การตรวจสอบฤทธิ์ต้านแบคทีเรียของสารสกัดหยาบจากเปลือกผลไม้ตระกูลส้มห้าชนิด

Investigation for the Antibacterial Activity of Five Citrus Fruits' Peel Extracts

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บทคัดย่อ

โรคติดเชื้อจากแบคทีเรียเป็นปัญหาที่สำคัญของประชากรโลกที่ทำให้หลายประเทศสิ้นเปลืองงบประมาณเป็นจำนวนมาก อย่างไรก็ตามในช่วงหลายปีที่ผ่านมาได้มีรายงานการพบสารต้านจุลินทรีย์ในพืชสมุนไพรรวมทั้งผลไม้อย่างต่อเนื่องและคาดว่าสารดังกล่าวจะมีศักยภาพในการช่วยบำบัดโรคติดเชื้อสำคัญๆ ได้มากมาย การศึกษาครั้งนี้มีวัตถุประสงค์ในการนำเปลือกผลไม้ตระกูลส้ม 5 ชนิด ที่ได้รับความนิยมในการบริโภคสูง ได้แก่ ส้มโอ ส้มเขียวหวาน ส้มสายน้ำผึ้ง มะกรูด และมะนาว มาศึกษาฤทธิ์ต้านแบคทีเรียชนิดที่สำคัญ โดยใช้วิธีการสกัด 4 วิธี ได้แก่ การต้มสกัด การหมักด้วยเอทิลแอลกอฮอล์ การปั่นผงแห้งเปลือกผลไม้ในน้ำและการปั่นเปลือกผลไม้สด นำสารสกัดหยาบที่ได้มาทดสอบฤทธิ์ต้านแบคทีเรีย 3 ชนิด ได้แก่ *Escherichia coli*, *Staphylococcus aureus* และ *Pseudomonas aeruginosa* โดยวิธี agar disk diffusion ผลการศึกษาพบว่าสารสกัดหยาบจากเปลือกส้มทั้ง 5 ชนิด โดยวิธีการหมักด้วยเอทิลแอลกอฮอล์มีฤทธิ์ต้านแบคทีเรีย *S. aureus* ได้ สารสกัดหยาบจากการสกัดด้วยวิธีการปั่นผงแห้งเปลือกผลไม้ในน้ำมีฤทธิ์ต้านแบคทีเรีย *P. aeruginosa* ได้ดี ส่วนสารสกัดหยาบจากการสกัดด้วยวิธีการต้มสกัดและการปั่นเปลือกผลไม้สดไม่พบฤทธิ์ต้านแบคทีเรียที่นำมาทดสอบ นอกจากนี้ยังพบว่าฤทธิ์ต้านแบคทีเรียของสารสกัดจากเปลือกผลไม้ตระกูลส้มขึ้นกับปัจจัยหลายประการ ได้แก่ สารละลายที่ใช้ในการสกัด วิธีการสกัด และชนิดของแบคทีเรียที่นำมาทดสอบ โดยสารสกัดหยาบจากเปลือกผลไม้ตระกูลส้ม 5 ชนิดที่นำมาศึกษานี้ที่ระดับความเข้มข้น 250 mg/200 µL มีฤทธิ์ต้านแบคทีเรีย *P. aeruginosa* ได้ในระดับใกล้เคียงยาที่ใช้เป็นตัวควบคุมบวก จึงควรศึกษาต่อไปถึงชนิดและปริมาณของสารออกฤทธิ์ เพื่อให้ได้ข้อมูลที่ชัดเจนสำหรับการนำเปลือกส้มเหล่านี้ไปประยุกต์ใช้เป็นสารต้านจุลินทรีย์ในผลิตภัณฑ์ต่าง ๆ ต่อไป

คำสำคัญ: ฤทธิ์ต้านแบคทีเรีย โรคติดเชื้อจากแบคทีเรีย ผลไม้ตระกูลส้ม สารสกัดหยาบจากเปลือกผลไม้

ABSTRACT

Bacterial infections are very important problems of world population. Many countries have to pay large budgets dealing with these diseases. However, there were lots of studies currently reported that fruits and folk plants were rich sources of medicinal compounds those have high potential as antimicrobial agents for alternative treatments. In this study, fruit peels of five citrus fruits including *Citrus maxima*, *Citrus reticulata* Blanco, *Citrus reticulata* Blanco cv. Sainampueng, *Citrus hystrix* DC and *Citrus aurantifolia* were extracted by

