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Comparison of Antimicrobial Effectiveness of Orange and Lime's Extracts on *Aeromonas sobria* and *Aeromonas hydrophila*

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Abstract

***Aeromonas* bacteria are often found in aquatic environments and can be isolated from various types of fish. Globally, the incidence of infection by *Aeromonas* reached 0.6–76 cases per 1 million people. Orange and lime are generally used as cooking spices to get rid of the fishy smell in fish, and also have antibacterial effects. The study compared the antibacterial effectiveness of lime and orange extracts on two *Aeromonas species; A. sobria* and *A. hydrophila*. Bacteria *Aeromonas* may emerge from fresh tuna bought in traditional markets. Lime and orange extracts were prepared using the maceration method using 96% methanol as solvent. The extract concentrations used were 6.25%, 12.5%, 25%, and 50%. Antimicrobial sensitivity test was carried out using the Kirby-Bauer method. The bacterial inhibition zone test revealed that the effectiveness of extracts on both oranges and limes was higher for the bacteria *A. hydrophila* compared to *A. sobria.* It is known from the average diameter of the inhibition zone, which is larger on *A. hydrophila* compared to *A. sobria,* except for the extract with a concentration of 6.25% orange and 50% lime. In a comparison between extracts, lime extract was found to be more effective as an antimicrobial than orange extract, except at a concentration of 6.5% in the bacterial test *A. sobria* and 50% concentration in the bacterial test *A. hydrophila*. In general, lime is more effective as an antimicrobial than orange.**

Keywords: *Aeromonas sobria, Aeromonas hydrophila*, Antimicrobial, Orange, Lime Extract

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INTRODUCTION

Indonesia is a region that has vast waters with a very abundant number of fish. This makes fish a food source that is often consumed either raw (e.g., nanjira) or cooked. Consumption of raw fish has potential risks to human health, which may cause several diseases, including bacterial infections.[1]

Aeromonas are a group of rod-shaped Gram-negative bacteria (0.3-1.0 x 1.0-3.5 m), facultative anaerobes, catalase- and oxidase-positive, could ferment glucose and break down nitrates into nitrites.[2] These bacteria are often found in aquatic environments and can be isolated from tap water, rivers, soil, various foods, and marine environment.[3] The number of these bacteria increases in aquatic environments when summer arrives.[4] Globally, the incidence of infection with *Aeromonas* ranges from 0.6–76 cases per 1 million people.[1] Other literature mentions that the infection *Aeromonas* alone (monomicrobial) occurs in 5.5% of traveler's diarrhea. *Aeromonas bacteria* generally cause disease in the form of diarrhea (common in healthy people), wound infections, and systemic opportunistic infections.[4] In addition, it can cause soft tissue infections with a mortality rate of up to 26.7–100%.[5]

The content of essential oils and secondary metabolites in citrus fruits can cause cell membrane permeability, causing cell membrane leakage.[6] In previous research, the use of orange and lime juice reduced the number of bacterial colonies found in goldfish. Sembiring, in his research, found that 40% of essential oil extract from orange juice has antibacterial properties against the bacteria *E. coli and B. Cereus.*[7] This study aims to compare the potential antimicrobial effectiveness of oranges and limes by testing both extracts using *Aeromonas* bacteria isolated from tuna (*Euthynnus spp. is related*) purchased at a traditional market in the city of Medan.

MATERIALS AND METHODS

Isolation of bacteria in fish

This study used the test bacteria *A. sobria* and *A. hydrophila*. Test for bacteria was performed in meat specimens by obtaining two fresh tunas purchased at traditional markets. The fish meat was then crushed using a blender, and a swab was taken using a sterile spoon. Specimens were cultured on blood agar and incubated for 24 hours at 35–37°C. Then, the colonies formed from the results of the blood agar culture were subcultured again on nutrient agar media for pure culture. The bacteria was identified using the VITEK® 2 machine.

Extract manufacturing

Lime and orange were blended and filtered through a Mesh-40 filter. The preparation of the extract was carried out by the maceration method, and the solvent used was 96% methanol. Maceration was carried out for three days, and every day the solution was stirred. Then, it is filtered with filter paper, and the remaining lime dregs are added to the methanol until the color of the methanol is almost the same as the color of the lime extract after stirring. The resulting extract was filtered again. Solvent separation was carried out using a rotary vacuum evaporator at 50°C. Then, the extract was put into the oven at 40°C until it was free of methanol. The available extracts of orange and lime were then diluted using the solvent *dimethyl sulfoxide* (DMSO) to obtain concentrations of 6.25%, 12.5%, 25%, and 50%.

Extract's antimicrobial sensitivity test

The antimicrobial effectiveness of oranges and limes were tested using the inhibition zone test with Kirby-Bauer method. Bacteria; *A. sobria* and *A. hydrophila* in nutrient agar media, were then plated in Mueller Hinton Agar (MHA) media. There were six discs used for measuring the inhibition zone, namely discs containing extracts of 6.25%, 12.5%, 25%, and 50% concentration, as well as a positive control in the form of ciprofloxacin and a negative control in the form of sterile distilled water. The repetition of the inhibition test was carried out three times. After the discs were placed in the MHA, the media were incubated for 24 hours at 37°C. The results of the inhibition zone formed were measured using a caliper.

