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The Number of Bacteria Colonies in Carp Fish (*Cyprinus carpio*) After Administration of Lime (*Citrus aurantifolia*) and Orange Extract (*Citrus jambhiri Lush*.)

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Abstract. Fish is one of the most commonly consumed protein sources. Fish are at risk as a medium for the growth of microbial contaminants that can cause foodborne diseases. This disease can cause diarrhea and even death.Contaminant microbes can be found in foods consumed raw, such as Naniura, a typical Batak cuisine in the form of raw carp. Various natural spices such as lime and orange also have potential as antimicrobials. This study aimed to determine the antimicrobial potential of lime and orange juice in fresh carp samples. This study used extracts of lime and orange with concentrations of 6.25%, 12.5%, 25%, and 50%, respectively. The sample used is carp meat. The bacteria present in the sample were counted using the Total Plate Count (TPC) method before and after lime and lime extract administration. The results showed that before administering the two extracts, the total colonies in the sample could not be counted. The antimicrobial effect of orange juice extract began to be seen at a concentration of 12.5%, while in lime, it was only seen at a concentration of 50%. From the results of this study, it was concluded that orange juice extract had a better antimicrobial effect than lime.

Keyword: Carp, extract, lime, rough lemon, total colonies

Abstrak. *Ikan sebagai salah satu bahan pangan sumber protein yang sering dikonsumsi, tak luput menjadi media pertumbuhan mikroba kontaminan yang dapat menimbulkan penyakit bawaan makanan yang menyebabkan diare hingga kematian. Mikroba kontaminan dapat dijumpai pada panganan yang dikonsumsi mentah, semisal Naniura, masakan khas Suku Batak yang berupa sajian ikan mas mentah. Berbagai rempah alami seperti jeruk nipis dan jeruk jungga memiliki potensi sebagai antimikroba. Tujuan dari penelitian ini adalah untuk mengetahui potensi antimikroba pada jeruk nipis dan jeruk jungga pada sampel ikan mas segar. Penelitian ini menggunakan ekstrak jeruk nipis dan jeruk jungga dengan konsentrasi masing-masing 6,25%, 12,5%, 25% dan 50%. Adapun sampel yang digunakan adalah daging ikan mas. Selanjutnya bakteri yang ada pada sampel dihitung dengan menggunakan metode Total Plate Count (TPC) sebelum dan sesudah pemberian ekstrak jeruk nipis dan jeruk nipis. Hasil penelitian memperlihatkan bahwa sebelum pemberian kedua ekstrak, total koloni pada sampel tidak dapat dihitung. Efek antimikroba pada ekstrak jeruk jungga mulai terlihat dari konsentrasi 12,5%, sedangkan pada jeruk nipis baru terlihat pada konsentrasi 50%. Dari hasil penelitian ini disimpulkan bahwa ekstrak jeruk jungga memiliki efek antimikroba yang lebih baik dibandingkan jeruk nipis.*

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1. Introduction

Fish is one of the food sources of protein commonly consumed in Indonesia as an archipelagic country. The Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia targets an increase in fish consumption from 56.39 kg/capita/year in 2020 to 62.50 kg/capita/year in 2024 [1]. This increase in consumption might increase foodborne diseases, mainly due to improper processing. Fish can be a growth medium for microbial contaminants such as Aeromonas hydrophila and Plesiomonas shigelloides bacteria, often found in fish and seafood [2].

The Batak people have a special dish in the form of carp served raw, called Naniura. Consumption of food like this is feared to cause food poisoning. Fish and fish products are often associated with human disease, especially when raw or undercooked fish and fish products are consumed. The presence of different bacteria species including human pathogenic bacteria in fish can be linked to direct contact with a contaminated water environment and ingestion of bacteria from sediments or contaminated feed [3]. In the research of Amelia et al. (2020), the number of colonies in fresh carp exceeds the safe limit for consumption [4]. In the Naniura food processing process, limes and oranges are also used as cooking spices. Various research results on the efficacy of this spice state that limes and oranges also have potential as antimicrobials. Hutapea, Leksono, and Sari (2019), on jelawat fish with lime, orange, and lemon treatment, found that the number of bacterial colonies was lower in the group given lime and orange juice compared to lemon [5]. Similarly, the results of the Sembiring, Sihotang, and Tampubolon research in 2019 [6] show that oranges also have an antimicrobial effect on Escherichia coli and Bacillus cereus bacteria. This antibacterial effect is moderate by using 40% orange juice extract [6].

In contrast, lime has an antimicrobial effect against the bacteria Streptococcus mutans and Lactobacillus casei. In bacteria S. mitis, S. sanguinis, S. salivarius and S. sobrinus, the antimicrobial effect was moderate. This antibacterial effect is due to the content of essential oils, secondary metabolites, and acidic pH in limes and oranges [7]. The addition of these spices can help reduce the number of microbial contaminants. This study aimed to compare the number of bacterial colonies in fresh carp given lime and orange juice extract.

