# The potential study of flavonoid compounds of lime peels against *Pseudomonas aeruginosa* isolated from pus

[1]**Evy Ratnasari Ekawati,** [2]**Win Darmanto\* and** [2]**Sri Puji Astuti Wahyuningsih**

[1]*Program Study of Medical Laboratory Technology, Faculty of Health Science, Maarif Hasyim Latif University, Sidoarjo, Indonesia*
[2]*Departement of Biology, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia*

### ABSTRACT

Giving an antimicrobial chemical is often used in the healing process of the skin lesions of infection. The bacteria that dominate in lesions of skin infections include *Pseudomonas aeruginosa*. Antibiotic resistance against pathogens is increasing, so many researchers concentrate on doing research to find antimicrobial plant. This study aimed to test the potential of flavonoids in lime peel against Pseudomonas aeruginosa isolates from pus on the skin lesions. This research was experimental laboratory. Samples collected from the plantations lime Bumi Aji, Batu and pus samples taken from three peoples with lesions of skin infections. Lime peels extract concentration of 80% and a 100% was response inhibits the growth of *Pseudomonas aeruginosa* with sensitive categories. Kruskal-Wallis test results obtained significance value of $0.005 \le \alpha = 0.05$, meaning that treatment of various concentrations of lime peel extract significant influence on the growth of *Pseudomonas aeruginosa*. Pus from three samples, one sample code $Pa_2$ were identified as *Pseudomonas aeruginosa* with similarity index > 99.9%.

## Introduction

Lime peels is often thrown away when lime is used as a juice, medicine, food seasonings or other uses. Lime peels contains flavonoids active substances, namely eriocitrin, nairutin, hesperidin, neohesperidin, neoponcirin, poncirin, isorhoifolin, diosmin, neodiosmin, sinensetin, nobiletin, tangeretin and heptatoxyflavone. The active substance in lime peels is hesperidin (Nogata *et al.,* 2006; Ekawati *et al.,* 2019). Lime peels, including parts albedo layer, flavedo and coating segment has a higher flavonoid content than the grain of the fruit juice (Ekawati *et al.,* 2019)

Active substances contained in the lime peels are able to work as an anti-microbial, anti-inflammatory, anti-virus, anti ulserogenic, anti-cancer, anti-neoplastic, anti-tumor, anti-platelet, anti-hepatotoxic, anti-hypertensive and cholesterol levels (Nogata *et al.,* 2006; Rathee *et al.,* 2009). For example, the active compound in lime peels is hesperidin and diosmin, either as single compounds or compound combination of both, can be used as an anti-carcinogenesis colon that they are due to azoxymethane (Nogata *et al.,* 2006),

The skin is the largest organ in the body and is the first defense against pathogens, toxins and trauma. Injuries or damage to the skin can cause

S222

*Eco. Env. & Cons. 27 (August Suppl. Issue) : 2021*

severe of morbidity and even mortality. The main causes lesions of the skin infection due to complications from surgery, trauma and insect bites on the mucosa or the skin surface. Skin infections wound healing process is a complex process, involving deposition of collagen in response to tissue injury, and at the final stage produce scar formation. Such mechanisms include inflammation, fibroplasia, and maturation of scar tissue (Forbes *et al.,* 2007; Rosique *et al.,* 2015),

Infectious lesions on the skin surface are easily colonized by various microorganisms. Some studies suggest several different kinds of bacteria from patients who live in areas with different geographic. Microorganism is a group of inflammatory pyogenic bacteria (Singh *et al.*, 2013; Ekawati *et al.,* 2018), Group pyogenic bacteria consists of many species that are widespread in the human body, among the most common and often are *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Streptococcus pyogenes, Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae, Salmonella typhi, Neisseria gonorrhoeae, Mycobacterium tuberculosis* (Singh *et al.*, 2013; Ekawati *et al.,* 2018),

*Pseudomonas aeruginosa* is one of the opportunistic pathogen bacteria were important because it shows a lot of resistance to antibiotics. *Pseudomonas aeruginosa* is one of the causes of nosocomial infections in immune-compromised patients and patients who are in intensive care unit. The bacteria can be isolated from various infections, such as respiratory infections, pyogenic infections, burns, urinary tract infections, surgical infections, etc. (Renuga *et al.*, 2016),

This study aimed to test the potential of flavonoids in lime peel against *Pseudomonas aeruginosa* isolates from pus on the skin lesions.

