BIOFARMASI J NAT PROD BIOCHEM
Volume 17, Number 1, February 2019
Pages: 47-54

E-ISSN: 2580-2550
DOI: 10.13057/biofar/f170105

Antibacterial and antifungal activities of essential oil of Tawangmangu sweet orange (*Citrus sinensis*) peel at different altitudes

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Manuscript received: 19 May 2019. Revision accepted: 26 July 2019.

Abstract. *Handayani ES, Nugraheni ER, Susilowati A. 2019. Antibacterial and antifungal activities of essential oil of Tawangmangu sweet orange (*Citrus sinensis*) peel at different altitudes. Biofarmasi J Nat Prod Biochem 17: 47-54. Staphylococcus aureus* and *Candida albicans* are human pathogenic microbes that cause skin infections. Essential oils are biologically active as antibacterial and antifungal. Environmental factors, e.g., temperature, lighting, and height, influence the synthesis of essential oils. This study aimed to determine the highest yield of essential oils of Tawangmangu sweet orange (*Citrus sinensis* L.) peel in different altitudes and to know the activity of essential oils of Tawangmangu sweet orange peel against bacteria *S. aureus* and fungi *C. albicans.* This study used essential oil of Tawangmangu sweet orange peel taken from four different altitudes of 1,000 ± 50; 1,200 ± 50; 1,400 ± 50, and 1,600 ± 50 m above sea level. First, the yield of essential oil was calculated then antibacterial and antifungal were tested using the Kirby Bauer method against *S. aureus* and *C. albicans*. Then, the diameter zones of inhibition were calculated. The results showed that Tawangmangu sweet orange peel at an altitude of 1,000, 1,200, 1,400, and 1,600 above sea level produced a different yield of essential oil. Tawangmangu sweet orange peel at an altitude of 1,600 m above sea level produced essential oil with the highest yield was 0.55%. Essential oil of Tawangmangu sweet orange peel at an altitude of 1,000, 1,200, 1,400, and 1,600 above sea level had different antimicrobial activity against bacteria *S. aureus* and fungi *C. albicans.* Essential oil at the altitude of 1,600 m asl in 100% concentration had the greatest antimicrobial activity with a diameter zone of inhibition of 23 mm against S. *aureus* and a diameter zone of 19 mm against *C. albicans.*

Keywords: Antibacterial, antifungal, *Citrus sinensis*, essential oil, Tawangmangu sweet orange

INTRODUCTION

Diseases caused by microbial infections are common in the tropics, like Indonesia, because of the dusty air conditions and warm and moist temperatures so that microbes can thrive. *Staphylococcus aureus* is a pathogenic bacterium in humans and causes symptoms ranging from localized wounds to life-threatening infections (Isbandrio 1999). The localized infection causes boils, pimples, and impetigo. When bacteria enter the bloodstream, bacteria can spread to other organs, causing diseases like pneumonia, infection of heart valves, leading to heart failure (endocarditis), inflammation of the bones (osteomyelitis), and even lead-to-dead infection. In the case of food poisoning due to S. aureus contamination, diarrhea, vomiting, and dehydration could occur, in which the symptoms appear approximately 1-6 hours after consuming contaminated food (Levinson 2004; Stroppler 2008). The *S. aureus* bacterial infection can be spread by contact with pus from an infected wound; direct skin-to-skin contact with an infected person in which *S. aureus* produces tissue-damaging hyaluronidase; and direct contact with objects used by an infected person such as towels, clothes, and so on. *S. aureus* can infect any tissue or organ in the body and cause characteristic signs, namely inflammation, necrosis, and abscess formation (Majid 2005).

The fungus *Candida albicans* usually lives as a saprophyte in the oral cavity, intestines, and vagina. In healthy people, this fungus is not pathogenic, but when the immune system decreases, this fungus can turn into a pathogen by causing various complaints. In the vagina, this fungus can cause symptoms of vaginal discharge known as vaginal candidiasis (Soemiati and Elya 2002). There is no factual data regarding the overall prevalence of candidiasis in Indonesia. However, the frequency of vaginal candidiasis is quite high among other sexually transmitted diseases in Indonesia, which is 43%. In Indonesia, it was reported that 84% of AIDS patients who were treated at the RSCM until 2000 also suffered from candidiasis caused by the opportunistic fungus *C. albicans.*

Handling that is often done in general to control the microbial disease uses chemicals such as potassium permanganate ($KMnO_4$) and methylene blue, antibiotics, and vaccinations. Infectious diseases due to *S. aureus* and *C. albicans* are generally treated with antibiotics (Majid 2005). Since their initial discovery in 1928, antibiotics have made an effective and positive contribution to controlling microbial infections in humans and animals. However, along with its development and use, pathogenic microbes become resistant to antibiotics. Some types of *Staphylococcus* have become resistant to the antibiotic methicillin and others previously used to treat infections. For example, infections caused by *Methicillin Resistant Staphylococcus Aureus* (MRSA) are difficult to treat because most antibiotics are difficult to kill the bacteria (Fitri et al. 2017).

