

Preliminary Study of Antimicrobial Activities on Medicinal Herbs of Thai Food Ingredients

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Abstract

To investigate the potential for use of medicinal herbs as natural antimicrobial additives for foods, the following fifteen medicinal herbs were examined for antimicrobial activity: coriander (*Coriandrum sativum* Linn), shallot (*Allium ascalonicum* L.), ginger (*Zingiber officinale* Roscoe), galangal (*Alpinia galanga* Swartz), lemon grass (*Cymbopogon citratus* Stapf.), kaffir lime leaves and peels (*Citrus hystrix* DC), lime peels (*Citrus aurantiifolia* Swing), bird chili (*Capsicum frutescens* L.), curcuma (*Curcuma longa* L.), sweet basil (*Ocimum basilicum* L.), holy basil (*Ocimum sanctum* Linn), bitter cucumber (*Momordica charantia* L.), devil's fig (*Solanum torvum* Sw.) and indian malberry (*Morinda citrifolia* L.). Fresh extracts and oil extracts of all medicinal herbs in this study were tested against *Bacillus cereus*, *Salmonella typhi* and *Staphylococcus aureus* by using Agar Diffusion Method. Six of them including *Zingiber officinale*, *Allium ascalonicum*, *Alpinia galanga*, *Citrus hystrix*, and *Citrus aurantifolia* were shown high activities in the first screening. A second screening for antimicrobial activities of six potential medicinal herbs were performed in duplication. All of six herbs exhibited antimicrobial activities against *Bacillus cereus* and *Staphylococcus aureus*, and all except *Z. officinale* exhibited significant activity against *Salmonella typhi*. Antimicrobial activities of oil extracts were higher than fresh extracts. *C. hystrix*, *A. ascalonicum*, and *C. aurantiifolium* were selected as potential antimicrobial food additives, and the identification of active compounds and suitable purification method in these medicinal plants have been suggested to further studies.

INTRODUCTION

Traditional Thai food has a very distinctive character because of the special combination of herb and spices in Thai food preparation. These herb and spice ingredients are more than just good taste but they make Thai food more healthful. Recently, the demand for medicinal herb products has begun to grow and gain popularity. Natural antimicrobial agent development from Thai food ingredients may respond to the national drug policy and "Thai Herbs for Health" promotion. The fifteen medicinal herbs of Thai food's ingredient were selected for antimicrobial screening are: coriander, shallot, ginger, galangal, lemon grass, kaffir lime leaves, kaffir lime peels, lime peels, bird chili, curcuma, sweet basil, holy basil, bitter cucumber, devil's fig and indian malberry (Thiengburanathum, 1996; Liangmaneehetp, 1997). The objective of this work was to investigate medicinal herbs of Thai food ingredients as natural antimicrobial agents.

MATERIALS AND METHODS

Fifteen selected medicinal herbs samples for Thai food's ingredients were collected from a local market in Bangkok. They were coriander (*Coriandrum sativum* Linn), shallot (*Allium ascalonicum* L.), ginger (*Zingiber officinale* Roscoe), galangal (*Alpinia galanga* Swartz), lemon grass (*Cymbopogon citratus* Stapf.) kaffir lime leaves and peels (*Citrus hystrix* DC), lime peels (*Citrus aurantiifolia* Swing), bird chili (*Capsicum frutescens* L.), curcuma (*Curcuma longa* L.), sweet basil (*Ocimum basilicum* L.), holy basil (*Ocimum sanctum* Linn), bitter cucumber (*Momordica charantia* L.), devil's fig (*Solanum torvum* Sw.) and indian mulberry (*Morinda citrifolia* L.). Each sample was prepared into fresh extracts and oil extract. Twenty grams of each chopped herbs were prepared for each extracts. Fresh extracts were prepared by using a manual extractor and squeezing. The oil extracts were extracted with alcohol by using Soxtec, Tecator model 1043. The preliminary study of antimicrobial activity on fresh and oil extracts of all herbs was performed by using agar diffusion method both in disk diffusion test and cup plate agar diffusion test (Isenberg, 1998; Mohon and Manuselis, 1995; Victor, 1991). The sensitivity of both extracts were tested against *Bacillus cereus* ATCC25923, *Salmonella typhi* ATCC19430 and *Staphylococcus aureus* ATCC25923 (TISTR culture collection, 1995). The sensitivity test was performed by using an antibiotic drug as a control. The antimicrobial activity was measured by the inhibition zones produced. All experiments were duplicated.

RESULTS AND DISCUSSION

This investigation of antimicrobial activity was performed on fifteen selected medicinal herbs. Six of them including ginger (*Zingiber officinale* Roscoe), galangal (*Alpinia galanga* Swartz), kaffir lime leaves and kaffir lime peels (*Citrus hystrix* DC) and lime peels (*Citrus aurantiifolia* Swing) showed high antimicrobial activity in the first screening step (Table 1). The second screening step in the preliminary study for antimicrobial activity was the Disk Diffusion Test on Agar Diffusion Method. This test was used in order to determine antimicrobial activity of fresh extracts and oil extracts. The diameter of the clear zone indicated the inhibition activity. All of the plants tested showed antimicrobial effect against *Bacillus cereus* in both fresh and oil extracts (Table 2). For antimicrobial activity against *Salmonella typhi*, all plants except ginger showed high activity in oil extracts but ginger, galangal and kaffir lime leaves showed no activity in fresh extracts (Table 3). All except ginger in fresh extracts showed antimicrobial activity against *Staphylococcus aureus* (Table 4). The confirmatory step was performed using the Cup Plate Agar Diffusion Method and gave the same results as in the second step (Table 5). It was also shown that the oil extracts exhibited higher antimicrobial activity than fresh extracts. Kaffir lime peels, shallot and lime peels showed high potential on antimicrobial activity against *Bacillus cereus*, *Salmonella typhi* and *Staphylococcus aureus*. The results from this study indicated the possibility of using medicinal herbs from Thai food ingredients as natural antimicrobial agents. Appropriate extracting conditions and identification of active compounds through several purification methods should be examined in future studies.

