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NATURAL ANTIBACTERIAL ACTIVITY OF THAI RED CURRY PASTE IN THAI RED CURRY (KANG-KATI AND KANG-PANAENG) MODEL AGAINST *Salmonella enterica* Enteritidis

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Abstract

Beginning in the 1970s, the incidence of *Salmonella enterica* serotype Enteritidis (SE) infection and the number of related outbreaks in the United States has increased dramatically. Foods as natural antibiotics itself might be considered as an alternative for food safety. Thai red curry is a Thai cultural dish which has become a well-known dish worldwide having fresh coconut milk and red curry paste as a main ingredient. The red curry paste main ingredients include various types of herbs; *Capsicum annuum* (chili), *Citrus hystrix* (Kaffir lime), *Cuminum cyminum* L. (Cumin), *Allium ascalonicum* L. (Shallot), *Allium sativum* (Garlic), *Cymbopogon citratus* (Lemongrass), *Alpinia galangal* (Galangal). All of the mentioned herbs have been investigated for their antibiotics activity. Therefore, the objective of this research is to investigate the antibacterial activity of red curry paste in Kang-Kati and Knag-Panaeng model against *Salmonella enterica* Enteritidis. The curry paste in-vitro antibacterial activity was evaluated by cell count serial dilution method on SS media every hour for 6 hrs at room temperature. Thai curry was prepared by Thai homemade authentic cooking method as it has been served in Thai cuisine. The result showed that the *S. enterica* Enteritidis log CFU/ml level in Kang-Kati was significantly lower than in nutrient broth (NB), as positive control, ($P < 0.05$) since 4th - 6th hour, 4th; 6.27±0.01 and 6.42±0.05, 5thhr; 6.36±0.04 and 7.20±0.07 and 6thhr; 7.13±0.05 and 7.39±0.03 log CFU/ml, respectively. For Kang-Panaeng was significant lower than those of positive control (NB), ($P<0.05$) at 5th and 6th hour: 5thhr; 6.45±0.017and 8.05±0.072, 6thhr;6.71±0.448 and 8.11±0.070 log CFU/ml, respectively. The T-test has been done by using SAS on log CFU/ml with $P < 0.05$.The curry paste in both Kang-Kati and Kang-Panaeng model showed promising antibacterial activity against food-borne pathogenic bacteria, *S enterica* Enteritidis.

Keywords: antibacterial, *S. enterica* Enteritidis, Kang-Kati, Kang-Panaeng

Introduction

Currypaste is a finely ground or pureed blend of aromatic spices and herbs. It is widely used as an ingredient in the cuisines of many cultures to make curries, stews, and other dishes. The ingredients that used for making Thai curry paste compost of variety types of herbs which are *Capsicum annuum* (chili), *Citrus hystrix* (Kaffir lime), *Cuminum cyminum* L. (Cumin), *Allium ascalonicum* L (Shallot), *Allium sativum* (Garlic) , *Cymbopogon citratus* (Lemongrass), *Alpinia galangal* (Galangal). At present, food safety is a fundamental concern to both consumers and food industries in particular as there are an increasing number of reported cases of food associated infections(1). Natural food antibacterial is considered as functional foods, which contain healing properties above and beyond basic nutritional value. Foods can stimulate immune function, increasing resistance to infection, and inhibit the action of pathogenic microorganisms. In addition, the herbs that used for making curry not only works as a food but also they can function as a medicinal food (1).

In 2011, there are many reports about the *Salmonella* sp. outbreaks which cause over 100 patients from salmonellosis (2). *Salmonella sp*.is rod-shaped, gram-negative, facultative anaerobic

bacteria. Therefore the objective of this experiment is to investigate the antibiotics activity of Thai curry paste in fresh coconut milk based curry model on *S. enterica Enteritidis*

Materials and Method

Preparation of curry paste for Kang-Kati

The curry paste formula were 40% w/w chilli (*C. annuum*), 20%w/w lemon grass (*C. citrates)*, 15% w/w garlic (*A. sativum)*, 10% w/w galangal (*A. galangal)*, 10% w/w shallot (*A. ascalonicum* L), 2% w/w shrimp paste, 1% w/w kaffir lime peel (*C. hystrix*), 0.5% w/w salt, and 1.5% of cumin powder (*C. cyminum* L. The raw materials were hand grinded by the mortar, approximately 100 rpm. In grinding, the raw materials were added in order and time as following; chili and salt for 10 min, garlic and shallot 5 min, galangal and lemongrass for 5 min, kaffir lime peel and cumin powder for 3 min, shrimp paste 2 min, and continue grinding for 5 minutes (3).

Preparation of Kang-Panaeng.

The curry paste formula were 25% w/w lemon grass (*C. citrates*), 20% w/w sugar, 15% w/w chili(*C. annuum*), 15% w/w garlic (*A. sativum*), 13.5% w/w shallot (*A. ascalonicum* L), 8% w/w peanuts, 3% w/w shrimp paste, 0.5% w/w salt. The raw materials were hand grinded by the mortar. In grinding, the raw materials were added in order and time as following; chili for 8minutes, lemongrass and garlic for 7 minutes, shallot and sugar for 5 minutes, peanuts for 2 minutes, shrimp paste and salt for 1 minutes. Then, grind every raw material about 4 minutes (3).

