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Potency of Lime (Citrus Aurantifolia) as Bio-Disinfectant Of Staphylococcus Aureus

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Abstract
Limes (Citrus aurantifolai) contain antiseptic. This study aimed to isolate, identify, and test the effectiveness of the active antiseptic substance of lime to inhibit the growth of Staphylococcus aureus. In the next step, it was expected to produce "Lime Oil" which was useful for the treatment of infectious disease that was caused by Staphylococcus aureus, particularly skin infections. The true-experimental research design was the posttest-only control design. The number of samples of each treatment group and control group were 24. The effectiveness of lime's active antiseptic substance was tested by using disc diffusion method. The collected data were analyzed by using Kruskal Wallis test. The result showed that lime (Citrus aurantifolai) positively contained an antiseptic such plavonoid and terpenoids with a total phenol of 285.80 GAE / ml. Statistical analysis showed that there was the difference of the effectiveness bio-desinfectant of lime in Staphylococcus aureus on various concentrations, but the effectiveness of bio-disinfectant had not been sensitive.

Keywords*: lime (Citrus aurantifolia), and Staphylococcus aureus, bio-disinfectant*

I. INTRODUCTION

Staphylococcus aureus bacteria has been resistant to a variety of antibiotics, hence, the medical treatment for infectious disease that is caused by *Staphylococcus aureus* cannot only focus on chemical treatment, especially antibiotics. Prevention by using medicinal plants is one of alternatives, because in Indonesia there are more than 100 species of medicinal plants. One of the plants which are used more as an antiseptic is lime. Lime contains many useful compounds such as citric acid, amino acid (*tryptophan and lysine*), essential oils (*limonene, acetate linalin, acetate geranil, fellandren, sitral, lime camphor, kadinen, aktialdehid,* and *anildehid),* vitamin A, vitamin B1, and vitamin C. The result of the study reported that lime was a medicine for various diseases (Haq et al., 2010). Antibacterial potency of lime's essential oils is played by *phenol* and its derivatives, which can denature bacterial cell protein. One of its derivatives is *kavikol*, which has bactericidal in five times stronger than *phenol*.

Farkhatul Afiyah (2004) reported that the essential oil of lime's rind had antibacterial activity through the formation of a clear zone at concentrations of 5%, 10%, 15%, 20% and 30% for both *Staphylococcus aureus* and *Escherichia Coli*. Erianto Fanani (2006) reported that the minimum inhibitory concentration of lime peel against MRSA (Methicillin Resistant Staphylococcus aureus) was at a concentration of 18%, while the minimum killing concentration was at concentration of 20% (Fitarosana, 2012).

According to the explanation above, it was necessary to identify the active antiseptic substance of lime extract and analyze the effectiveness of lime's bio-disinfectant through *Staphylococcus aureus*, by looking at the growth inhibition of *Staphylococcus aureus* bacteria in various concentrations of lime extracts.

II. METHODS

Design of this true-experimental research was posttest only-control design. Members of each group were chosen randomly. The sample size was 24, which were obtained by making four treatments of lime extract with the concentration of 5%, 10%, 15% and 20%. The number of repetition was twice for each concentration and 3 times replication, added with positive and negative controls (Hanafi and Pack, 2005).

Identification of active antiseptic substance was conducted at the Laboratory of Pharmacy, Faculty of Mathematics and Natural Sciences, Udayana University, while the bio-disinfectant effectiveness test was conducted at the Laboratory of Microbiology, Faculty of Medicine, Udayana University. The procedures, which were conducted, were: 1) preparation of tools and media, 2) the manufacture of lime extract, 3) identification of active antiseptic substances, 4) setting up the concentration of lime extracts, 5) examination of samples. The data were analyzed by using Kruskal Wallis test in order to test the difference of the effectiveness bio-desinfectant of lime in *Staphylococcus aureus*, on various concentrations.

III. RESULTS

A. Lime Antibacterial Substances

Qualitative tests that utilized phytochemical screening of antibacterial compound of lime with positive result were: *flavonoids*, *terpenoids*, citrate acid, and essential oil. The result of quantitative test showed the total *phenol* was 285.80 GAE / mL. *Hesperidin* activity test that was conducted in order to see the activity of antibacterial lime extract was showed by the peak fluctuation (substance 5) in Figure 1.