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using four methods including boiling, maceration in absolute ethanol, blending of fruit peels powder in water and blending of fresh fruit peels in water. The antibacterial activities of citrus fruits' peels were evaluated using the agar disk diffusion method towards *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The results showed that crude extracts of these five fruits' peels from ethanolic extraction showed antibacterial activity to *S. aureus* and those using dry powder blending extraction had good antibacterial activity to *P. aeruginosa*. No inhibition zone was found in all five extracts that used boiling extraction and fresh peel blending extraction.This study also showed that the activities of those extracts depended on many factors such as species of fruits, solvent, and the method of extraction. This preliminary study demonstrated that these citrus fruit peels crude extracts at concentration 250 mg/ 200 µL had antibacterial activity against *P. aeruginosa* comparable to that of the positive control. Further study for the type and quantity of those important substances should be performed. This will lead to proper applications in pharmaceuticals or other antimicrobial products in future.

Keywords: Antibacterial activity, Bacterial infections, Citrus fruits, Peel extracts

Introduction

Infectious diseases are nowadays major threats of world population. They caused by a vast variety of pathogens with difference virulence levels (Ramadan et al., 2017). Those pathogens may be bacteria, fungi, protozoa or viruses. For bacterial groups there are lots of important pathogens such as *Staphylococcus aureus*, *Streptococcus* sp., *Salmonella* sp., *Shigella* sp., *Escherichia coli*, *Bacillus cereus* and *Listeria monocytogenes* (Ranibar et al., 2018; Barco et al., 2014; Choi et al., 2014; Bloch et al., 2012; Campion et al., 2004).

The rapidly emergence of antibiotic resistant bacterial pathogens has made infectious diseases be more terrible to human and animals than previously (Bengtsson-Palme et al., 2018; Sharma et al., 2018; WHO, 2014; Levy and Marshall, 2004; Anderson, 1999). Chemical drugs have been generally used for treatment of infectious diseases but adverse side effects in some of them and high cost still be problems (Tagel et al., 2018; Tayel et al., 2013). So there has been much attempt to search for another sources of antimicrobial agents. Searching for high potential but lower problem drugs is a big challenge that gives benefit to human. In Thailand, a great deal of plants are interesting as new candidates for sources of antibacterial agents (Ramadan et al., 2018; Van Vuuran and Holl, 2017; Panda et al., 2016). In this study we aimed to investigate for the antibacterial activity of fruit peels of five citrus fruits those were most common and widely consumed in Thailand including *Citrus maxima* (Pomelo), *Citrus reticulata* Branco (Mandarin orange), *Citrus reticulata* Branco cv. Sainumpueng (Sainumpueng orange), *Citrus hystrix* DC (Kaffir) and *Citrus aurantifolia* (Lime). The research data will be very useful for efficient uses of these fruit peels as antimicrobial agents in future.

Methods

Bacterial strains

Antibacterial activity of five citrus fruits' peels was investigated against three bacterial strains including *Staphylococcus aureus*ATCC25923, *Escherichia coli*ATCC25922, *Pseudomonas aeruginosa* ATCC27853. All strains were maintained intrypticase soy broth containing 15 percentglycerol at -70°C and subcultured onto blood agar before used.

Plant materials and preparations

Plant materials to be studied were fruit peels of five citrus fruits from local markets in Bangkok and nearby areas. The species of those citrus fruits were *Citrus maxima* (Pomelo), *Citrus reticulata* Branco (Mandarin orange), *Citrus reticulata* Branco cv. Sainumpueng (Sainumpueng orange), *Citrus hystrix* DC (Kaffir) and *Citrus aurantifolia* (Lime).

Those fruit peels were washed in tap water and rinsed with distilled water followed by cutting into thin pieces with 0.2 cm long. After being dried by a hot air oven at 50°C for 8 hrs, then they were made to powder by using electrical blender and kept at 4°C in sterile airtight bottles.

Extract preparation

In order to compare the biological activity of fruit peels using different extraction methods, we prepared crude extracts by using four methods including boiling in water, maceration in absolute ethanol, blending of fruit peel powder in water and blending of fresh fruit peels in water.

For boiling method, 40 g of fruit peel powder was soaked in 200 mL distilled water for 20 minutes followed by boiling for 45 minutes then evaporated to get the crude extracts. In maceration method 40 g of peel powder was soaked 120 mL absolute ethanol for 7 days with everyday agitation then collected the extracts and evaporated. The last two methods also used 40 g of peel materials mixed with 200 mL sterile distilled water followed by blending for 5 minutes.