## Materials and Methods

### Sample Collection

Lime juice collected from the BumiAji, Batu, Malang, East Java, Indonesia. Lime peels of flavedo and albedo and flavedo separated from the flesh with a knife of stainless steel, then dried. Lime peel separation process until extraction were done in an organic chemistry laboratory, Faculty of Science and Technology, Airlangga University Surabaya. Meanwhile, pus samples obtained from three people who experience skin lesions, and identification in micro-

biology laboratories, Faculty of Health Sciences, UMAHA.

### Instruments, Reagents, and Medium

The instrument used in this study is a knife stainless steel, blender, glass jars, funnels, evaporator, balance digital, Erlenmeyer 100 ml, scoop samples, petri dishes, test tubes, steel armor (cylinder cup), tweezers, micro pipette 100 ml, micro pipette 1000 ml, pipette drops, Mc Farland 0.5 standard, ciprofloxacin 500 mg, distilled water, pus, media NB, MC, EMB, NAS, Rapid NF Plus.

### Extraction of Lime Peels Preparation

Powdered lime peels soaked with 96% ethanol until submerged whole, then covered with aluminum foil. Left for five days with occasional stirring evenly. After five days, filtered through filter paper, so the resulting filtrate parts 1 and dregs 1. Dregs 1 soaked with 96% ethanol, then covered with aluminum foil, left for two days. After two herring filtered, so the resulting filtrate 2 and dregs 2. filtrate 1 and filtrate 2 mixed together, then evaporated using a rotary evaporator at a temperature of $80^0$C, the solvent is evaporated until all ethanol is lost and the pure extract obtained viscous. Extract weighed and stored in jars before being used for testing (Mpila *et al.,* 2012).

### Identification of *Pseudomonas aeruginosa* in Pus

Pus planted in the NB medium, and then planted in the MC and EMB medium. Colonies that grew on media MC Gram stained. If obtained morphology Gram-negative rods, then continued to be tested in Rapid NF Plus.

### Preparation of Variation Concentration Lime Peels Extract

Created variation lime peels extract concentration of 20%; 40%; 60%; 80% and 100% [w / v], by means of weighed 0.2 g; 0.4 g and 0.6 g, 0.8 g and 1 g of lemon peel extract, then each dissolved in 100 ml of sterile distilled water (Mpila *et al.,* 2012),

### Preparation of Negative Control

We prepared 1 ml of sterile distilled water and taken as much as 100 ml for inclusion in the well (Ekawati, 2018).

### Preparation of positive control

Antibiotic ciprofloxacin 500 mg was dissolved in

100 mL of sterile distilled water and then 100 mL was taken to be put into the well (Ekawati, 2018).

### Test Potential of Lime Peels Extract

Five mL of NA medium was poured into a petri dish and allowed to solidify, then three stainless pickers were placed with a spacing of between 1.5 cm. One ml of bacterial suspension was put into 15 ml of NA medium and homogenized, then poured into a petri dish containing a steel tray as a second layer and allowed to solidify. Steel pickers were aseptically removed from the petri dish so that the wells are formed.

Each of the wells was filled with 100 ml of lime peels extract in various concentration and controls, then incubated at 37 ᵒC for 24 hours. Observations we taken for the formed inhibitory zone (Ekawati, 2018).

### Research design

The study design used was completely randomized design (CRD) with 7 treatments and each treatment consisted of 3 replicates

## Results and Discussion

The results of the identification of *Pseudomonas aeruginosa* in a sample of pus from skin lesion are presented in Table 1 while the results of the test potential lime peels extract on the growth of *Pseudomonas aeruginosa* are presented in Table 2 and Figure 1.

**Table 1.** Identification results of *Pseudomonas aeruginosa* in pus from skin lesions

| Sample code | Result | Similarity Index (%) |
|---|---|---|
| $PA_1$ | *Klebsiella pneumonia* | 86.2 |
| $PA_2$ | *Pseudomonas aeruginosa* | > 99.9 |
| $PA_3$ | *Pseudomonas putida* | 77.7 |

The identification results of three samples of pus, in the sample code $Pa_1$ found was *Klebsiella pneumonia* with a similarity of 86.2%, $Pa_2$ found was *Pseudomonas aeruginosa* with a similarity of > 99.9%, and $Pa_3$ found was *Pseudomonas putida* with a similarity of 77.7%.