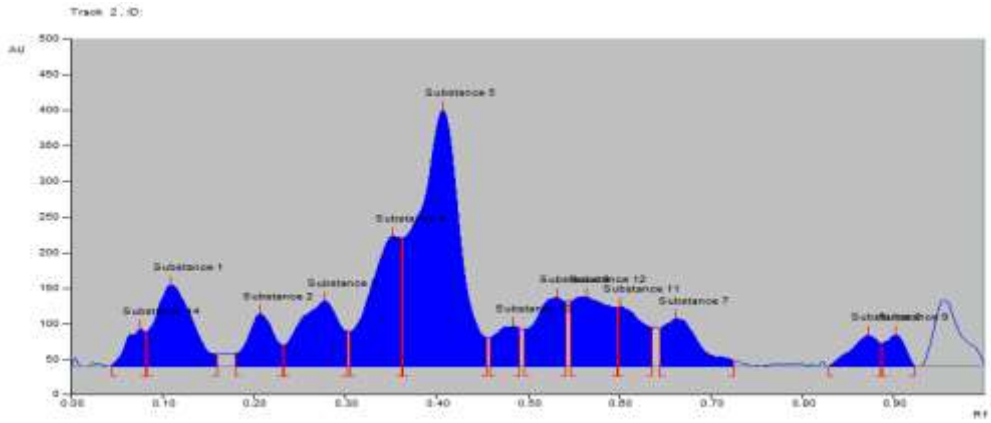

Figure 1.
The result of *hesperidin* identification with KLT- *Spectrofotodensitometer*

B. Test of Lime Inhibition

1. Positive control

Positive control was the antibiotics disc (*Chloramphenicol* 30 mcg) which resulted inhibition zone on the first repetition until the third replication. The diameter of inhibitory zone on replication I, II and III were not significantly different. The average inhibition zone diameter was 18 mm (Table 1).

Table 1. Growth Inhibition Zone of *Staphylococcus aureus* at Positive Control

Repetition	Inhibition zone diameter (mm)		
	Replication I	Replication II	Replication III
I	17	17	18
II	19	20	17
Total	36	37	35
Average	18	18,5	17,5

2. Negative control

Negative control was sterile *ethanol* that did not produce inhibition zone diameter because it did not contain any antibacterial substances. In replication I, II, and III, inhibition zone diameter was 0 mm.

3. Lime extract with concentration of 5%

In replication I to III, lime extract with concentration of 5% was grown on *Mueller Hinton* in order to show inhibition zone. In replication I, II, and III, inhibition zone diameter was 0 mm.

4. Lime extract with concentration of 10%

In replication I, the average of inhibition zone diameter was 9 mm and the average of inhibition zone in replication II was 9.5 mm. Meanwhile, the average of inhibition zone in replication III was 9 mm. if it was compared with inhibition zone diameter of *Chloramphenicol* 30 mcg in the table NCCLS, this inhibition zone diameter was in the category of resistance (Table 2).

Table 2. Growth Inhibition Zone of *Staphylococcus aureus* on Lime Extract with Concentration of 10%

Repetition	Inhibition zone Diameter (mm)		
	Replication I	Replication II	Replication III
I	9	9	10
II	9	10	8
Total	18	19	18
Average	9	9,5	9

5. Lime Extract with concentration of 15%

In replication I and replication II, the average of inhibition zone was 11 mm. Whether, inhibition zone average in replication III was 10.5 mm. If it was compared with inhibition zone diameter of *Chloramphenicol* 30 mcg in the table NCCLS, this inhibition zone diameter was in the category of resistance (Table 3).

Table 3. Growth Inhibition Zone of *Staphylococcus aureus* on Lime Extracts with Concentrations of 15%

Repetition	Inhibition zone Diameter (mm)		
	Replication I	Replication II	Replication III
I	10	10	11
II	12	12	10
Total	22	22	21
Average	11	11	10,5

6. Lime extract with concentration of 20%

In replication I, inhibition zone diameter Average was 12 mm and the average of Inhibition zone diameter of replication II was 13 mm. Meanwhile, the inhibition zone average in replication III was 12 mm. If it was compared with inhibition zone diameter *Chloramphenicol* 30 mcg in table NCCLS, this inhibition zone diameter was in category of resistance (Table 4).