Table 1 List of Thai herbs used in this experiment

Scientific name	Common name	Used part
Capsicum annuum	Chili	Fruit
Citrus hystrix	Kaffir lime	Peel
Cuminum cyminum L.	Cumin	Seed
Allium ascalonicum L.	Shallot	Tuber
Allium sativum	Garlic	Tuber
Cymbopogon citrates	Lemongrass	Stem
Alpinia galangal	Galangal	Tuber

Preparation of Kang-Kati

The fresh coconut milk was prepared by weight 1 coconut: 1 water; mixed it together, soaked for 5 minutes and separated coconut part out of coconut milk by cheesecloth.

The prepared fresh coconut milk was boiled for 5 minutes using hot plate (VELP SCIETIFICA, model Are2) as heater. At the step, oil and water phase in coconut milk was separated then the 45 grams prepared curry paste was added, and stirred for 5 minutes. Then, coconut milk solution (1 coconut milk: 2 water) was added and continue boiling until 1 hr. The curry was stirred every 5 minutes. The temperature is in the range of 90-92 ˚C. (3)

Preparation of the culture

The stock culture was prepared by inoculating one loopful of *Salmonella enteric Enteritidis* into 50 ml fresh NB and shake on the shaker (IKA LABORTECHNIK, model KS 501 Digital) with 100 rpm at room temperature overnight. Then 1 % v/v overnight culture was inoculated into 50 ml of fresh NB, at room temperature by Culture tube Rotator SCI (Stuart Scientific), until OD600 reach 0.1 (SPECTRONIC, model GENESYS 5) which is early log phase. (3)

Antibacterial Assay

1% v/v of early log phase *S. enterica Enteritidis* was inoculated in 100 ml curry inoculated curry then incubated at room temperature. The cell count serial dilution method was used to evaluate antibacterial activity by using the Salmonella- Shigella (SS) agar. The curry was taken every hour for 6 hrs. The colony forming unit was observed after 24 hours incubation at room temperature. The

control was done in the same way in NB, inoculated at room temperature, 100 rpm, and to show the real growth pattern of *S. enterica* Enteritidis.(3)

Statistical analyses

The experiment was performed in duplicate and repeated three times. The independently two-sample t-test was used to study the effect of the antibiotic from the curry paste on the growth of *S. enterica* Enteritidis, at different time by using SAS program. (3)

Result and Discussion

From Table 2 and 3, it showed that curry paste had promising in antibacterial activity.

Table 2: The logCFU/ml count of *S. enterica* Enteritidis growth in Kang-Kati and control

Time (Hours)	log CFU/ml	
	Kang-Kati	**Control(NB)**
0	ND	ND
1	ND	ND
2	5.70 ± 0.04^{a}	5.77 ± 0.06^{a}
3	6.14 ± 0.02^{a}	6.30 ± 0.11^{a}
4	6.27 ± 0.01^{a}	6.42 ± 0.05^{b}
5	6.36 ± 0.04^{a}	7.20 ± 0.07^{b}
6	7.13 ± 0.05^{a}	7.39 ± 0.03^{b}

Table 3: The logCFU/ml count of *S. enterica* Enteritidis growth in Kang-Panaeng and control

Time (Hours)	log CFU/ml	
	Kang-Panaeng	**Control(NB)**
0	ND	ND
1	6.32 ± 0.068^{a}	ND^{b}
2	6.38 ± 0.104^{a}	6.25 ± 0.044^{a}
3	7.05 ± 0.587^{a}	6.19 ± 0.206^{a}
4	7.05 ± 0.545^{a}	6.38 ± 0.038^{a}
5	8.05 ± 0.072^{a}	6.45 ± 0.017^{b}
6	8.11 ± 0.070^{a}	6.71 ± 0.448^{b}

*: there is significantly different ($P < 0.05$)
ND: less than 30 colonies

It showed that there is no significantly different of *S. enterica* Enteritidis level ($P>0.05$) between Kang-Kati and control during first 3 hours. However, since the fourth hour to the sixth hour, the result showed that there is significantly different the log CFU/ml *S. enterica* Enteritidis level ($P>0.05$) between curry comparing with control since 4^{th} - 6^{th} hour, 4^{th}; 6.27±0.01 and 6.42±0.05, 5^{th}hr; 6.36±0.04 and 7.20±0.07 and 6^{th}hr; 7.13±0.05 and 7.39±0.03 log CFU/ml, respectively. For Kang-Panaeng, the results showed that the *S. enterica* Enteritidis was significant lower than those of positive control (NB). ($P<0.05$) at 5^{th} and 6^{th} hour, 5^{th}6.45±0.017and 8.05±0.072, 6^{th}hr;6.71±0.448 and 8.11±0.070 log CFU/ml, log CFU/ml, respectively.

