Comparison of Antibacterial Activity in Ethanol Extract and Essential Oil of *Citrus sinensis* (L.) Peels Obtained by Sohxlet and Distillation Methods

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Abstract. Ethanol extract and essential oil of *Citrus sinensis* (L.) peels have been characterized and compared for its antibacterial activity. Ethanol extract of *C. sinensis* (L.) peels was obtained by Soxhlet extraction method in ethanol with purity of 96% and the essential oil was obtained by distillation method for 5 hours at 100°C. The antibacterial activities of the extracts were investigated against *Staphylococcus aureus*. The experimental results showed that the ethanol extract yielded 29.50% (w/w), with total flavonoid compounds of 4.74 mg/g and polyphenolic of 20.54 mg/g, while the water extract (essential oil) yielded 6.80% (w/w) with total polyphenolic compounds of 0.65 mg/g. The flavonoid compound was not observed in the essential oil obtained from distillation method. The bioactive compounds in the ethanol extract obtained by Soxhlet extraction are larger than those found in the essential oil obtained by distillation method. The ethanol extract showed stronger growth inhibitory effect of *Staphylococcus aureus* with zone inhibition diameter of 18 mm, whereas the essential oil and the commercial d-limonene extract had similar zone inhibition diameter of 15 mm.

1. Introduction

The amount of dust and pollution in Jakarta, Indonesia, causes some health problems to human such as, skin infections, respiratory tract disorders, and other diseases. One of the most common skin infection is acne. Acne has been known to affect all gender, men and women, as well as attacking various ages after puberty. Hormonal changes after puberty can cause the occurrence of acne, in addition to that, acne can also arise because of skin pore clogging by dust and pollution, worsen by bacteria infection. One of the culprit that causes acne is bacteria *Staphylococcus aureus* [1]. In addition to causing acne, bacteria from the genus *Staphylococcus* also causes carbuncle and furuncle on the skin [2].
Although there are many medicines used for acne, there are some of them that do not suitable for certain people. Side effects and allergies can occur by applying these medicines. Acne medicines usually contain antibacterial agent that inhibit the formation and/or destroy of pathogenic bacterial walls. Thus, it encourages us to search natural antibacterial agent that is safer for acne medicine application.

Previously, the extract of *Citrus limon* peel has been reported as antimicrobial and antioxidant that applied for skin diseases [2]. Another reported that extract of myrtle lemon had an active antibacterial agent, but myrtle anise essential oil did not have any inhibitory activity against bacteria [3]. The application of natural resource as antibacterial agent has the advantages of being save and generally accepted by the consumer [4]. In Indonesia, production of *Citrus sinensis* (L.) is about 2.6 million tons in a year. One fruit of *Citrus sinensis* (L.) can yield about 10 - 20% (w/w) of peels, thus we assumed that the availability of *C. sinensis* (L.) peel is about 0.25 million tons in a year. Citrus peel is also a potential source for vitamin C, alkaloids compounds, and essential oil which is used as ingredients of perfume, refresher [5], and applied for skin rash treatments. By utilizing citrus peels, we could reduce the disruptive effects of organic waste to the environment.

Based on the literature study, *Citrus sinensis* (L.) peels contained flavonoid and polyphenolic compounds. These compounds are used in topical medicines for skin diseases. *Citrus limon* (Rutaceae), well-known as lemon, was reported to have anticancer and antibacterial activities [6].

In this paper, we extracted the peels of *C. sinensis* (L.) using Soxhlet extraction and distillation method to obtain the antimicrobial agent and characterized its chemical and biological properties. The aim of this study is to investigate and compare the antibacterial activity of these extracts. This study will provide important data for the utilization of *C. sinensis* peels as the source of bioactive ingredients and natural compounds for health care products. This study is also to show which extraction method is more feasible for production of antibacterial agent from *C. sinensis* peels in real application based on the antibacterial activity of each extract.

