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EFFECTIVENESS TEST OF ANTI-BACTERIAL LIME JUICE (CITRUS AURANTIFOLIA) ON SALMONELLA SP BACTERIA IN SALMON FISH (ONCORHYNCHUS NERKA)

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Abstract

Salmonella Sp is a gram-negative pathogens that are often found in marine foods,one of process of serving salmon is channeling lime juice, where lime juice is acidic and has phytochemical properties that have anti-bacterial properties. This study aims to explore anti-bacterial effect lime juice on Salmonella thypii bacteria in Salmon. This research is an experimental study using disc diffusion method. Lime samples that were used were purchased from one of the modern markets in Medan which were made in several series of concentrations using distilled water as a solvent, while the salmon samples used in this study were purchased from one of the Japanese dining restaurants located in Medan City. Meanwhile, isolation of Salmonella thypii bacteria from salmon samples was carried out using nutrient broth and SSA media. The results of this study indicate that differences in concentration indicate the differences in the diameter of the inhibitory zone (P value = 0.000). With the diameter of the widest inhibition zone formed by a concentration of 100% is 15.40 ± 0.44 mm and the smallest diameter is formed by a concentration of 5% which is 7.23 ± 0.55 mm. Thus, it can be concluded that the lime juice has anti-bacterial activity against Salmonella thypii bacteria from salmon.

Keywords*: Lime juice, Citrus Aurantifolia, Salmonella Sp*

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INTRODUCTION

Salmonella Sp is a rod-shaped Gram-negative bacteria that do not form spores. This bacteria has harmful pathogens for humans and animals. Salmonellosis is the example of disease caused by Salmonella bacteria contamination can be transmitted through food products and become one of the world's problems. Salmonellosis case that becomes the cause of the disease is known has number range 95% that can spread through various types of food such as chicken carcasses, meat, eggs, fish, dairy products. Salmonellosis may cause health problems such as bactericemia, gastroenteritis, fever, abdominal cramps, nausea, vomiting, and dizziness (Razak, Djamal, and Revilla, 2013).

Most of salmonella source comes from animal foods including fishery food material. Unclean handling or imperfect food product processing or the contamination of the environment is one of the causes of human Salmonella infections (BPOM RI, 2014; Morgan, 2005).

In fishery products it is obtained a quite high prevalence report associated with the case of extraordinary events (KLB), such as nausea, vomiting, fever and diarrhea. As an example is fresh fishery material consists of 23 samples of fish, 4 samples of squid, 110 samples of shrimp and 4 samples of scallops and immunity to antibiotics. Samples got from traditional markets and modern markets in five municipalities of DKI Jakarta and Bogor by using purposive random sampling method conducted in the period March 2013 to October 2014. It indicates the result of the research was the prevalence of Salmonella Sp. fresh fishery is about 32% (45/141 samples) (Ajizah, 2004; Benigna, 2015).

Information about the prevalence of *Salmonella Sp*. in fishery products has been reported several times, but it was still limited. (Ajizah, 2004; Weinstein et al., 2019) stated that the prevalence of *Salmonella Sp*. on shrimp ponds in Tangerang is 26%. In addition, it ws also identified in Karawang by 70%. Another information indicates that 36% (9/25) of the product of fresh white shrimp (Penaeus merguiensis) in the traditional market in Surabaya were known to have been contaminated with Salmonella sp (Fajarwati, 2013).

Lime is one of fruits used directly in food serving of Salmon fish by flushing lime juice. Lime juice contains a variety of phytochemicals such as saponins and flavonoids which are compounds of oilfenol which have antioxidant and

antibacterial activity. Antibacterial activity of this juice is derived from 5,8-dimethoxypsoralen, 5-geranyloxypsoralen, palmitic acid, linoleic acid, oleic acid, 4-hexan-3-one and citral. In addition to the phytochemical content(Miguéis, Moura, Saraiva, & Esteves, 2016; Morgan, 2005), lime juice has a content of citric acid which causes the pH of lemon juice is low. The average pH of lime juice with a concentration of 100% is 2.3. The low pH value of lime juice hampers the process of delivery of amino acids from RNA so that it inhibits the growth of several types of bacteria (Liew, Ho, Yeap, & Sharifudin, 2018; Yahya, 2016).

