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Antimicrobial Activities of Crude Extracts from Pomelo Peel of Khao-nahm-peung and Khao-paen Varieties

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Abstract

This study aimed to evaluate crude extracts from pomelo peels of Khao-nahm-peung and Khao-paen varieties for their antimicrobial activities against tested pathogenic and food spoilage microorganisms, *Staphylococcus aureus* TISTR 118, *Bacillus cereus* TISTR 5040, *Bacillus subtilis* TISTR 6633, *Listeria monocytogenes* DMST 17256, *Escherichia coli* DMST 4212, *Salmonella* Typhimurium DMST 0562, *Pseudomonas aeruginosa* ATCC 27853, *Saccharomyces cerevisiae* TISTR 5019 and *Zygosaccharomyces rouxii* TISTR 5044. Susceptibility test by disk diffusion method revealed that crude hexane extract did not inhibit any investigated bacteria, while the crude hexane extract of flavedo of Khao-nahm-peung variety inhibited the tested yeasts. The crude ethyl acetate extract of flavedo inhibited the growth of Gram-positive bacteria and yeasts but this crude extract did not show any inhibition of the tested Gram-negative bacteria. The crude ethanol extract had no antimicrobial activities against any investigated microorganisms at specified concentration in this study.

Keywords: antimicrobial activity, crude extracts, pomelo peel, Khao-nahm-peung, Khao-paen

1. Introduction

Plant extracts attract growing interest in both industry and scientific research because of their antimicrobial, antifungal, antiviral and anti-parasitical activities. Several antimicrobial compounds occur naturally in plants [1-3] and are known to retard the growth of or to kill food-borne pathogen [4]. Citrus (Citrus L.) is one of the most important world fruit crops and is consumed mostly as fresh produce or juice, and most of the peel is discarded. Citrus peels are subdivided into the epicarp or flavedo and mesocarp or albedo [5]. The flavedo is the colored peripheral surface of the peel while the albedo is the white soft middle layer of the peel. Citrus fruits (flavedo and albedo) contained various compounds, not only antioxidant, but also those that may have antimicrobial effects [6]. The health benefits of citrus fruits have mainly been attributed to the presence of bioactive compounds, such as phenolics (e.g., flavonoid, glycoside, hydroxycinnamic acids) vitamin C and carotenoid [7]. Citrus peels are reported to possess the highest amounts of flavonoid compound and hydroxycinnamic acids compared to other parts of the fruit [5, 8].Pomelo (*Citrus grandis* Osbeck) is the largest member of the citrus family. It is native in Southest Asian region including Thailand. The pomelo is one of the popular citrus fruits in

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Thailand's domestic market.The most common varities grown in Thailand are Khao-nahm-peung, Khao-paen, Thong-dee and Khao-hom. The growth of food spoilage and food-borne pathogens on or in food can decrease nutritional quality of the food by consuming fat, protein and carbohydrate that are presented in the food, subsequently causing food discoloration, biochemical changes and weight loss and toxicity to the consumer. Some species of them are able to produce highly toxic compounds which can adversely affect the health of humans [9]. The aim of this study was to evaluate the antimicrobial activities of crude extracts from pomelo peels, flavodo and albedo, and tested on their ability to inhibit microorganisms associated with food spoilage and food-borne pathogens.

2. Materials and Methods

2.1 Preperation of pomelo peel

Pomelos (*Citrus grandis* Osbeck), Khao-nahm-peung and Khao-paen varieties, were purchased from Nakhon Pathom Province, Thailand. The pomelos were washed thoroughly to remove dust. After washing, the yollow-green peel (flavedo) and a layer of white spongy peel (albedo) were removed from the segment of fruit. The fruit peels, flavedo and albedo, were cut into small pieces and dried overnight in a tray dryer at 45 ^{o}C. They were then ground with a blender to make powder.

2.2 Preparation of pomelo peel extracts

Ten grams of pomelo peel powder (albedo and flavedo) were extracted with 60 ml of organic solvent at room temperature for 3 days. The peel powder was subjected to sequential extraction using hexane, followed by ethyl acetate and finally with 95% ethanol. The filtrates were dried under reduced pressure at 40 ^{o}C using a rotary evaporator (Buchi, V-800), and left in desiccators to remove the residue solvent for approximately one week. The dried and semi-dried crude extracts were cold stored at 4 ^{o}C until use [10].