CONCLUSIONS

Six of the fifteen selected medicinal herbs used as Thai food ingredients, ginger, galangal, kaffir lime leaves, kaffir lime peels and lime peels showed antimicrobial activity against *Bacillus cereus*, *Salmonella typhi* and *Staphylococcus aureus* on the first screening step. In the second and confirmatory steps, kaffir lime peels, shallot and lime peels showed high sensitivity for all studied microbials. The oil extracts exhibited higher antimicrobial activity than fresh extracts. The potential of kaffir lime peels, shallot and lime peels as natural antimicrobial agents have been confirmed. Further studies on active compound identification and suitable purification of these medicinal plants are suggested.

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Tables

Table 1. Antimicrobial activity of selected medicinal herbs on the first screening step.

Medicinal herbs	<i>Bacillus cereus</i>		<i>Salmonella typhi</i>		<i>Staphylococcus aureus</i>	
	Oil extracts	Fresh extracts	Oil extracts	Fresh extracts	Oil extracts	Fresh extracts
Coriander	-	-	-	-	-	-
Shallot	+	+	+	-	+	+
Ginger	+	-	-	-	+	-
Lime peel	+	+	+	-	+	-
Galangal	+	-	-	-	+	-
Kaffir lime leaves	+	-	-	-	+	-
Kaffir lime peels	+	-	+	-	+	-
Curcuma	+	-	-	-	-	-
Bird chili	-	-	+	-	-	-
Sweet basil	-	-	-	-	-	-
Holy basil	-	-	-	-	-	-
Bitter cucumber	+	+	-	-	-	-
Lemon grass	+	-	-	-	-	-
Devil's fig	-	-	-	-	-	-
Indian malberry	+	-	-	-	-	-

(+ = with clear zone, - = no clear zone).

Table 2. Showed antimicrobial activity against *Bacillus cereus* of 6 potential medicinal herbs on second screening step.

No.	Medicinal herbs	Fresh Extracts		Oil Extracts	
		Test 1	Test 2	Test 1	Test 2
		dia. of clear zone (mm)	dia. of clear zone (mm)	dia. of clear zone (mm)	dia. Of clear zone (mm)
1	Ginger	8	8	10	10
2	Shallot	9	9	13	13
3	Galangal	11	11	15	15
4	Kaffir lime leaves	8	8	11	12
5	Lime peels	8	8	11	11
6	Kaffir lime peels	10	11	17	18
Control	Chloramphenicol	32	32	32	32

Table 3. Showed antimicrobial activity against *Salmonella typhi* of 6 potential medicinal herbs on second screening step.

No.	Medicinal herbs	Fresh Extracts		Oil Extracts	
		Test 1	Test 2	Test 1	Test 2
		dia. of clear zone (mm)	dia. of clear zone (mm)	dia. of clear zone (mm)	dia. of clear zone (mm)
1	Ginger	6*	6*	6*	6*
2	Shallot	10	10	12	12
3	Galangal	6*	6*	8	8
4	Kaffir lime leaves	6*	6*	8	8
5	Lime peels	8	8	8	8
6	Kaffir lime peels	10	10	12	12
Control	Penicillin	13	13	14	13

* = no clear zone and report as R resistant

Table 4. Showed antimicrobial activity against *Staphylococcus aureus* of 6 potential medicinal herbs on second screening step.

No.	Medicinal herbs	Fresh Extracts		Oil Extracts	
		Test 1	Test 2	Test 1	Test 2
		dia. of clear zone (mm)	dia. of clear zone (mm)	dia. of clear zone (mm)	dia. of clear zone (mm)
1	Ginger	6*	6*	12	12
2	Shallot	13	13	24	24
3	Galangal	8	8	11	11
4	Kaffir lime leaves	10	9	12	12
5	Lime peels	10	10	15	16
6	Kaffir lime peels	14	15	18	19
Control	Gentamycin	15	15	15	15

* = no clear zone and report as R resistant

Table 5. Showed antimicrobial activity against *Bacillus cereus*, *Salmonella typhi* and *Staphylococcus aureus* of 6 potential herbs on confirmatory step.

No.	Medicinal herbs	<i>Bacillus cereus</i>		<i>Salmonella typhi</i>		<i>Staphylococcus aureus</i>	
		Oil extracts	Fresh extracts	Oil extracts	Fresh extracts	Oil extracts	Fresh extracts
		1	Ginger	21	19	12*	12*
2	Shallot	24	16	18	12*	32	20
3	Galangal	21	19	12*	12*	21	19
4	Kaffir lime leaves	18	14	14	12*	18	17
5	Lime peels	20	16	20	19	28	18
6	Kaffir lime peels	25	25	27	25	28	24
Control	Deoxytetracycline	42	42	56	56	45	45

* = no clear zone and report as R resistant