Given the evidence about the current use of antibiotics, which often causes resistance, it is necessary to conduct research on natural antibiotics contained in plants. One of the plants that can be used as natural antibiotics is sweet orange (*Citrus sinensis* L.). Sweet orange peel contains essential oils that can be used for treatment. Recently, essential oils have attracted worldwide attention because essential oils from several plants are biologically active as antibacterial and antifungal agents. Therefore, they can be used as preservatives in food and as natural antibiotics. In addition, essential oils can inhibit several types of harmful bacteria such as *Escherichia coli, Salmonella* sp., *S. aureus, Shigella*, and *Pasteurella* (Agusta 2000). Based on the research of Chanthaphon et al. (2008), the diameter of the inhibitory power of *Citrus hystrix* DC. peel essential oil against *S. aureus* was 11 mm, while that of *E. coli* was 8 mm. From the research of Gulay et al. (2009), it is known that the diameter of the inhibition of "Turkish Citrus peel oils" against *C. albicans* is 12 mm.

Sweet orange peel essential oil contains the monoterpene compounds group, namely limonene (91.6%), *α-pinene* (0.9%), *sabinene* (1.0%) and *myrcene* (1.3%); the sesquiterpene group, namely *α-copaene* (0.1%) and *β-caryophyllene* (0.1%); the aldehyde group includes *octanal* (1.4%), *decanal* (0.2%) and *geranial* (0.2%); alcohol groups i.e. linalool (0.4%), α-terpineol (0.1%) and geraniol (0.1%); ester groups, i.e., *geranyl acetate* (0.1%) and *neryl acetate* (0.1%) (Gulay et al. 2009). Essential oil from sweet orange peel is also a rich source of bioactive compounds such as coumarins, flavonoids, carotenes, terpenes, linalool, limonene, and pinene (Mondello et al. 2005). Recently, orange peel essential oil has been studied for its natural antioxidant and antimicrobial properties. The antioxidant and antimicrobial abilities are related to the bioactive compounds such as phenolics and terpenoids (Viuda-Martos et al. 2008).

The environment can affect the content and quality of essential oils produced as secondary metabolites of plants. Bruneton (1995) and Rosman et al. (2004) stated that environmental factors could affect the yield of secondary metabolites. These environmental factors include air temperature, lighting (intensity of sunlight), lighting duration, and altitude where it grows (Ketaren 1987). Research on the effect of plant growth on the content and chemical content of essential oils has been carried out. Agustina et al. (2009) reported that the essential oil of *Cinnamomum burmannii* (Nees &Th. Nees) (cinnamon) obtained from 3 different growing sites had different chemical components. Arniputri et al. (2007) also reported that the essential oil of *Temu Kunci* taken at different heights of growing places showed differences in the main components that make up the essential oil. In connection with the indications that sweet orange peel has antimicrobial power, it is necessary to research the antibacterial and antifungal activity of the essential oil of Tawangmangu sweet orange (*Citrus sinensis* L.) grown at different altitudes.

The aims of this study were: (i) to determine the highest yield of essential oil of Tawangmangu sweet orange peel (*C. sinensis*), which grew at an altitude of 1,000 m asl, 1,200 m asl, 1,400 m asl, and 1,600 m asl. (ii) to determine the inhibitory activity of essential oil of Tawangmangu sweet orange peel (*C. sinensis*) growing at an altitude of 1,000 m asl, 1,200 m asl, 1,400 m asl, and 1,600 m asl against *S. aureus* and *C. albicans* bacteria.

MATERIALS AND METHODS

The materials used in this study were Tawangmangu sweet orange peel, as much as 450 grams for each altitude, *S. aureus* isolates, NA (Nutrien Agar) media, chloramphenicol, *C. albicans* isolates, PDA (Potato Dextrose Agar) media, ketoconazole and DMSO.

Sampling

Tawangmangu sweet orange peel samples were taken from Tawangmangu Sub-district, Karanganyar District, Central Java, Indonesia at different altitudes, namely 1,000 ± 50 m above sea level (Tawangmangu Village), 1,200 ± 50 m above sea level (Kalisoro Village), 1,400 ± 50 m above sea level (Blumbang Village) and 1,600 ± 50 m above sea level (Gondosuli Village). Four hundred fifty grams of Tawangmangu sweet orange peel from 3 different trees were taken from each altitude. Then the essential oil was distilled using Stahl distillation.