2. Materials and Method
2.1. Preparation of ethanol extract of C. sinensis (L) by Soxhlet extraction
Fresh orange fruits (*C. sinensis* (L.)) (1 kg) was obtained from the local supermarket in Depok, Indonesia. The peel of *C. sinensis* (L.) fruit (30.01 g) were cut and dried in oven at 70°C for 6 hours. The dried peels were grinded to size of 0.1 µm. The dried peels were put into conical flasks containing 200 mL ethanol (96%) for 4 hours. The suspension was separated and evaporated to obtain the filtrate. The yield of ethanol extract of *C. sinensis* (L.) was 29.5% (w/w) with yellowish brown and smells of citrus.

2.2. Preparation of essential oil by Distillation method
The dried peels of *C. sinensis* (L.) (30.01 g) were put in the conical flasks containing aquoues 300 mL and evaporated for 5 hours at 100°C in a heating mantle. The essential oil was separated from water layer using a drop pipette. The colourless essential oil with yield of 6.8% (w/w) was obtained.

2.3. Antibacterial test
This assay was done according to the procedure of [7]. The growth of bacterial in each sample were analysed by microbiological test using agar well-diffusion method against *Staphylococcus aureus*. The pure cultures of bacteria were sub-cultured on nutrient agar (NA) medium. After incubation at 37°C for 24 hours, the difference of zone inhibition diameter of bacteria was observed.

2.4. Total polyphenolic content
The total polyphenolic content of the ethanol extract and essential oil were determined by the Folin–Ciocalteu method. This method was according to the method that reported by Baba and Malik [8]. The total phenolic content was calculated from the calibration curve. This result was represented as mg of gallic acid equivalent per g dry weight.
2.5. Total flavonoid content
The total flavonoid content of ethanol extract and essential oil were determined by the aluminium chloride colorimetric method. This method was according to the method that reported by Baba and Malik [8]. The result was revealed as mg rutin equivalent per g dry weight. Rutin was used as calibration standard.

3. Results and Discussion
Both preparations of ethanol extract and essential oil are shown in Figures 1 and 2. The ethanol extract of C. sinensis (L.) from Soxhlet method in 96% ethanol had total flavonoid compounds of 4.74 mg/g and polyphenolic compound of 20.54 mg/g. The flavonoid content observed in this study was smaller when compared to the reported ethanol extract of Citri reticulatae in 80% ethanol solvent with flavonoid of 233.4 ± 4.1 mg/g and polyphenol of 79.5 ± 7.7 mg gallic acid equivalents/g [9]. The yield of essential oil obtained from distillation method is 6.80% (w/w) and had total polyphenolic compounds of 0.65 mg/g, less than the ethanol extract. Flavonoid compound was not observed in the essential oil extract of C. sinensis.

![Step 1. C. sinensis (L) peels was dried in oven at 70°C](image1)
![Step 2. The C. sinensis (L) powder](image2)
![Step 3. The C. sinensis (L) powder was mixed with 200 mL ethanol by Soxhlet extraction](image3)
![Step 4. The ethanol extract of C. sinensis (L.)](image4)

**Figure 1.** Schematic representation process for Soxhlet extraction

The inhibition zone of ethanol extract and essential oil of C. sinensis (L.) against Staphylococcus aureus is shown in the Table 1. Higher antibacterial activity against Staphylococcus aureus was observed from the ethanol extract with zone inhibition of 18 mm (see Table 1). On the other hand, the essential oil showed the zone inhibition of 15 mm, less effective than the ethanol extract. The essential oil of C. sinensis (L.) as antifungal activity was also reported by Viuda-Martos and co-workers [10].

The antimicrobial agent contained in citrus extract killed S. aureus due to the change in the S. aureus cell wall and membrane structure. The changes were caused by the interaction between bioactive compounds and polyphenolic compounds which contain a lot of aromatic and hydroxyl groups.

Zone inhibition of ethanol extract and essential oil of C. sinensis (L.) are presented in Figure 3A and B. Compared to the ethanol extracts and essential oil of C. sinensis (L.), the essential oil had only
polyphenolics compounds (see Table 1). The growth inhibition on *Staphylococcus aureus* was clearly associated with the content of the total phenolic.