2.3 Microorganisms and their culture

Six bacterial and two yeast species of food spoilage and food-borne pathogens were selected, these were *Staphylococcus aureus* TISTR 118, *Bacillus cereus* TISTR 5040, *Bacillus subtilis* TISTR 6633, *Saccharomyces cerevisiae* TISTR 5019, *Zygosaccharomyces rouxii* TISTR 5044, obtained from Thailand Institute of Scientific and Technological Research and *Escherichia coli* DMST 4212, *Listeria monocytogenes* DMST 17256, *Salmonella* Typhimurium DMST 0562, obtained from Culture Collection for Medical Microorganism Department of Medical Sciences Thailand. Bacteria were cultured in Nutrient Agar (HIMEDIA) excepted *Sal*. Typhimurium was cultivated in Trypic Soy Agar (Merck) at 37 ^{o}C for 24 h. Yeasts were cultured in Potato Dextrose Agar (HIMEDIA) at 30 ^{o}C for 48 h.

2.4 Determination of antimicrobial activity of pomelo peel extracts

The antimicrobial activity of each extract was determined by paper disk diffusion method according to Mayachiew and Devahastin [11] and CLSI [12]. The concentrations of crude extracts were 100, 150 and 200 mg/ml of extracted solvent. Mueller Hinton Agar (Merck) and Sabouraud Dextrose Agar (Merck) were the media used for bacteria and yeasts, repectively. An aliquot of 0.1 ml of inoculum containing 10^{8} CFU/ml was spread onto the agar plates. After drying in a sterile hood, 6 mm diameter disks were each impregnated with 20 µl of the different extract dilutions, dried and then placed on the culture medium. Ceflazidine (1.5 mg/ml), vancomycin (1.5 mg/ml) and ketoconazole (1.5 mg/ml and 0.05 mg/ml) were used as positive control. The extracted solvents were used as negative control. The dishes were then incubated for 24 h at 37 ^{o}C for

bacteria and for 48 h at 30 ^{o}C for yeasts. All experiments were performed in duplicate and the antimicrobial activity was expressed as the mean of inhibition diameter (mm) produced.

3. Results and Discussion

Preliminary screening of antimicrobial activities against food spoilage and pathogenic microorganisms using the filter paper disk agar diffusion method of the crude extracts of pomelo peels from Khao-nahm-peung and Khao-paen varities showed various degrees of growth inhibition against test bacteria and yeasts. Compared to reference antibiotics, crude extracts showed low to moderate activity. The diameter of the inhibition zone ranged from 7.0-14.0 mm. The antimicrobial activity of ethyl acetate extract was better than that of the extracts obtained by hexane and 95% ethanol.

The results given in Table 1 indicate that hexane extracts showed no inhibition against Gram-positive and Gram-negative bacteria strains. However, the flavedo hexane extracts of Khao-nahm-peung exhibited inhibition activities of 8.2 - 12.2 mm against all yeast strains examined. Ketoconazole showed antimicrobial activities of 10.9 mm and 15.6 mm for *Sac. cerevisiae* and *Z. rouxii*, respectively.

Table 2 shows that flavedo ethyl acetate extracts of Khao-nahm-peung and Khao-paen varities exhibited better inhibition activities against Gram-positive bacteria than Gram-negative bacteria. *Baillus cereus* and *B. subtilis* are most susceptible and *E. coli* is least susceptible to the extract. Its effective antimicrobial concentration is 150-200 mg/ml. The obtained results were similar to the results reported by Mokbel and Hashinaga [10]. Their work indicates that ethyl acetate extracts of flavedo from buntan pomelo (*Citrus grandis* Osbeck) showed a higer activity than albedo extracts toward paper disk methods. Furthermore, ethyl acetate extracts of flavedo had stronger antimicrobial activities on the Gram-positive bacteria (*S. aureus*, *B. subtilis* and *B. cereus*) than on the Gram-negative bacteria (*Sal.* Enteritidis and *E. coli*). The reason for different sensitivity between Gram-positive and Gram-negative bacteria could be ascribed to the morphological differences between these microorganisms. Gram-negative bacteria have an outer phospholipidic membrane. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da [13]. The Gram-positive bacteria should be more susceptible since they have only an outer peptidoglycan layer which is not an effective permeability barrier [14]. Only the flavedo ethyl acetate extracts of Khao-nahm-peung showed good activity againt *Z. rouxii* at the concentrations of 150 and 200 mg/ml, inhibition activities were 9.5 and 10.2 mm, repectively.

None of the ethanol extracts was found to inhibit the growth of any test microorganism, indicating that they did not posses any antimicrobial effect on the spoilage and illness. It is possible that the concentrations of active compounds in the ethanol extract are not enough to inhibit the growth of these microorganisms.

Table 1. Antimicrobial activities of the hexane extracts from pomelo peels against the microorganism.