Statistical analysis

Data will be presented in descriptive method and described accordingly; however, to further produce a proper conclusion, statistical analysis was performed using SPSS v. 25. ANOVA test were conducted to measure statistical difference of inhibition zone diameters.

RESULTS AND DISCUSSION

Test results of orange and lime extracts on bacteria *A. sobria* and *A. hydrophila* are depicted in Figure. From the results of the inhibition zone test, it was found that the greater the concentration of the extract, the greater the diameter of the inhibition zone formed. In addition, the effectiveness of the extracts of orange and lime also seemed to be more effective against bacteria *A. hydrophila* compared to *A. sobria*. This could be seen from the larger mean diameter of the inhibition zone formed on *A. hydrophila* compared to *A. sobria* except for the extract with a concentration of 6.25% oranges and 50% concentrations of lime. When compared between extracts, lime extract appears to be more effective as an antimicrobial than orange extract. This could be inferred from diameter of the inhibition zone, which was larger at various concentrations except at a concentration of 6.5% in the test bacteria *A. sobria* and 50% concentration in the test bacteria, *A. hydrophila* (Table). ANOVA test confirms difference within all groups either in *A. sorbia or A. hydrophilia.*

This study is the first to compare the antimicrobial effectiveness of oranges and limes against the bacteria *Aeromonas*. In our previous study, we compared the antimicrobial effectiveness

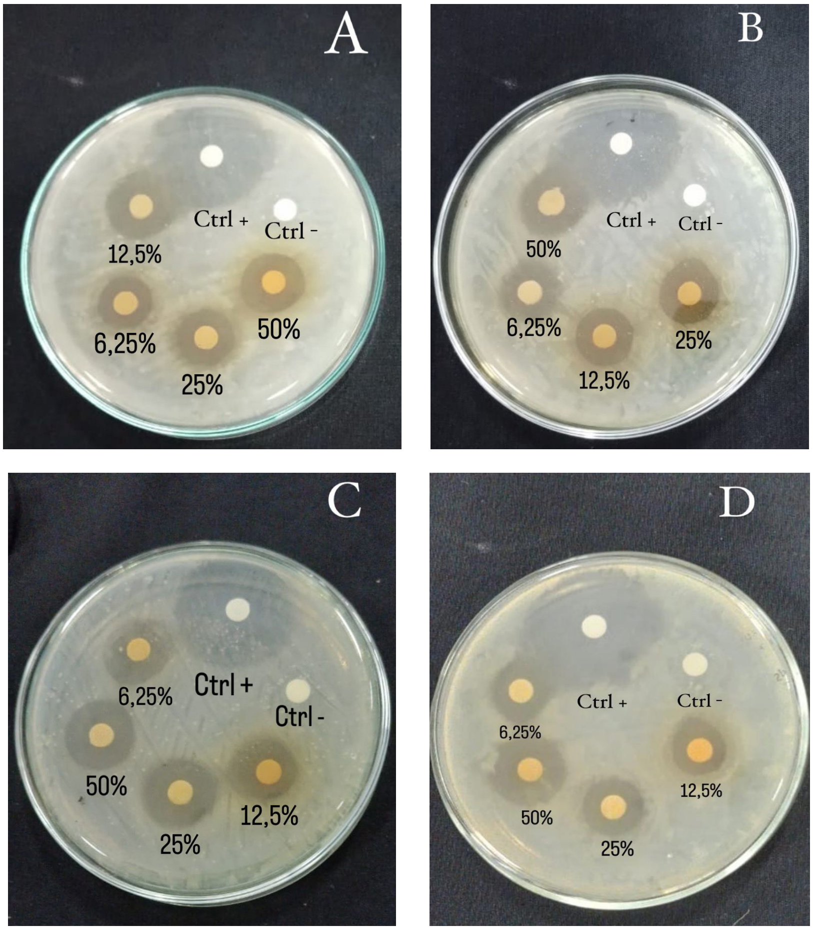

Figure. Inhibition zone diameter test results; A. Lime extract on *A.sobria*; B. Orange extract on *A. sobria*; C. Lime extract on *A. hydrophila*; D. Lime extract on *A. hydrophil*

Table. Inhibition zone diameter (mm)

Bacteria	Reps.	Control +	Orange extract (%)				Lime extract (%)			
			6,25	12,5	25	50	6,25	12,5	25	50
A.sobria	1	33	14,1	15	20	25	14	16	15	20
	2	33,2	14	15	16	18	15	17	20	26
	3	33,5	15	15	18	21	12	14	19	24
Mean ($\bar{x}$)		33,2	14,3	15	18	21,3	13,6	15,6	18	23,3
p-value		< 0,001*								
A.hydrophila	1	32,2	14,2	16	20	24	15	18	20	21,2
	2	33	13,5	15,7	18	23	14,7	20	20	23,1
	3	33,4	14	18	18	23	14,9	20	20	23
Mean ($\bar{x}$)		32,8	13,9	16,5	18,6	23,3	14,8	19,3	20	22,4
p-value		< 0,001*								