Some previous studies have been conducted to explore the antibacterial activity possessed by lime juice to *Staphylococcus aereus* and *Enterococococcus feacalis* bacteria, as well as various other pathogens (Khanifah, 2015, the Minister of Health, 2011). With the development of future consumption of salmon in the community, the society are at risk contaminated with *Salmonella sp*. The period is growing in many areas without exception in Medan so that it raises the author ‘s interest to prove whether lime juice is effective on *Salmonella Sp* bacteria in salmon.

MATERIALS AND METHODS

The type of this study was experimental research which was done in the room Laboratory of Microbiology University of Prima Indonesia, in March 2019 - June 2019. Population affordable of this study were all salmon served at one of the restaurants of Japanese food in Medan and lime from whole modern market in Medan city. The samples were taken from each of the sampled salmon and lime in Medan by using purposive sampling technique. Where the selected modern market and Japanese food restaurant are the closest to the location of the study.

The instruments used in this study were a petri dish, autoclave, aluminum foil, measuring cup, flask, funnel filter, filter paper, paper disc, analytical balance, a inoculating loop, test tubes, test tube rack, bunsen, tweezers, microscopes, glass objects, a pipette, camera, cotton, and stationery.

Materials used in this study were salmon, freshly lime juices (Citrus aurantifolia), sterile distilled water, Salmonella Shigella Agar (SSA) media, Nutrient Agar (NA), distilled water and 70% alcohol.

As much as 25 gram samples of salmon which have been obtained finely were pounded with a mortar. As much as 100 mg was taken and placed in a test tube, then was added nutrient broth to

reach a volume of 10 ml. Nutrient broth was then incubated for approximately 18-24 hours at 37 ° C. The results of enrichment was diluted to 10^3 and was grown using the selective media (SSA), sterilized and incubated at 37° C for 36-48 hours. The shape of colonies that grow in isolation form SSA later were identified by gram staining to confirm the bacteria *Salmonella sp* (Olaimat & Holley, 2013; Rahayu, 2000).

Lime was cut into 2 parts. The next step was to squeeze the water into the tube flask then was filtered using filter paper until get liquid lime juice as much as 5 ml. Making the concentration of lime according to Yahya (2016), determination the concentration of lime juice by using a formula of dilution that is; V1xM1 = V2xM2 using a volumetric flask(Sati, Dhyani, Bhatt, and Pandey, 2019; Yahya, 2016),

Antimicrobial activity test method was done by using disc diffusion method. The testing step was carried out by taking bacteria *Salmonella sp* that had grown to SSA media, was swabben to the rest of the agar media. Sterile paper disc was dip into each of concentration and distilled water as a negative control, sterile paper disc then put on agar media that has been swabbed by Salmonella sp bacteria, after all sterile paper disc containing the concentration has been placed, agar media was wrapped and incubated for 36-48 hours (Schwarz et al., 2010),

Determination of MIC did after getting the test results of antimicrobial activity. Lime juice which has the greatest inhibition diameter was chosen for determination of MIC. The step of MIC value determination was done by paper disc given marinade solution discs, and the size of disc was measured by using a caliper(Olaimat & Holley, 2013).

Processing and Data Analysis. Data obtained were incorporated and processed in the data processing software using the program Statistical Program and Service Solution (SPSS) with statistic test using One Way ANOVA and Post Hoc Test Tukey HSD.