Table 4. Growth Inhibition Zone of *Staphylococcus aureus* on Lime Extracts with Concentrations of 20%

Repetition	Inhibition zone Diameter (mm)		
	Replication I	Replication II	Replication III
I	12	11	12
II	12	15	12
Total	24	26	24
Average	12	13	12

C. Statistical Analysis

Lime extracts with concentrations of 5%, 10%, 15%, and 20% were not sensitive or not effective to inhibit the growth of *Staphylococcus aureus*. The diameter of inhibition zone that was formed on all concentrations of lime extract was classified as resistant categories. The result of *Kolmogorov Smirnov* statistical test showed that *asymp sig* value was 0.046 (<0.05), which meant the data was not normally distributed, thus, the process was continued by using *Kruskal Wallis test*, with p-value of 0.000 (<0.05). This result indicated that there were differences in growth inhibition zone in *Staphylococcus aureus* on various concentrations of pure lime extract. In this case, there were differences in effectiveness bio-disinfectant on various concentrations of lime extract toward *Staphylococcus aureus*. The result of LSD (Least Significant Deference) test showed that there were differences in effectiveness bio-disinfectant on various concentrations of lime extract toward *Staphylococcus aureus*. It was based on P-value of each concentration with values of <0.05.

IV. DISCUSSION

A. The Differences of effectiveness bio-disinfectant on various concentrations of pure lime extract toward Staphylococcus aureus.

The result showed that the lime extract with various concentrations did not entirely produce diameter of inhibition zone, especially on concentration of 5%. The difference of inhibition zone arose from differences on concentration of lime extract that contained antibacterial substance toward *Staphylococcus aureus.* In each concentrations of lime extract contained different levels of active substances, due to extract volume and diluent volume of each different concentration. The higher concentration of lime extract, the higher active substance that made the larger diameter of inhibition zone which was formed. This was appropriate with the research conducted by Hasbi (2012), which was about the effects of avocado leaf distillation toward *Pseudomonas Sp*. In this case, the higher concentration of avocado leaf distillation, the bigger inhibition zone that was formed.

The result of qualitative phytochemical screening test, the active substances contained in lime extracts were *flavonoids*, *terpenoid*, *citrate acid* and essential oil. The test result of *hesperidin* identification showed antibacterial activity. *Flavonoid* was the active substance of *hesperidin* and acted in causing permeability damage of cell *membrane*, *microsomes* and *lysosomes* as the result of interaction between *flavonoid* and bacterial DNA (Pratiwi, 2014).

Essential oils in lime had bioactivity as antimicrobial, insecticidal, and antioxidants (Astarini, 2010). Essential oils contained volatile compounds such as *monoterpenes* and *sesquiterpene*, one of them was a *phenol* (Aryadi, 2014). *Phenol* and its derivatives could denature bacterial cell protein (Fitarosana, 2012). Quantitative test showed that total phenol was 285.80 GAE / mL. The roles of phenol as an antibacterial agent were to poison protoplasmic, damage and penetrate cell membrane, and precipitate proteins from bacterial cell. Large molecular phenolic compounds were able to make an essential enzyme in bacterial cells to become inactive, although in very low

concentrations. In addition, phenol could damage bacterial cells, denature proteins, make enzyme to become inactive and cause leakage of the cell (Aryadi, 2014).

Other factors which could affect inhibition zone diameter were incubation temperature, incubation time, the sterility of instruments, contamination, turbidity of bacterial suspension, thickness of media, and disc distance (Dwijayanti, 2012). Furthermore, these factors had been controlled in order to have no influence to the result of this study.

B. The concentration of lime extracts which were effective as bio-disinfectant of Staphylococcus aureus

Based on the result, the entire lime extract concentrations were not sensitive to the growth of *Staphylococcus aureus* (all diameters of inhibition zone were in category of resistance). This was occurred because the concentrations which were used were still relatively low, thus, the numbers of active antibacterial substances were still low. Therefore, this amount had not been able to inhibit the growth of *Staphylococcus aureus.*

Lime extract could inhibit the growth of *Staphylococcus aureus,* but it was not better than positive control. We could say that four concentrations, which were tested, had not been sensitive enough to inhibit the growth of *Staphylococcus aureus*. Thus, further research was needed to be conducted by using lime extract with higher concentration.

The difference of inhibition zone was occurred due to amount variations of active substance on each component, which was in lime. It depended on several parameters, including: ripeness, vegetative phase of plant, and storage conditions (Astarini, 2010). Maturity index of lime could be determined based on size and shape of fruit, overall color of fruit, basic color of rind, flesh color, hardness (firmness) of flesh, dissolved sugar content (*soluble solid content*), acidity (*acidity*), and *ethylene* concentration. Fruit which was picked too early or too late also affected its color, texture, flavor and aroma as well as chemical compounds. These things affected physical and chemical changes during maturation, because after picking, the fruit was still experiencing metabolic reactions. The optimum temperature for fruit storage was 5-10^{o}C. Temperature that was too low could cause fruit damage (*chiling injury*). The result showed that limes which were carefully picked, and stored in room temperature (23-31^{o}C) for 3 weeks, decayed as much as 7%. Limes which were dropped on the floor would decay as much as 12%. Limes which were picked wet would decay 21%. Limes which were picked too ripe would decay as much as 29%. Limes which were exposed to sun for a day, would decay 38% (Kristiani, 2011).