Essential oil distillation

Essential oil distillation was carried out using a Stahl distillation apparatus. Tawangmangu sweet orange peel was cut into small pieces and then put into a two-necked round bottom pumpkin. A total of 150 grams of Tawangmangu sweet orange peel was distilled using a Stahl device with 500 mL of distilled water at 95°C. The distillation process of Tawangmangu sweet orange peel essential oil was carried out in three repetitions. Furthermore, the resulting essential oil can be seen in the measuring cup in the Stahl tool. Data on sample weight and volume of essential oil will be obtained from the measuring cup, and then the yield will be determined. The obtained yield of essential oil was assumed to be 100% concentration. Based on Ashok et al. (2011) the yield of essential oils is stated as follows:

$$R = V/B \times 100\ \%$$

Note: R = essential oil yield (%), V = essential oil volume (mL), B = Weight of sweet orange peel sample (grams)

The essential oil was put in a closed flakon and covered with aluminum foil. Then, the obtained essential oil was separated from the distilled water using anhydrate sodium.

Making of microbial growth curves and standard curves

Microbial growth curves were made using the turbidimetric method using a spectrophotometer. First, 1 ose of cultured *S. aureus* and *C. albicans* were transferred to an Erlenmeyer flask containing 10 mL of NB medium for bacteria and PDB medium for fungi. Then it was incubated in an incubator shaker at a speed of 120 rpm at 37°C for 24 hours. After 24 hours, every 5 mL of the

microbial suspension was taken and then mixed into 95 mL of NB and PDB media in a larger Erlenmeyer flask and homogenized for 5 minutes. Then, the NB and PDB media were prepared as blanks, and 3 mL of the media was put into a cuvette tube. Finally, 3 mL of microbial suspension was put in another cuvette to measure the absorbance value every 2 hours for bacteria and 4 hours for fungi for 24 hours at a wavelength of 530 nm. From the OD value obtained at each age of growth, a growth curve can be made that shows the relationship between the magnitude of the OD value and the age of the microbe so that the logarithmic growth phase of the test microbe can be known.

The relationship between OD and the number of microbes per mL was obtained by constructing a standard curve and determining the OD and plate count plot. One ose of microbes was grown in 10 mL of NB media and PDB media, and then it was shaken in an incubator shaker at a speed of 120 rpm for 24 hours. After 24 hours, a serial dilution of 5 tubes containing NB and PDB media was carried out.

Five milliliters of each microbial culture was added to 5 mL of NB and PDB media, respectively, and it was expressed as the first dilution /PI (10^{-1}). Then the PI culture was vortexed, and then 5 mL of this culture was added to 5 ml of new NB and PDB media and expressed as PII (10^{-2}). Next, PII was vortexed, and 5 mL of it was taken and added to 5 mL NB and PDB and expressed as PIII (10-^{3}). And so on until the PV (10^{-5}) was created. Furthermore, the absorbance value of each test tube containing the media and microbial culture was calculated using a spectrophotometer at a wavelength of 530 nm. Based on the experimental results, this wavelength can show the highest absorbance value in the medium used, surpassing other wavelengths.

After knowing the OD value, a serial dilution of 7 test tubes containing 9 mL of 0.85% physiological salt was carried out in the 10^{-3} (PIII). A total of 1 mL of the microbial culture from the PIII tube was put into 9 mL of 0.85% physiological saline, and it was expressed as the first dilution/EI (10^{-1}).

Then the EI culture (10^{-1}) was vortexed, and then 1 mL of it was taken and added to 9 mL of new 0.85% physiological salt and expressed as EII (10^{-2}). Next, EII was vortexed, and then 1 mL of it was taken and added to 9 mL of new 0.85% physiological saline and then expressed as EII (10-^{3}). And so on, until EVII (10^{-7}) was created. Then, 100 µL of the microbial culture was added from each dilution. Next, NA and PDA media were added and poured on a pour plate, and then it was incubated for 24 hours at 37ºC. The calculated number of bacterial colonies after incubation was a colony with a total of 30-300 colonies. The number of microbes in a test tube containing NB and PDB media is obtained in each tube. A standard curve can be obtained with a regression value of $y = bx + a$ with y = value of absorbance and x = number of microbes.

Antibacterial and antifungal activity testing

The antibacterial and antifungal activity of Tawangmangu sweet orange peel essential oil was tested using the paper disc method (Kirby Bauer). First, 15 mL of NA media for bacteria and PDA media for fungi were put into a petri dish until solid. The suspension of *S.aureus* and *C. albicans* bacteria in the log phase was taken with a cotton swab and swapped over NA media for bacteria and PDA media for fungi evenly. Next, 6 mm diameter filter paper spheres were dripped with 5 µL of essential oil samples at four concentrations (100%, 75%, 50%, and 25%) which had been dissolved in DMSO solution for each essential oil at different altitudes of 1,000 ± 50 m asl, 1,200 ± 50 m asl, 1,400 ± 50 m asl, and 1,600 ± 50 m asl. With sterile tweezers, the filter paper circles were placed on top of the NA and PDA media inoculated with the test microbes. Press the disc paper gently to ensure it adheres to the media. The same was done for chloramphenicol (antibacterial) and ketoconazole (antifungal) as positive controls and DMSO as negative controls. The culture was stored in the refrigerator for ± 2 hours so that the diffusion process went well. After that, all the petri dishes were incubated for 24 hours at 37ºC, and the Inhibitory Power Diameter (IPD) was measured, which was shown by the clear zone around the paper disc with a caliper. This test was repeated three times.

