHT coconut milk, dry chili showed the highest antibacterial activity (the lowest MIC, 50 µl/ml). In Kaeng Pa, the galangal showed the highest antibacterial activity (the lowest MIC 50 µl/ml) against *S. Typhimurium* DT104b.

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) on herbs contained in Thai red curry paste under different extraction models against *S. Typhimurium* DT104b.

Extraction	Herbs	MIC (µl/ml)	MBC (µl/ml)
Kaeng Kathi (fresh coconut milk)	Galangal	25	100
	Shallot	75	>150
	Kaffir lime	50	50
	Cumin	125	125
	Dry chili	50	50
	Lemongrass	100	>150
	Garlic	100	125
Kaeng Kathi (UHT coconut milk)	Galangal	75	150
	Shallot	75	150
	Kaffir lime	>150	>150
	Cumin	75	75
	Dry chili	50	50
	Lemongrass	125	125
	Garlic	25	25
Kaeng Pa (Water)	Galangal	50	125
	Shallot	>150	>150
	Kaffir lime	>150	>150
	Cumin	75	>150
	Dry chili	75	75
	Lemongrass	100	>150
	Garlic	>150	>150

4. Conclusion

The obtained results indicate that the different extraction methods have significantly different effects on the antimicrobial activities of herb extracts. Generally, Kaeng Kathi using fresh coconut milk is the best extraction model among the three models, followed by Kaeng Kathi using UHT coconut milk and then Kaeng Pa. Concerning the individual herbs, Kaeng Kathi using fresh coconut milk extracted cumin has the best inhibition effect against *S. Typhimurium* DT 104b.

It can be concluded that herbs contained in Thai red curry paste have a great potential to become the alternative source of natural antibacterial activity in order to combat *S. Typhimurium* DT 104b.

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