V. CONCLUSIONS AND RECOMMENDATIONS

Based on result and discussion of the research, it could be concluded that: (1) Antibacterial substances of lime extract were *plavonoid* and *terpenoids,* citric acid, and essential oil with total *phenol* of 285.80 GAE/mL, (2) The effectiveness Bio-disinfectant in lime toward *Staphylococcus aureus* on concentrations of 5%, 10%, 15% and 20% were classified as resistance, (3) There was the difference of the effectiveness bio-desinfectant of lime in *Staphylococcus aureus* on various concentrations, but all of them were not sensitive.

Suggestions based on conclusion of this research are: (1) The researchers should conduct further research by using higher concentrations and it was developed *in vivo*, (2) the society could utilize lime extracts as a precaution against infection which was caused by *Staphylococcus aureus.*

References

1. Ahmad S.A., Hakim, E.H., and Makmur, L. 2009, *Ilmu Kimia dan Kegunaan Tumbuh-tumbuhan Obat Indonesia.* Bandung: ITB.
2. Awang, Messyliana, 2014, Pengaruh Berkumur Larutan Air Perasan Jeruk Nipis (Citrus aurantifolia) Terhadap Akumulasi Plak, Medicines Faculty. Denpasar University.
3. Ansel, H.C., 2005, *Pengantar Bentuk Sediaan Farmasi*. Edisi keempat. Jakarta: UI Press.
4. Darmadi, 2008, *Infeksi Nosokomial Problimatika dan Pengendaliannya*. Jakarta: Salemba.
5. Dwijayanti, 2012. *Perbedaan Berbagai Konsentrasi Ekstrak Bawang Putih (Allium sativum linn) Terhadap Zona Hambat Pertumbuhan Escherichia coli.* KTI Department of Health Analyst. Denpasar Health Polytechnic.
6. Ernawati. Dyah, 2008, Pengaruh Penggunaan Ekstrak Jeuk Nipis (*Citrus aurantifola*) Terhadap Residu Nitrit Daging Curing Selama Proses Curing. Faculty of Agriculture. Sebelas Maret University, Surakarta.
7. Fauziah, 2007, Tanaman Obat dan Keluarga (Revisi), Jakarta: Niaga Swadaya.
8. Fitarosana, 2012, Pengaruh Pemberian larutan Ekstrak Jeruk Nipis (Citrus aurantifolia Terhadap Pembentukan Plak Gigi, Undergraduate Medical Education Program, Medicines Faculty, Diponegoro University, Semarang.
9. Hanafiah, Kemas, A., 2005. *Rancangan Percobaan Aplikatif*, Jakarta : Grafindo.

10. Haq, G. Istifany, Anna Permanasari, Hayat Sholihin, 2010, Efektivitas penggunaan Sari Buah Jeruk Nipis Terhadap Ketahanan Nasi. Jurnal Sains dan Teknologi Kimia1 (1) : 44-58. Chemical Department of FPMIPA UPI, Bandung.
11. Hariana, Arief, 2006. *Tumbuhan Obat & Khasiatnya seri 1*. Jakarta: Penebar Swadya.
12. Jawetz, E., Melnick, J. L., dan Adelberg, E. A., 2005. *Mikrobiologi Kedokteran*, 23rd edition. Jakarta: UI.
13. Noor, 2011. *Metodologi Penelitian*, Jakarta: Kencana Group.
14. Nurkalimah, Cut, 2011. Daya Antibakteri Air jeruk Nipis *(Citrus aurantifolia)* Terhadap Pertumbuhan *Staphylococcus aureus* dan *Escerichia coli* yang diuji secara in Vitro, Medicines Faculty, North Sumatra University, Medan.
15. Pelczar, M.J., dan Chan, 2006. *Dasar-dasar Mikrobiologi Jilid 2*, Jakarta: UI Press.
16. Sugiyono, 2013. *Metode Penelitian Pendidikan (Pendekatan Kualitatif, Kuantitatif, dan R&D).* Bandung: Alfabeta.
17. Supardi, dan Sukamto., 2003. *Mikrobiologi Dalam Pengolahan Dan Keamanan Produk Pangan*. Bandung: Alumni