2. Method

This research is an experimental study with a pretest-posttest design. The extracts used in this study were lime and orange juice extracts that had been made at the ASPETRI Medicinal Plant Research and Development Laboratory by maceration method [8]. The lime and orange juice extracts concentration was created using the dilution formula (1).

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$$M1 \times V1 = M2 \times V2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

M1 : Initial concentration (100%)

V1 : Volume of required 100% concentration solution (ml)

M2 : Desired final concentration (100%)

V2 : Volume of concentration solution (ml)

The solvent used was DMSO (Dimethyl sulfoxide) to obtain various extract concentrations, namely 6.25%, 12.5%, 25%, and 50% for either lime and orange extract.

Carp fish were bought at the market and brought in containers that have been sterilized using an autoclave beforehand. A total of 10 grams of fish meat was taken aseptically and mixed with ± 90 ml sterile water, then crushed using a blender to form a solution. Researchers used the multilevel dilution method to minimize or reduce the number of microbes suspended in the liquid. A ratio of 1: 9 is used for the sample and the first and subsequent dilutions so that the successive dilution contains 1/10 of the microorganism cells from the previous dilution. Researchers prepared 11 test tubes, each containing 9 ml of nutrient broth. As much as 1 ml of the fish meat solution was put into the first tube and stirred using a vortex to make the solution homogeneous. From the first tube, 1 ml of a solution that has been mixed using a vortex is taken and transferred to tube 2. From the 2nd tube, 1 ml of a homogeneous solution is retaken to be inserted into the third tube until the 10th tube. The 11th tube is only containing only distilled water as a negative control.

The posttest stage is carried out after the pretest stage is completed. The posttest used the same method as in the pretest. The difference in treatment lies in adding lime and orange juice extracts in various concentrations. After all the transfer processes are complete, they will then be incubated for 24 hours before being applied to Plate Count Agar to count the number of colonies on raw carp.

Total plate count is a method used for the cultivation of bacteria by using solid media, whose working principle based on homogenization of dilution series samples with multiples of 10. Calculation result with this method is in the form of Colony forming units (CFU). This CFU shows the number of colonies that grows per gram or milliliter of the sample calculated of the number of cups, dilution factor, and volume used [9]. After 24 hours, the number of colonies count manually. If the number of colonies >300 colonies, it is declared as Too Numerous Too Count (TNTC).

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3. Results and Discussion

The TPC test carried out in this study showed that the total colonies in the pretest group could not be counted (TNTC). Similar results were also obtained for lime extract with a concentration of 6.25%, 12.5%, 25%, and orange juice extract with a concentration of 6.25%. The total number of colonies that could be compared in both extracts was 50%, with the number of bacterial colonies in the orange group being less than that of lime (2.3 × 106 vs. 1.1 × 1012). The results can be seen in table 1.

Table1. The number of colonies bacteria in lime and orange extract

Treatment	The number of colonies bacteria c			
	6.25%	12.5%	25%	50%
Lime extract	TNTC	TNTC	TNTC	TNTC
Orange extract	TNTC	2.2×10^8	1.6×10^8	2.3×10^6

This study found a decrease in the number of good bacteria colonies in lime and orange juice extracts. However, even though they were given treatment in the form of extracts from lime and orange, the number of bacterial colonies in both groups still exceeded the safe limit for consumption, namely 5.0 × 105 CFU/ml [4]. The condition of the number of bacterial colonies that exceed this safe limit can cause foodborne diseases. The condition of the outbreak of fish consumption poisoning is caused by a bacterial infection (such as Salmonella sp., Clostridium sp., Shigella, and so on). Poisoning due to fish consumption accounts for about 10% of all food poisoning in the United States [10]. Fish are known to carry bacterial colonization on fish scales and gills, because the area is constantly exposed to contaminated water. Generally, the muscles and internal organs in fish are sterile places. If contaminant microbes are found in fish meat, it may be caused by weakened immunity in fish, making it is easier for contaminant microbes to grow in fish meat [11].

The effect of decreasing the number of bacterial colonies is thought to come from the active substances found in oranges and limes. Lime contains four essential oil components: limonene, linalool, citronellal, and citronellol. While oranges also have the main component of essential oil is limonene [6]. This antibacterial mechanism will generally increase the permeability of the bacterial membrane, resulting in cell membrane leakage [3]. Another study also suggested that essential oils had a better antibacterial effect against Gram-positive than Gram-negative; this was associated with the more complex structure of the cell wall of Gram-negative bacteria in the presence of an outer membrane. This structure can complicate the penetration of essential oils into bacterial cells so that the antibacterial function of essential oils is lower in Gram-negative bacteria [12].

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4. Conclusion

In processing raw carp, the use of oranges is also more effective in reducing the number of microbes compared to limes

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