Data analysis

Essential oil distillation using Stahl distillation produced essential oil yield data with the assumption of 100% concentration. The essential oil yield data were statistically analyzed using one-way analysis of variance (ANOVA) with the help of the Statistical Product and Service Solutions (SPSS) 17 analysis tool.

Antibacterial and antifungal tests were carried out using the Kirby Bauer method to obtain the Inhibitory Power Diameter (IPD) of bacterial and fungal growth. Then the average Inhibitory Power Diameter of the antibacterial and antifungal activity test results was statistically analyzed using a two-way analysis of variance (ANOVA) with the help of the Statistical Product and Service Solutions (SPSS) 17 analysis tool.

RESULTS AND DISCUSSION

Essential oil distillation of Tawangmangu sweet orange peel

Essential oils have been widely used in the health and beauty fields as aromatherapy, relaxation, and a mixture of cosmetic ingredients. In addition, it is also used in the food sector as a flavoring agent. Tawangmangu sweet orange peel is a source of essential oils. This essential oil can be obtained by distillation, pressing, and solvent extraction. In this study, the extraction process of sweet orange peel essential oil was carried out by distillation using a Stahl distillation apparatus. The working procedure of Stahl distillation is the distilled material is in direct contact with boiling water so that hydrodiffusion or water penetration occurs in plant tissues. The water vapor causes the oil glands to rupture so that the essential oil can be carried by the water vapor and then cooled in the condenser to obtain a solution of essential oils. In this study, the essential oil

produced was in the form of a clear yellow liquid with a distinctive smell of Tawangmangu sweet orange peel, as shown in Figure 1.

One hundred fifty grams of Tawangmangu sweet orange peel from different altitudes produced essential oils with different volumes and levels. It is presented in Table 1. The results of ANOVA on the yield of essential oil of Tawangmangu sweet orange peel growing at different altitudes showed a significant difference ($P< 0.05$).

Table 1 shows an increase in the volume of Tawangmangu sweet orange peel essential oil according to the increase in the height of the sampling site. Tawangmangu sweet orange peel taken at an altitude of 1600 m above sea level produces the highest essential oil volume with 0.83 mL. This result is in accordance with the research of Ebrahimi et al. (2011) about the effect of temperature on the essential oil of chamomile. Chamomile grown at the lowest temperature (12ºC) produced the largest volume of essential oil compared to those grown at 15ºC, 20ºC, and 25ºC. According to Fitter and Hay (1991), the temperature is one of the plant stress factors. Therefore, the location with an altitude range of ± 200 meters will affect the environmental conditions, as listed in Table 2.

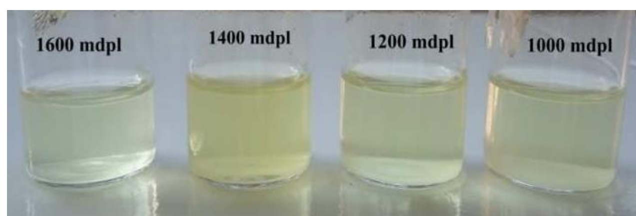

Figure 1. Essential oil of Tawangmangu sweet orange peel (*Citrus sinensis* L.) from different altitudes

Table 1. Volume and yield of Tawangmangu sweet orange peel (*C. sinensis*) essential oil from different altitudes

Altitude (m asl)	Sweet orange peel weight (gram)	The volume of essential oil (mL)	The yield of essential oil (%)
1000	150	0.23	0.15^a
1200	150	0.40	0.26^b
1400	150	0.46	0.30^c
1600	150	0.83	0.55^d

Note: Different letter notations in the same column based on the DMRT test with a 5% confidence level show a significant difference ($p>0.05$)

Table 2. Environmental parameters at each altitude

Parameter	Altitude (m asl)			
	1000	1200	1400	1600
Air temperature (ºC)	35-38	35-38	27-30	23-28
Light intensity (lux)	920 x 100	1014 x 100	1086 x 100	1197 x 100
Humidity (%)	50	55	64	67
Soil moisture (%)	3	3.5	4.9	5.3
Soil pH	7	7	6.5	6.5

The increase in altitude will cause a decrease in soil temperature and pH and an increase in light intensity and air humidity. The cold environmental conditions affect the formation of secondary metabolites in Tawangmangu sweet orange, especially in forming essential oils. The formation of the essential oil is the response of the Tawangmangu sweet orange plant to temperature stress in its surrounding environment. The lower the temperature, the more essential oils produced by the Tawangmangu sweet orange plant.