In Table 4, the specific growth rate in Kang-Kati and Kang-Panaeng was less than control, The reducing of specific growth rate indicated that curry paste inhibited *S. enterica* Enteritidis

Table 4: Specific growth rate of *S. enterica Enteritidis* growth in curry and control

Specific Growth Rate (hour^{-1})	
Kang-Kati/Control	**Kang-Panaeng/Control**
0.18/0.252	0.158/0.495

These results showed that Thai curry paste in Kang-Kati and Kang-Panaeng model, which was kept at room temperature (Thai traditional style, approximately 30 °C), inhibited the growth of *S. enterica* Enteritidis. This indicated that both curry paste showed the combination effect of natural antibacterial activity against *S.enterica* Enteritidis. And the curry paste is source of natural antibacterial compounds against *S. enterica* Enteritidis growth.

Coconut milk contain 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 (5,6). The higher fat content means the solvent become more non-polar. These polarity properties may effect on the extraction process and cause different extraction rate for the compound on herbs, and the amount of antimicrobial compound extracted may be different among these herbs (6).

The curry paste is made from many kind of herbs as followed; *C. annuum* (chili) which was investigated that the main chemical component was capsaicin. Capsaicin is a hydrophobic molecule with boiling point of 210-220°C (7,8). Capsaicin constitutes of paper and gives its taste, also has pharmacological and physiological effect (7,8). It has also been show that capsaicin species and also capsaicin have antimicrobial effects with broadly activities on both bacterial and fungal such as *Fusarium* (9) , *Helicobacter pylori* (10), *Botrytis cinerea*, and *Aspergillus niger* (11). Capsaicin might be a possible antibacterial agent against *S. enterica Enteritidis*in this experiment. *C. hystrix* (Kaffir lime), its main essential oil was β-pinene which can inhibit the growth of many microorganisms; *S. Enteritidis* (12*S. aureus*, *B. cereus*, *Listeria monocytogenes*,*Saccharomyces cerevisiae* var. sake and *Aspergillus fumigatus* TISTR 3180 (13).

C. cyminum L. (Cumin) ,mostly compost of oil was cuminaldehyde (20-72%) and monoterpene hydrocarbons (e.g. β-pinene, γ-terpinene, p-cymene), which showed that they can inhibit the growth of about 20 serotypes of *Salmonella* sp. (inhibition zone range of 8-10 mm.) by the ethanolic extracts (12). The cumim using fresh coconut milk extracted, 1.6 ± 0.22 cm, against *S. enterica*EnteritidisTyphimurium DT104b (14). *In vitro* antibacterial screening results, individual cumin in Kang-Kati model using fresh coconut milk showed highest antibacterial; 0.90±0.14cm, against *S. enterica* Enteritidis US clone (15).

A. ascalonicum L. (Shallot) was flavanols and phenolic compounds (16). Shallot also has antimicrobial activity; it has been reported to have a heat stable antimicrobial activity against bacteria and fungi (17). Moreover, they also have broad spectrum against both fungal and bacterial such as *Syncephalastrum*, *A. niger*, *Penicillium* sp., *Paecilomyces* sp., *Scopulariopsis* sp. (18), *B. cereus*, *Escherichia coli* O157:H7, and *S. enterica* (17).

Allium sativum(Garlic) which is one of the condiments in chili paste contains allicin. It is one of the active ingredients found during crushing garlic. Allicin has variety of antimicrobial activities (19). Thus by making chili paste, mechanic mortar will be able to extract allicin out. Also from the work of Kumar & Berwal (20) and Singh (21) reported that garlic was found to be effective against *L. monocytogenes*. Lis-Balchin and Deans (22) studied 93 commercial essential oils against 20 *L. monocytogenes* strains. *In vitro* antibacterial screening results, individual garlic in Kang-Kati model using fresh coconut milk showed highest antibacterial; 0.90±0.14cm, against *S. Enterica* 4,5,12:i:- (human) US clone (23).

Duan and Zhao (22) reported that Lemongrass essential oil inhibit the growth of *E. coli* O157:H7 and *S. enterica* Enteritidis completely when the concentration of lemongrass was increased to 3µl/ml. Nanasombat and Lohasupthawee (12) studied crude extracts and essential oils of many herbs including crude ethanolic extract of lemongrass which was active against 17 strains of *Salmonella* spp. (7-11 mm) from total 25 strains including *S. Enteritidis*. Lazuardi and others 2012, lemongrass extracted in fresh coconut milk gave antibacterial activity, 1.4 ± 0.24 cm against *S. enterica* Typhimurium DT104b.

It has been shown that essential oils from both fresh and dried rhizomes of galangal have antimicrobial activities against bacteria, fungi, yeast and parasite (24). Rattanakom and Yasurin, 2012 (25), studied antibacterial activity of red curry paste in Kang-Pa as real food model showed that the levels of *L. monocytogenes* 10403S in Kang-Pa was significant lower than of positive control (BHI) ($P < 0.05$), since 1st-6th hour. This indicated that Thai curry paste in Thai red curry showed promising antibacterial activity.

Conclusion

The curry paste in Kang-Kati and Kang-Panaeng model (as read food model) that was made by Thai homemade authentic cooking method showed natural antimicrobial combination effect against *S. enteric* Enteritidis.

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