RESULTS AND DISCUSSION

Results of analysis of inhibitory concentrations of lime juice

Inhibitory activity of lime juice on the bacterium Salmonella sp on agar media are following data:

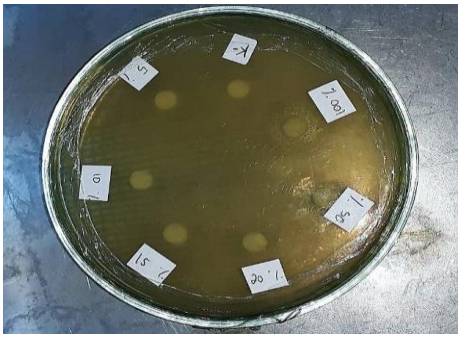

Figure 1. The inhibitory concentration of lime juice

Results of analysis of data normality

Based on analysis of data normality by the Shapiro-Wilk (on output) and homogeneity of data by Levene (on output) found that the data were normally distributed and homogeneous, with a P value> 0.05. Because the data were normally distributed and homogeneous, then the analysis was followed by analysis of One-Way ANOVA and Tukey HSD as advanced test. The results of the analysis of One-Way ANOVA and Tukey HSD followed by Post-Hoc Test are shown in the following table.

Table 1. Results of Analysis of One Way ANOVA and Post Hoc Test (Tukey HSD)

Concentration	Inhibition Zone Diameter (mm) [Mean±SD] *	value P
5%	7:23 ± 0.55a	
10%	8:23 ± 0.51a, b	
15%	9:40 ± 0.75b, c	
20%	10:57 ± 1.00c, d	0:00
25%	11:13 ± 0.68c, d	
100%	15:40 ± 0.44e	
Negative control	5:50 ± 0.00a	

*Data presented in the form of Mean ± SD. Different lowercase letters in the same column indicate significance at P> 0.05.

From the table above it indicates that there was significant statistical difference inhibition zone diameters that were formed from each concentration. Because the value of P <0.05 at each concentration there was a tendency difference in the diameter of inhibition zone, where in the diameter of inhibition zone formed at lower concentrations of 5% and 10% has a statistically significant difference compared to the diameter of inhibition zone which is formed at higher concentration, they are 15 %, 20% and 25%, and at the highest concentration - 100%. At all concentrations samples, it had statistically significant differences compared to the negative control, except at the lowest concentration of 5%. All differences were assessed with a confidence level of 95% (α= 0,05).

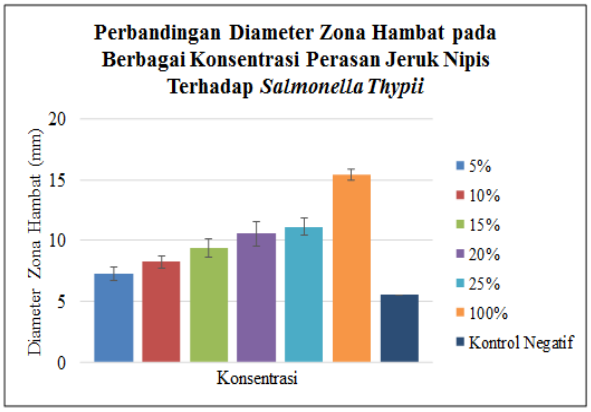

Figure 2. Comparison of inhibitory concentrations of lime juice

Preliminary test of the effects of lime juice to the bacterium *Salmonella Typhi* indicates positive results. Test of antibacterial effect has been done by disc diffusion method was to prove the existence of an antibacterial effect in lime juice supply to *salmonella typhi* and show the difference by using distilled water control. The result observed was the diameter of inhibition zone created around the disk containing trial materials on agar media that were inoculated with *Salmonella Typhi*. The result was positive if there was zone diameter inhibited. Measurement of inhibitory zone was done after media were incubated for 36-48 hours using a measuring instrument with measurement calipers millimeters (mm). (Image 1)

Fresh lime juice has a lactic acid content of 0.09% citric acid 6.15% as well as a small amount of tartaric acid [8]. The antibacterial activity of lime caused by the content of a number of organic acids, one of them is citric acid as main content, then lactic acid, malic acid and tartaric acid. The resistance process as antibacterial of the organic acid for pH decrease below the range of the microorganisms growth and was inhibited through metabolism by conditioned acidic substances (Yahya, 2016).