Growth curve and standard curve of *S. aureus* bacteria

The purpose of making the growth curve of *S. aureus* is to determine the pattern and time of achieving the logarithmic phase. The logarithmic phase (log phase) is a phase of the rapid growth of bacterial cells because the nutritional needs and ideal conditions for growth are optimally met.

On the growth curve in Figure 2, it can be seen that the logarithmic (log) phase of *S. aureus* bacteria occurred at 0 to 12 hours, and the stationary phase occurred at 12 to 18 hours. Therefore, the age of the *S. aureus* bacteria used for the antibacterial test was the age of the 8th hour with an OD value of 2.50. A standard curve was made to determine the equation in calculating the number of bacterial cells to be used in the antibacterial test. From the standard curve, a regression equation was obtained so that the number of bacteria tested at an OD of 2.50 could be determined as 7.3 x 108 cfu/mL.

Growth curve and standard curve of the fungus *C. albicans*

The purpose of making a growth curve for *C. albicans* is to determine the pattern and timing of *C. albicans* experiencing good and optimal growth by dividing rapidly. On the growth curve of *C. albicans* shown in Figure 3, it can be seen that *C. albicans* has good growth at 0 to 12 hours. *C. albicans* experienced good growth with increased cells, so the 8th hour was determined for an antifungal test at OD 2.00 with the number of fungal spores as much as 1.7 x 108 cfu/mL determined from the standard curve.

Antibacterial and antifungal activity of sweet orange peel (*C. sinensis*) Tawangmangu essential oil

The results of testing the antibacterial and antifungal activity of Tawangmangu sweet orange peel essential oil at each altitude at various concentrations using the Kirby Bauer method showed inhibition of microbial growth, which was indicated by the Inhibitory Power Diameter (IPD). IPD is a clear zone that indicates the inhibition of microbial growth around the paper disc, and a cloudy color on the media indicates the presence of microbial growth, which can be seen in Figures 4 and 5.

According to Ganjar (2006), antimicrobial strength is classified into 3, namely strong activity if it produces IPD more than 8 mm, moderate activity if it produces 7-8 mm IPD, and weak activity if it has IPD less than 7 mm. The essential oil of Tawangmangu sweet orange peel from an altitude of 1,600 m asl and 1,200 m asl can be said to have strong antimicrobial activity against *S. aureus* and *C. albicans* bacteria because it can inhibit microbial growth with an IPD of more than 8 mm, at the lowest concentration. On the other hand, the essential oil of

Tawangmangu sweet orange peel from an altitude of 1,400 m asl has weak antimicrobial activity against *S. aureus* and *C. albicans* bacteria. At the smallest concentration, it inhibits microbial growth with an IPD of less than 8 mm. The essential oil of Tawangmangu sweet orange peel from an altitude of 1,000 m asl has weak antimicrobial activity in inhibiting *S. aureus* bacteria. Still, it is strong in inhibiting *C. albicans* fungi.

The results of ANOVA (Appendix 6 and Appendix 7) showed that Tawangmangu sweet orange peel essential oil at each altitude with different concentrations had a significant effect ($p<0.05$) on the growth of *S. aureus* and *C. albicans*. It means that the concentration of Tawangmangu sweet orange peel essential oil affects the size of IPD against *S. aureus* and *C. albicans*. Furthermore, the height of the growing site also had a significant effect on the size of IPD on the growth of *S. aureus* and *C. albicans.*

The antibacterial activity of Tawangmangu sweet orange peel essential oil obtained from each altitude showed significant differences. It meant that there were differences in antibacterial activity at each altitude. For example, the antifungal activity of Tawangmangu sweet orange peel essential oil obtained from an altitude of 1,400 m asl and 1,600 m asl showed significantly different results. It indicated a difference in antifungal activity at an altitude of 1,600 m asl and 1,400 m asl compared to the other 2 altitudes. In comparison, at an altitude of 1,000 m asl and 1,200 m asl, there was no difference in antifungal activity because the ANOVA results showed no significant difference ($p>0.05$) presented in Table 3 and Table 4.