Assay for antibacterial activity of citrus fruits' peel extract

The antibacterial activity was evaluated using agar disk diffusion method (CLSI, 2012). Bacterial suspension at the concentration of $1.5x10^8$ cells/ mL was spread on Mueller Hinton agar and allowed to dry for 5 minutes. Sterile 6 mm diameter paper discs, each containing 25 μL of each extract dissolved in dimethyl sulfoxide (DMSO) at 250 mg/200 μL, were put on surface of the culture. A positive control (Chloramphenicol, 30 μg) and a negative control (10% DMSO) were included. All culture plates were incubated at 37°C for 24 hrs then examined for inhibition zone and recorded the zone diameter.

Statistical analysis

All experiments were replicated three times and results were repressed as means ± SD

Results

Fruit peels' crude extracts prepared from those four methods including boiling in water, maceration in absolute ethanol, blending of fruit peels powder in water and blending of fresh fruit peels in water were assayed for the antibacterial activity using chloramphenicol 30 μg and 10% DMSO as positive control and negative control, respectively. The results summarized in Table 1 showed that antibacterial activity of citrus fruit peels depended on the species of citrus and methods of extractions and these factors should also be concerned in further applications of citrus fruit peels in order to get best activity

Table 1 Antibacterial activity as determined by inhibition zone diameter of citrus fruit peels' crude extracts from different extraction methods against *E. coli*, *S. aureus* and *P. aeruginosa*

Citrus peel Samples	Inhibition zone diameter (mm)											
	E. coli				*S. aureus*				*P. aeruginosa*			
	boiling	abs ethanol	blending	fresh peel blending	boiling	abs ethanol	blending	fresh peel blending	boiling	abs ethanol	blending	fresh peel blending
Pomelo	-	-	-	-	-	9.0±0.0	-	-	-	-	14.3±4.0	-
Mandarin orange	-	-	-	-	-	10.0±0.0	-	-	-	-	16.7±3.5	-
Sainumpu - eng orange	-	-	-	-	-	8.0±0.0	-	-	-	-	8.3±0.6	-
Kaffir	-	-	-	-	-	11.0±1.0	-	-	-	-	10.0±0.0	-
Lime	-	-	-	-	-	11.3±1.5	-	-	-	-	11.7±2.5	-
Pos. ctrl.	28.8	28.8	28.8	28.8	27	27	27	27	8.5	8.5	8.5	8.5
Neg. ctrl.	-	-	-	-	-	-	-	-	-	-	-	-