From the results of the study, trial materials of lime juice has antibacterial effects against Salmonella Typhi with different resistor power capability of each concentration.

The results indicates the average inhibition zone in distilled water as a negative control 5.5 mm, at a concentration of 5% inhibition of the average of 7.23 mm, at a concentration of 10% inhibition of the average 8,23 mm, the 15% inhibitory concentration average 9.40 mm, at a concentration of 20% inhibition of average 10.57 mm, at a concentration of 25% inhibition of the average 11.13 mm, at a concentration of 100% inhibition of the average 15.40 mm (table 1).

Based on the criteria of inhibitory zone CLSI guideline 2013, the inhibition zone formed by the lime juice is categorized as weak.

Table 2. Classification of Response Inhibition Bacteria Growth (Suandy, Girsang, Nasution, and Lister, 2020)

Inhibition Zone Diameter	Inhibition activity
> 20 mm	very potent
16-20 mm	potent
10-15 mm	Enough
<10 mm	Weak

This is supported by research (Razak et al., 2013) which reported that the inhibition of lime juice against the growth of Staphylococcus aureus bacteria in vitro shows the existence of inhibition activity against Staphylococcus aureus at a concentration of 25%, 50%, 75% and 100% with the average zone of inhibition created by 5,167mm, 6,167mm, 7,7mm and 10.5 mm (Razak et al., 2013),

While the results of study conducted by Hilmi Yahya shows that the antibacterial activity of lime juice (Citrus aurantifolia S.) affect on the inhibition of bacterial growth Enterococcus faecalis. It is proved by the existence of inhibition zone around the wellbore contains lime juice (Citrus aurantifolia S.) concentration of 25%, 50% and 100%, have an average diameter of inhibition zone in succession by 5.09 mm, 8.72 mm and 13.52 mm (Sumono & SD 2008; Yahya, 2016),

Based on the results of study, it is found out that their inhibition zone diameter variation was formed due to the differences in the concentration of lime juice. The higher concentration of lime juice, the greater the inhibition zone diameter was formed as well as the greater the ability of inhibition caused. It indicates that by increasing concentrations of lime juice, the greater the active ingredient contained in the lime juice acts as an antibacterial, so the greater its ability to inhibit the growth of bacteria(Olaimat & Holley, 2013).

Bacterial growth can be inhibited by flavonoids compounds and essential oils contained in the lime juice. The essential oil works by destroying the bacterial cell proteins and damaging cell cytoplasmic membrane. The proteins which have denaturation process will lose physiological activities and an imbalance in the cell wall will increase the cell permeability, the transport function becomes active, the arrangement of protein molecules control bacterial cells disrupted resulting in the loss of macro-molecules and ions from the cell. Cells will lose its shape so it is lysis and damaged. While flavonoids has bacteriostatic or bactericidal depending on the concentration, inhibits the growth of bacteria by interacting directly with DNA, causing the DNA of bacteria was broken so the bacteria die(BPOM RI, 2014),

In addition to the phytochemical content, freshly lime juice has citric acid which causes the pH of lime juice is low. The average pH of lime juice with a concentration of 100% is 2.3. The low pH value of lime juice inhibit the process of delivery amino acids of RNA so that it inhibits the growth of several types of bacteria(Liew et al., 2018; Yahya, 2016).

CONCLUSION

The conclusions of this study is amino acids is proved to have anti-bacterial and can inhibit the growth of bacteria Salmonella Typhi, the maximum of antibacterial activity of lime juice is found out at 100% concentration of 15.40 mm.

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