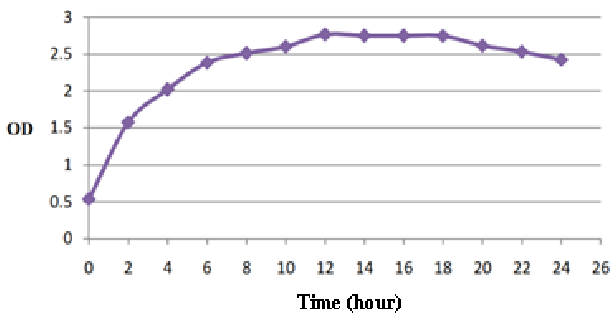

Figure 2. Bacterial growth curve *S. aureus*

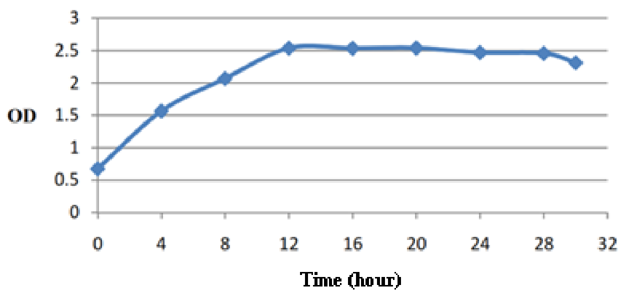

Figure 3. Growth curve of *C. albicans*

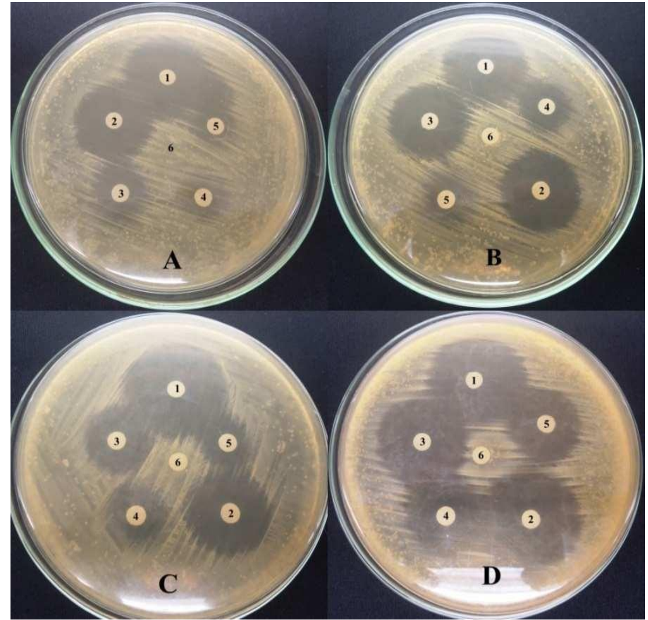

Figure 4. Inhibitory power diameter of essential oil of Tawangmangu sweet orange peel obtained from an altitude of 1,000 m asl (A), 1,200 m asl (B), 1,400 m asl (C), 1,600 m asl (D) with chloramphenicol (1), concentration 100% (2), 75 % (3), 50 % (4), 25 % (5), DMSO (6) against *S. aureus*

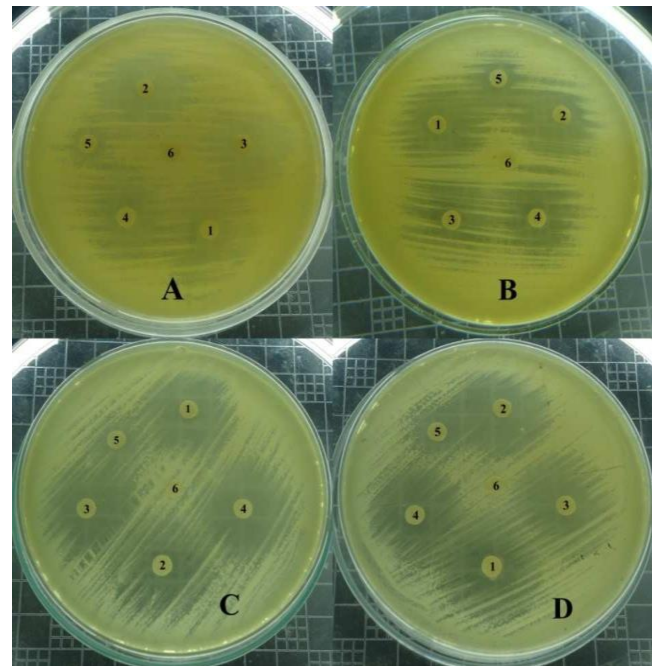

Figure 5. Inhibitory power diameter of essential oil of Tawangmangu sweet orange peel obtained from an altitude of 1000 m asl (A), 1200 m asl (B), 1400 m asl (C), 1600 m asl (D) with ketoconazole (1), concentration 100% (2), 75 % (3), 50 % (4), 25 % (5), DMSO (6) against *C. albicans*

Table 3. Inhibitory power diameter of Tawangmangu sweet orange peel (*C. sinensis*) essential oil on the growth of *S. aureus* bacteria

Concentration (%)	IPD *(mm) at altitude (m asl)			
	1000	1200	1400	1600
100	18aa	18ab	18ac	23ad
75	16ba	14bb	11bc	20bd
50	10ca	12cb	9cc	14cd
25	6da	9db	5dc	12dd
Chloramphenicol 30 mg/mL		27e		
DMSO		0f		

Note: Different letter notations in the same column and row based on the DMRT test with a 95% confidence level showed a significant difference (p>0.05). *: The size of the Inhibitory Power Diameter has been reduced by the diameter of the 6 mm paper disc.