(–): No inhibition zone

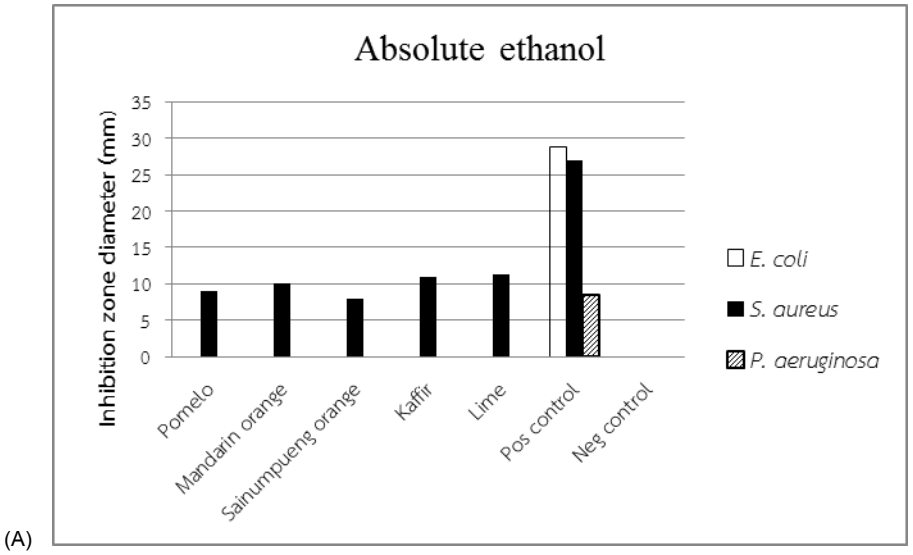
abs ethanol: Absolute ethanol

Pos ctrl: Positive control

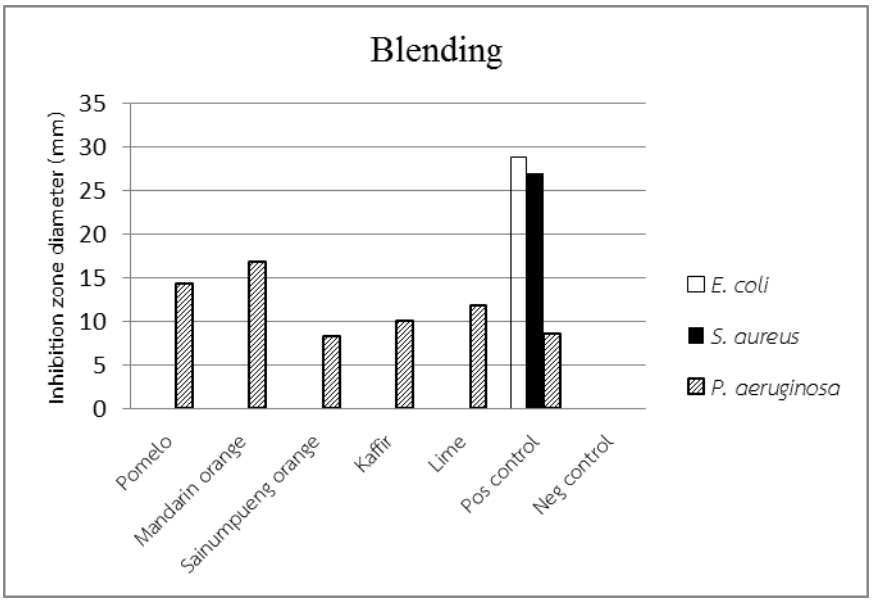
Neg ctrl: Negative control

The ethanolic extracts of *Citrus aurantifolia* , *Citrus hystrix* DC and *Citrus reticulata* Branco exhibited antibacterial activity against *S. aureus* with the inhibition zone diameter of 11.3 mm, 11.0 mm and 10.0 mm respectively. Whereas the blending extraction of peels' powder of *Citrus reticulata* Branco and *Citrus maxima* showed very good antibacterial activity against *P. aeruginosa* with the inhibition zone diameter of 16.7 mm and 14.3 mm respectively and was higher than the positive control. However none of crude extract had antibacterial activity against *E. col*

Figure 1 showed the comparison of antibacterial activity of different citrus fruits' peel ethanolic extracts (Figure 1A) and blending extracts (Figure 1B) against *S. aureus*, *E. coli* and *P. aeruginosa*. Antibacterial activity expressed by inhibition zone diameter obviously showed that two ethanolic extracts and three blending extracts had a good antibacterial activity against *S. aureus* and *P. aeruginosa* respectively.



(A)



(B)

Figure 1 Antibacterial activity as determined by inhibition zone of five citrus fruits' peel ethanolic extracts(A) and blending extractsof peels' powder (B) against *E. coli*, *S. aureus* and *P. aeruginasa*

Discussion and Conclusion

Traditional medicine of Thailand has used citrus fruits for some treatments for long time. Citrus peels have also been used in many aspects such as antimicrobial agents and anti inflammatory agents. The extract of Pamelo's peels was recorded for treatments of some skin infections, abscesses and inflammation. Other citruses such as lime and kaffir were also recorded for traditional uses (Otang and Afolayan, 2016, Suryawanshi, 2011). Scientific research data is necessary for supporting the used of all citruses in future.

In this study fruit peel crude extract of *Citrus reticulata* Branco, one citrus species that was used in traditional medicine to treat some skin infections, showed the outstanding antibacterial activity to *P. aeruginasa* which was an important skin infectious agents at inhibition zone diameter of16.7 mm, higher than the positive control. Furthermore it was also found that peel extract of

Pamelo, another citrus species that was also used in traditional medicine, showed an interesting antibacterial activity against *P. aeruginasa* at inhibition zone diameter of 14.3 mm. Ethanolic peel extracts of these two citrus fruits also showed antibacterial activity to *S. aureus*, a very important skin infectious agent, corresponding to that reported by Chen et al. (2018) who reported the significant antibacterial effect of Pamelo oil to *S. aureus*. Lime and kaffir are also citrus fruits of interest those habor antibacterial activity to both *S. aureus* and *P. aeruginosa*, consistent with that reported by Makni et al. (2018) on the significant inhibition of lemon peels against these two pathogenic species.

However, the level of activities were found to depended on species of citrus fruits, method of extraction and the species of tested bacteria. Many factors revealed to concerned in efficient uses of these plants. The consistent of traditional applications and bioactivity data will provide more confirmation for treatments uses and for the potential of citrus fruits' peels as antimicrobial agent in developments of household products, medical products and other value added applications.

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