*ANOVA test

of orange and lime on carp using the Total Plate Count (TPC) method. The results obtained showed that the use of orange extract resulted in a better reduction in the number of bacterial colonies than lime extract. [6] In research conducted by Sembiring in 2018, they found that the essential oil in orange juice has moderate antibacterial effectiveness at a concentration of 40% with test bacteria E. coli and *B. cereus.*[7] In another study, orange juice had high antibacterial effectiveness against the bacteria *B. subtilis*, *S. aureus, and P. multocida* but weak antibacterial effectiveness against *E. coli.*[8] In previous research on lime, it was found that by giving an extract concentration of 80% to the test bacteria *S. mutans, it could* produce an average inhibition zone diameter of 22.6 mm.[9] In other studies, different diameters of the inhibition zone were found, namely at *E. coli with a* concentration of lime extract of 100% (20.75 mm) and on *S. aureus* with an extract concentration of up to 100% (18.9 mm).[10]

Antimicrobial potential of orange and lime is thought to originate from their acidic nature and posess of bioactive substances. Orange has a pH of around 2.50 and lime has a pH of 2.48, while the bacteria *Aeromonas* is known to have slower growth rate at low pH (<6).[11,12] Apart from acidic nature, bioactive substances such as the essential oils in oranges and limes can also act as antimicrobials. The mechanism of essential oils as antimicrobials is through its effect of causing damage to the inner and outer membranes of bacterial cells by means of penetration of essential oils into the plasma membrane (due to the lipophilic nature of essential oils), this will form holes or gaps in the cells thus triggering the release of electrolytes and result in the death of bacteria.[13-15]

While study with specific settings were not available as of yet there are several studies identifying both orange and lime antimicrobial activities. Study by Shehata determine through UPLC-ESI-MS/MS analysis of sweet orange peel extract that these extract had great potential as natural antioxidant and antimicrobials. The predominant polyphenols were narirutin, naringin, hesperetin-7-O-rutinoside naringenin, quinic acid, hesperetin, datiscetin-3-O-rutinoside and sakuranetin.[16]

Oikeh *et al.* analysis of fresh and dry ethanol extracts obtained from sweet orange peels were consistent with our result. Sweet orange peels extract were able to work against 5 common bacterial strains and 3 fungal strains by observing zone of inhibition using disc diffusion method. Oikeh further underlined that fresh extract might be more effective compared with dry extract.[17]

Anwar's study in 2023 was intriguing. By extracting sweet orange peel oil through soxhlet apparatus they were able to found its antimicrobial activities against *E. coli* and *A. flavus*. *C. sinensis* peel oil demonstrated antimicrobial capabilities, implying that it could be used as a natural preservative in food or as an effective treatment against a variety of pathogenic organisms.[18]

Study performed by Liew *et al.* on lime were also consistent with our result. Freshly prepared calamansi lime EO nanoemulsion was the most effective against *Escherichia coli, Salmonella spp,* and *Staphylococcus aureus* by exhibiting the largest diameter of inhibition zone (8.34, 7.71, and 9.98 mm, respectively). Liew further concluded that lime EO nanoemulsions showed great potential to be incorporated into water-based food products and beverages as flavouring and antimicrobial agents.[19]

Sreepian study with makrut lime essential oil for inhibiting MRSA isolates were quite interesting. The MIC values of CHEO are 18.3 ± 6.1 mg/mL in MSSA isolates and 17.9 ± 6.9 mg/mL in MRSA isolates ($p > 0.05$). The antibacterial activity of CHEO demonstrated the bactericidal effect with MIC index 1.0–1.4. This underlined lime extract potential as antimicrobial agents.[20]

Lau reports were interesting too in which they were able to use lime as mouthwash and hand sanitized. They were able to provide counselling for common folk in methodology to prepare lime as mouthwash and had sanitizer. While Lau couldn't report any immediate health benefit result in term of infection or public health observation. This study is still quite interesting and proof the applicability of our study.[21]

Lastly, a study performed by Selahvarzi *et al.* in 2021 attempted to investigate the antimicrobial activity of arange and pomegranate peels extract. The results show that the minimum inhibitory concentration (MIC) for PPE samples was 1.5 and 2.5 times that of OPA against Gram-positive and Gram-negative bacteria, respectively. Increasing the levels of extracts in beverages, especially OPE, can reduce the pH during 27-day in refrigeration storage and also significantly reduce the growth of total bacterial, molds, and yeasts.[22]

CONCLUSION

Overall, lime has a better antimicrobial potential than orange in the Aeromonas test. This study can be a reference for further research to test the antimicrobial effect before and after giving orange and lime juice directly to food, or it can also be used to test the antimicrobial effect on other bacteria.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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