Table 4. Inhibitory power diameter of Tawangmangu sweet orange peel (*C. sinensis*) essential oil on the growth of *C. albicans* bacteria

Concentration (%)	IPD *(mm) at altitude (m asl)			
	1000	1200	1400	1600
100	18aa	18aa	18ab	19ac
75	11ba	12ba	17bb	17bc
50	11ca	10ca	13cb	15cc
25	10da	10da	6db	9dc
Ketoconazole 30 mg/mL		22e		
DMSO		0f		

Note: Different letter notations in the same column and row based on the DMRT test with a 95% confidence level showed a significant difference (p>0.05). *: The size of the Inhibitory Power Diameter has been reduced by the diameter of the 6 mm paper disc.

Tawangmangu sweet orange peel essential oil with concentrations of 100%, 75%, 50%, and 25% can inhibit the growth of *S. aureus* and *C. albicans* bacteria. It is in accordance with the research conducted by Roy et al. (2012). They conducted an antibacterial test of lemon peel essential oil against *S. aureus* bacteria with a concentration of 100% having an inhibitory diameter of 8 mm. Meanwhile, in the study of Mathur et al. (2011), sweet orange peel essential oil with a concentration of 100% was reported to inhibit *S. aureus* with an IPD of 12 mm. This study is also in accordance with the research of Jwanny et al. (2012), who tested the antifungal and antibacterial activity of sweet orange peel from Egypt. This research shows that sweet orange peel essential oil has strong antibacterial activity against *Bacillus subtilis, E. coli, Pseudomonas aeruginosa, Salmonella typhi, Proteus mirabilis, Candida tropicals, Saccharomyces cerevisiae* including *S. aureus* and *C. albicans*. It shows that sweet orange peel essential oil has a strong antibacterial and antifungal potential. Based on Table 3 and Table 4, a diagram depicting the IPD of Tawangmangu sweet orange peel essential oil was made, which is presented in Figure 6 and Figure 7.

In general, Figure 6 and Figure 7 showed that the decrease in the concentration of Tawangmangu sweet orange peel essential oil was in accordance with the decrease in the amount of IPD. The increasing concentration of Tawangmangu sweet orange peel essential oil causes more antimicrobial content, so microbial growth inhibition is greater. Still, an increase follows not all increases in concentration in IPD. For example, Figure 7 showed that the essential oil of sweet orange peel at an altitude of 1,000 m above sea level for concentrations of 75% and 50% had the same strong antifungal activity, i.e., 11 mm. On the other hand, Tawangmangu sweet orange peel essential oil at an altitude of 1,200 m above sea level for concentrations of 50% and 25% had the same strong antifungal power as 10 mm. The possibility of an equally strong antifungal activity was indicated by the same large IPD, which appeared due to the difference in the rate of diffusion of the essential oil on the agar medium and the volatilization of the essential oil on the disc paper because it was left out in the air for too long.

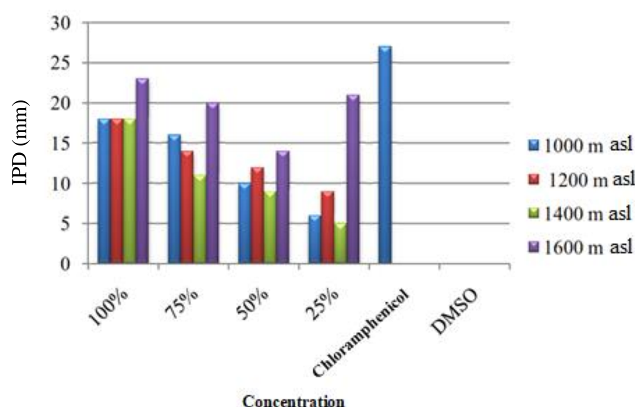

Figure 6. Inhibitory diameter of Tawangmangu sweet orange peel essential oil obtained from each altitude against *S. aureus*

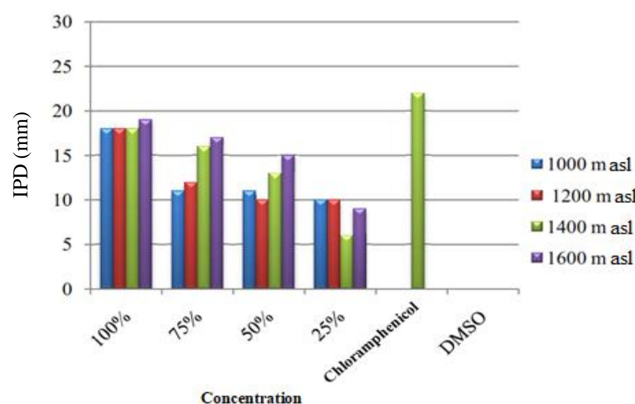

Figure 7. Inhibitory power diameter of Tawangmangu sweet orange peel essential oil obtained from each altitude against *C. albicans*