Test microorganisms	inhibition zone (mm)[1]												
	Flavedo (Khao-nahm-peung)				Flavedo (Khao-paen)			Albedo (Khao-nahm-peung)			Albedo (Khao-paen)		
	Positive[2] control	100 mg/ml	150 mg/ml	200 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml
Gram-positive bacteria													
S. aureus	13.8±5.1	-	-	-	-	-	-	-	-	-	-	-	-
B. cereus	16.4±1.3	-	-	-	-	-	-	-	-	-	-	-	-
B. subtilis	19.0±0.1	-	-	-	-	-	-	-	-	-	-	-	-
L. monocytogenes	14.8±0.4	-	-	-	-	-	-	-	-	-	-	-	-
Gram-negative bacteria													
E. coli	21.0±0.4	-	-	-	-	-	-	-	-	-	-	-	-
Sal. Typhimurium	22.2±7.0	-	-	-	-	-	-	-	-	-	-	-	-
Yeasts													
Sac. cerevisiae	10.9±0.8	8.4±0.5	9.5±0.7	12.2±1.5		-	-	-	-	-	-	-	-
Z. rouxii	15.6±8.1	8.2±1.2	9.8±2.5	10.5±4.1		-	-	-	-	8.5±3.2	-	-	-

[1] Diameter of inhibition zone (mm) including disk diameter of 6 mm.

[2] Vancomycin (1.5 mg/ml) was used as positive control for *S. aureus*, *B. cereus*, *B. subtilis* and *L. monocytogenes*.
Cefrazidine (1.5 mg/ml) was used as positive control for *E. coli* and *Sal.* Typhimurium.
Ketoconazole (1.5 and 0.05 mg/ml) was used as positive control for *Sac. cerevisiae* and *Z. rouxii*.

- no inhibition.

Table 2. Antimicrobial activities of the ethyl acetate extracts from pomelo peels against the microorganism.

Test microorganisms	inhibition zone (mm)[1]												
	Flavedo (Khao-nahm-peung)				Flavedo (Khao-paen)			Albedo (Khao-nahm-peung)			Albedo (Khao-paen)		
	positive[2] control	100 mg/ml	150 mg/ml	200 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml
Gram-positive bacteria													
S. aureus	13.8±5.1	-	8.2±3.36	10.0±0.53	-	-	7.5±2.23	-	8.0±3.21	8.0±2.22	-	-	-
B. cereus	16.4±1.3	8.0±2.22	10.0±1.41	11.2±1.26	7.0±3.14	8.8±1.43	10.2±1.31	-	8.8±1.25	9.2±1.65	-	-	7.2±3.33
B. subtilis	19.0±0.1	8.2±3.11	10.8±4.22	14.0±3.23	7.5±2.53	9.0±2.26	10.8±4.42	-	9.0±3.33	9.2±5.03	-	-	7.5±2.65
L. monocytogenes	14.8±2.7	-	7.8±1.36	11.0±2.41	-	-	8.0±1.23	-	8.2±5.21	9.0±4.35	-	-	-
Gram-negative bacteria													
E. coli	21.0±0.4	-	-	8.0±0.33	-	-	-	-	-	-	-	-	-
Sal. Typhimurium	22.2±7.0	-	-	-	-	-	-	-	-	-	-	-	-
Yeasts													
Sac. cerevisiae	10.9±0.8	-	-	-	-	-	-	-	-	-	-	-	-
Z. rouxii	15.6±8.1	-	9.5±0.13	10.2±0.22	-	-	-	-	-	-	-	-	-

[1] Diameter of inhibition zone (mm) including disk diameter of 6 mm.

[2] Vancomycin (1.5 mg/ml) was used as positive control for *S. aureus*, *B. cereus*, *B. subtilis* and *L. monocytogenes*.
Cefrazidine (1.5 mg/ml) was used as positive control for *E. coli* and *Sal.* Typhimurium.
Ketoconazole (1.5 and 0.05 mg/ml) was used as positive control for *Sac. cerevisiae* and *Z. rouxii*.
- no inhibition.

4. Conclusions

The results of the paper disk diffusion method indicate a difference in antimicrobial activities among the extracts. The selective extraction of antimicrobial from natural sources by appropriate sovent is very important in obtaining components with high antimicrobial activity. In general, the pomelo peel extracts had better antimicrobial activities on the Gram-positive bacteria than on Gram-negative bacteria and yeasts. Crude hexane extracts could not inhibit the growth of any tested bacteria. The flavedo ethyl acetate extract of Khao-nahm-peung showed high antimicrobial activities, especially to *B. cereus* and *B. subtilis*. The ethanol extracts had no antimicrobial activities against any investigated microorganisms at all three concentration levels tested (from 100-200 mg/ml).

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