Based on Table 2, lime peel extract at a concentration of 20% already inhibits responded to the growth of *Pseudomonas aeruginosa* but with the resistant category. At a concentration of 40% to 100%

**Table 2.** Potential test results of lime peels extract on the growth of *Pseudomonas aeruginosa*

| The concentration of lime peels extract | Average resistor zone (mm) | Note |
|---|---|---|
| $D_0$ | 0 | R |
| $D_1$ | 16:46 | R |
| $D_2$ | 7.66 | R |
| $D_3$ | 14:06 | I |
| $D_4$ | 16.92 | I |
| $D_5$ | 17:07 | S |
| $D_6$ | 17:27 | S |

Information: $D_0$ = negative control; $D_1$ = positive control; $D_2$ = Concentration of 20%; $D_3$ = Concentration of 40%; $D_4$ = Concentration of 60%; $D_5$ = Concentration of 80%; $D_6$ = concentration of 100%; R (resistance) = ≤ 10 mm; I (Intermediate) = 11-16 mm; S (susceptible) = ≥ 17 mm (Soleha, 2015).
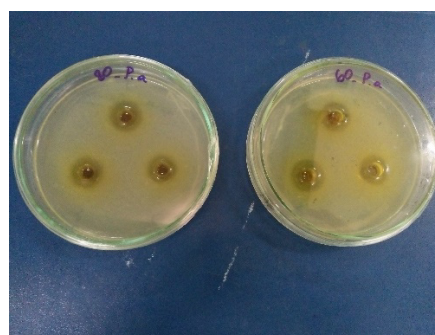


**Fig. 1**. Potential test of lime peels extract on MHA medium.

was response impediments to the growth of *Pseudomonas aeruginosa* with intermediate categories. For the negative control ($D_0$) does not inhibits respond against *Pseudomonas aeruginosa*, whereas the positive control ($D_1$) to inhibit respond against *Pseudomonas aeruginosa* with resistance categories. The area of an inhibitory zone is formed

The Data above were tested using the Kruskal-Wallis test to Determine the differences between lime peels extract given to *Pseudomonas aeruginosa* growth. The Kruskal Wallis test results obtained p-value 0.000> α = 0:05, the which means there was difference in the outcome of providing lime peels in different doses of *Pseudomonas aeruginosa* growth. Based on the data presented in the table and statistical tests, the optimum dose of lime peels roommates extract can inhibit *Pseudomonas aeruginosa* was 40% with intermediate categories.

A new antimicrobial indispensable to overcome the increased resistance of *Pseudomonas aeruginosa*.

S224

*Eco. Env. & Cons. 27 (August Suppl. Issue) : 2021*

Lime peel contains flavonoids, that are naringin, hesperidin, naringenin, hesperitin, routine, nobiletin and tangeretin (Choy *et al.*, 2007). Flavonoids are polyphenolic compounds the largest of which can work as antioxidants and also as a way of antibacterial proteins denature bacterial cell and cell-damaging bacteria (Adindaputri *et al.,* 2013),

Multidrug - resistant *Pseudomonas aeruginosa* specimens in our study had Categorized as resistant to Several classes of antibiotics like aminoglycosides, beta-lactam penicillin, sulfa trimethoprime, tetracycline, chlorampenicol, macrolide, and quinolones. These bacterial specimens are sensitive only to the beta-lactam class of 3rd generation cephalosporins, such as cefoperazone - sulbactam. Various antibiotics had been tested against the Multidrug - resistant *Pseudomonas aeruginosa* roomates specimens used in our experiment. We conclude that the bacteria Showed diverse mechanisms of resistance to antibiotics such as: protein synthesis inhibition (aminoglycosides, tetracycline, chlorampenicol, macrolide); cell wall synthesis inhibition (beta-lactam penicillin); inhibition of folic acid metabolism pathway (sulfa - trimethoprime) (Zharfan *et al.,* 2017).