**Table 1.** Comparison of antibacterial activities of *Citrus sinensis* (L.) extracts against *Staphylococcus aureus*

| Sample               | Total of polyphenolics (mg/g) | Total of flavonoids (mg/g) | Zone of inhibition (mm) |
|----------------------|------------------------------|---------------------------|------------------------|
| Ethanol extract      | 20.54                        | 4.74                      | 18                     |
| Essential oil        | 0.65                         | 0 (not observed)          | 15                     |
| Commercial d-limonene| 0.14                         | 0 (not observed)          | 15                     |

**Figure 2.** Schematic representation process for distillation method
Figure 3. Zone inhibition of ethanol extract of *C. sinensis* (L.) from Soxhlet extraction (A) and the essential oil from distillation method (B)

For comparison, the antibacterial activity of *Citrus paradise* extract from ethyl acetate, methanol, and water solvents against *Staphylococcus aureus* was reported to have zone inhibition of $8 \pm 0.3$, $10 \pm 0.7$ and $12 \pm 0.5$ mm [4]. Other reported that the antibacterial activity of silver nanoparticles synthesized using *Citrus sinensis* (L.) extract showed zone inhibition of $7.8$ mm at $25^\circ C$ and $9.2$ mm at $60^\circ C$ [11]. However, the zone of inhibition against *S. aureus* using *C. sinensis* (L.) in this study are smaller than those found in the acetone extract of *Citrus limon* with zone of inhibition against *E. faecalis* and *B. subtilis* was 23 and 20 mm, respectively [2]. In this study, we also investigated the antibacterial activity of the commercial d-limonene extract of *Citrus terpene* against *S. aureus*. The zone inhibition of $15$ mm was observed for d-limonene. It is similar value with the prepared essential oil of *C. sinensis* (L.). The d-limonene has polyphenolic compounds of $0.14$ mg/g. The phenolic compounds are well-knowns as antimicrobial activity [4]. As we know that limonene is the major component (96.1% w/w) of the volatile fraction from the orange essential oil [12].

4. Conclusion
We evaluated the extracts of *Citrus sinensis* (L.) peels obtained by Soxhlet extraction and distillation methods. Both extracts showed antibacterial activities against *Staphylococcus aureus* contributed by its polyphenolic compound. Ethanol extract has more polyphenolic compound than the essential oil, thus showed better antibacterial activity compared to that of the essential oil. Based on this study, Soxhlet extraction method would be more feasible for production of antibacterial agent from *C. sinensis* peels in real application.

5. References
[1] Dunlavey E S 2000 *Current Problems in Dermatology* 12 216-221
[2] Otang W M and Afolayan A J 2016 *South African Journal of Botany* 102 46–49
[3] Nirmal N P, Mereddy R, Li L and Sultanbawa Y 2018 *Food Chemistry* 254 1-7
[4] Sayari N, Sila A, Balti R, Abid E, Hajlaoui K, Nasri M and Bougatfee A 2015 *Biocatalysis and Agricultural Biotechnology* 4 616–623
[5] Nekvapil F, Brezestean I, Barchewitz D, Glamuzina B, Chiş V and Pinzaru S C 2018 *Food Chemistry* 242 560-567
[6] Kawai S, Tomono Y, Katase E, Ogawa K, Yano M, Koizume M, Ito C and Furukawa H 2000 *Journal of Agriculture and Food Chemistry* 48 3865–3871
[7] Kusrini E, Usman A, Wisakanti C D, Arbiante R, and Nasution D A 2015 *Makara Journal of Technology* 19 148-152
[8] Baba S A, and Malik S A 2015 *Journal of Taibah University for Science* 9 449–454
[9] Yi Z B, Yu Y, Liang Y Z, and Zeng B 2008 *LWT* 41 597–603
[10] Viuda-Martos M, Ruiz-Navajas Y, Fernández-Lo´pez J and Pe´rez-A´lvarez J 2008 Food Control 19 1130–1138
[11] Kaviya S, Santhanakshmi J, Viswanathan B, Muthumary J and Srinivasan K 2011 Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 79 594–598
[12] Gonçalves D, Costa P, Rodrigues C EC and Rodrigues AE 2018 J. Chem. Thermodynamics 116 166–17

Acknowledgments
This work was funded by the IbM grant No. 602/H2.R12/PPM.01/2015. The authors are grateful to Mr Diki Firdaus for his technical help.