Nursal and Juwita (2006) stated that phenolic compounds, terpenoids, and flavonoids are secondary metabolic products of plants that actively inhibit the growth of bacteria and fungi. According to Lota in Vasudeva and Sharma (2012), the three main elements in orange peel essential oil are generally limonene, terpinene, and linalyl acetate. Jwanny et al. (2012) reported that the essential oil of sweet orange peel contains ascorbic acid, which causes a sour taste. In addition, this essential oil also contains bioactive compounds such as phenolic compounds, alkaloids, flavonoids (especially flavone glycosides), and limonene which is a terpenoid derivative and the most abundant. Essential oil from sweet orange peel is also reported to be a rich source of bioactive compounds such as saponins, tannins, coumarins, flavonoids, carotenes, terpenes, linalool, limonene, alkaloids, and pinene (Mondello et al. 2005; Ashok et al. 2011). Soković et al. (2007) reported that *C. aurantium* and *C. limon* essential oils contained the largest secondary metabolite compound, limonene, respectively 90.01% and 59.68%. In this study, limonene from *C. aurantium* and *C. limon* showed positive results in inhibiting S. *aureus* bacteria with IPD of 14 mm and 16 mm, respectively. In the study of Siddique et al. (2011), it was known that *C. aurantium* essential oil contained 98.17% limonene, tested for antibacterial, and showed positive results in inhibiting *S. aureus* with an IPD of 12 mm.

The working procedure of essential oils in inhibiting the growth or killing of microbes is by interfering with forming membranes and/or cell walls. The compounds contained in Tawangmangu sweet orange peel essential oil inhibit bacteria by damaging the peptidoglycan (protein) structure in the cell wall through protein denaturation. The *S. aureus* is a Gram-positive bacterium with a cell wall structure with a thick peptidoglycan layer and a thin layer of teichoic acid. Gram-positive bacteria are more sensitive to essential oils than Gram-negative bacteria due to their different outer membrane structure (Chanthaphon et al. 2008). Gram-positive bacteria do not have a lipopolysaccharide layer that protects the membrane, causing essential oils to damage the porin protein more easily, causing cell lysis (Mulyani et al. 2009). Flavonoids can inhibit bacterial growth by damaging the cell walls and cytoplasmic membranes of bacteria and preventing bacterial division so that bacteria cannot grow (Robinson 1995).

The cell wall of *C. albicans* contains 6-25% protein, the rest is carbohydrates (80-90%) and fat (1-7%), and the outer part of the wall is covered with a layer of fibrils in the form of fibers (Chaffin et al. 1998). Non-polar compounds can induce changes in the membrane permeability of *C. albicans* through the interaction between the active site of the compound and the active site of the cell membrane, especially the cholesterol and ergosterol parts. This interaction results in changes in permeability and causes the cell membrane to become unstable, causing fungal cell death (Kusumaningtyas et al. 2008). In addition, alkaloids work by inhibiting the biosynthesis of nucleic acids (Mc-Charty et al. 1992).

Flavonoids have antifungal activity in *C. albicans* by interfering with the formation of pseudohyphae during the pathogenesis process and denaturing proteins so that they can damage cell membranes that are irreversible (cannot be repaired) (Cushnie and Lamb 2005). The mechanism of action of inhibition of antibacterial and antifungal compounds in Tawangmangu sweet orange peel is not known for certain because to find out how these antibacterial and antifungal compounds work, and molecular research is needed. According to Jawetz et al. (2001), the combined activity of several antibacterial compounds can be more effective than the work of each compound. On the other hand, the combined working activity of several antibacterial and antifungal compounds can be less effective than the work of each compound. Judging from the antibacterial and antifungal activity of the active compound of Tawangmangu sweet orange peel essential oil, the inhibition of the growth of S. aureus and C. albicans bacteria may be carried out by all active compounds or only one active compound. It is uncertain and requires further research.

The research can be concluded: (i) Sweet orange peel (*C. sinensis*) Tawangmangu growing at an altitude of 1,000 m asl, 1,200 m asl, 1,400 m asl, and 1,600 m asl produced different yields of essential oils. Tawangmangu sweet orange peel, which grows at an altitude of 1,600 m asl, produces essential oils with the largest yield of 0.55%. (ii) Sweet orange peel essential oil (*C. sinensis*) Tawangmangu at an altitude of 1,000 m asl, 1,200 m asl, 1,400 m asl, and 1,600 m asl had different antimicrobial activities against *S. aureus* and *C. albicans*. Tawangmangu sweet orange peel essential oil at an altitude of 1,600 with a concentration of 100% had the greatest antimicrobial activity with an Inhibitory Power Diameter of 23 mm against *S. aureus* and an Inhibitory Power Diameter of 19 mm against *C. albicans*.

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