## Conclusion

Lime peels extract concentration of 80% and 100% was response impediments to the growth of *Pseudomonas aeruginosa* with sensitive categories. Kruskal-Wallis test results obtained significance value of $0.005 \le \alpha = 0.05$, meaning that treatment of various concentrations of lime peel extract significant influence on the growth of *Pseudomonas aeruginosa*. Pus from three samples, one sample code $Pa_2$ is identified as *Pseudomonas aeruginosa* with similarity index > 99.9%

## Acknowledgment

## References

Adindaputri, Z., Purwanti, N. and Wahyudi, I. A. 2013. Pengaruh Ekstrak Kulit Jeruk Nipis (*Citrus aurantifolia* Swingle) Konsentrasi 10% Terhadap Aktivitas Enzim Glukosiltransferase *Streptococcus mutans. Majalah Kedokteran Gigi Indonesia.* 20(2) : 126. doi: 10.22146/majkedgiind.6803.

Choy, S. 2007. Correlatio Between Flavonoid Content and The No Production Inhibitory Activity of Peel Extract from Various Citrus Fruits. *J. Biol. Pharm. BUll*, 30(4) : 772–778.

Ekawati, E. R. 2018. *Uji Potensi Senyawa Antimikroba metode Sumuran.*

Ekawati, E. R., Pradana, M. Sungging and Darmanto, W. 2019. Lime (*Citrus aurantifolia*) peel as natural antibacteria for wound skin infection caused by *Staphylococcus aureus. International Journal of Pharmaceutical Research*. 11(1). doi: 10.31838/ijpr/2019.11.01.042.

Ekawati, E. R., Pradana, Muhammad Sungging and Darmanto, W. 2019. Lime (*Citrus aurantifolia*) peel as natural antibacteria for Wound Skin Infection caused by *Staphylococcus aureus. International Journal of Pharmaceutical Research*. 11(1) : 363–366. Available at: https://doi.org/10.31838/ijpr/2019.11.01.042.

Ekawati, E. R., Yusmiati, S. N. H. and Herawati, D. 2018. Identifikasi kuman pada pus dari luka infeksi kulit. *Jurnal SainHealth*. 2(1) : 31–35.

Forbes, B. A., Sahm, D. F. and Weissfeld, A. S. 2007. *Bailey & Scott's Diagnostic Microbiology*. New York: ELSEVIER, Mosby.

Mpila, D. A., Fatimawali and Wiyono, W. I. 2012. Antibacterial activity test of ethanol extract of mayana leaf (*Coleus atropurpureus* [L] Benth) on *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* in *In-vitro. Journal Pharmacon*. pp. 13–21. Available at: https://ejournal.unsrat.ac.id/index.php/pharmacon/article/download/440/351.

Nogata, Y. 2006. Flavonoid Composition of Fruit Tissues of Citrus Species. *Biscience Biotechnology and Biochemistry*. 70(1) : 178–192. doi: 10.1271/bbb.70.178.

Rathee, P. 2009. Mechanism of Action of Flavonoids as Anti-inflammatory Agents: A Review. *Inflammation and Allergy-Grugs Targets*. 8(3) : 229–235. doi: 10.2174/187152809788681029.

Renuga, 2016. Prevalence of *Pseudomonas aeruginosa* and its antibiotic susceptibility pattern in a Tertiary Care Hospital. *International Journal of Research in Pharmaceutical Sciences*. 6(1) : 27–30.

Rosique, R. G., Rosique, M. J. and Junior, J. A. F. 2015. Curbing Inflammation in Skin Wound Healing/: A Review. 2015(iv).

Singh, S. 2013. Antibacterial Activities Against Pyogenic Pathogens. *International Journal of Pharmaceutical Sciences and Research*. 4(8) : 2974–2979.

Soleha, T. U. 2015. Uji Kepekaan Terhadap Antibiotik', *Jurnal JUKE Unila*. 5(9) : 119-123. Available at: http://juke.kedokteran.unila.ac.id/index.php/juke/article/view/644.

Zharfan, R. S., Purwono, P. B. and Mustika, A. 2017. Antimicrobial activity of Pineapple (*Ananas comosus* L. Merr) extract against multidrug-resistant of *Pseudomonas aeruginosa*: An *in vitro* study. *Indonesian Journal of Tropical and Infectious Disease*. 6(5) : 118–123.