Antibacterial effect of Andaliman (*Zanthoxylum acanthopodium*) against contaminant in raw common carp (*Cyprinus carpio* Linnaeus)

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Abstract. One of the well-provided fishery products in Indonesia are common carp (*Cyprinus carpio* Linnaeus). The consumption rate of common carp is highly prevalent among the people; however, improper handling of fishery product could support food-borne disease transmission. Andaliman (*Zanthoxylum acanthopodium*) is a herbal product used as an additional flavor in Batak traditional food; it has been postulated that the plant has antimicrobial properties. We conduct an experimental study using pretest-posttest design to determine the crude antimicrobial activity of Andaliman. Bacterial counting examination was determined after 24 hours incubation period in the microbiology laboratory, Department of Microbiology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. There is a descriptive decrease of bacterial colonies followed by the application of Andaliman based on total plate count test whereas the statistical analysis failed to show the significant difference. The use of Andaliman in daily life basis must be encouraged to reduce the likelihood of food-borne disease transmission.

1. Introduction

Indonesia is the largest archipelago island in the world, with an area of 3.25 million km² of water [1]. With the substantial amount of water in the country supported by its tropical climate conditions, Indonesia is rich with fishery products and resources. In Indonesia, fish consumption increased in 2016 reaching 43.9 kg per capita per year, up from 2015 and 2014, which were 41.1 kg and 37.2 kg per capita per year, respectively [2]. One of the most frequently consumed fish species by the people is common carp, as much as 2.59% in 2013 [3]. The common carp is processed traditionally in the Batak province including North Sumatera which is commonly raw-prepared. Naniura, translated to Indonesia as uncooked-fish, is a traditional type of delicacy served in Batak region using distinctive flavor. The carp will be soaked into a kaffir lime acid until the bad smell of the fish will decrease [4]. Therefore, the preparation and handling process makes this traditional delicacy mentioned as the sushi of the Batak people.
However, the threat of various pathogenic and opportunistic microorganisms in fish products can provoke bacterial transmission, particularly for food-borne disease-causing debilitating symptoms, such as diarrhea, abdominal pain, and vomiting. It is estimated that there were 600 million infected by food-borne pathogen and its associated mortality rate reaches 420,000 deaths in 2010 [5]. In a study using intestinal and gill specimen of carp as well as sediment and water in a pond farming of carp, it was discovered that the number of colonies exceeding the acceptable standard of SNI or so-called Indonesia National Standard. SNI regulates regarding the definition of fresh fishery product must not exceed 5.0CFU/g (reference number SNI 01-2729.1-2006).

Andaliman is a herbaceous plant of the family Rutaceae, genus Zanthoxylum, and species of Zanthoxylum acanthopodium. Its fruit is widely used as a spice or additional flavor for traditional delicacy by the Batak tribe [7]. There are several active substances in an andaliman plant including alkaloids, terpenoids, flavonoids, saponins, tannins, while its fruit also contains essential oils [8]. Its antimicrobial activity of andaliman fruit with different concentration of andaliman extract has been studied against three bacterial species, such as Escherichia coli, Salmonella typhi, and Staphylococcus aureus, it was proven that only 0.25% of the extract could inhibit S.aureus colonies while higher concentration, 0.5%, for E.coli and Salmonella sp. [9].

The study aimed to observe the antibacterial effect of Andaliman fruit which applied to raw common carp based on the reduction of bacterial colonies in a total plate count agar, both pre and post administration of crude andaliman.

2. Method
The study is an experimental research study designed with one group pretest-posttest which conducted in Laboratory of Microbiology, Universitas Sumatera Utara, Medan, Indonesia between May and June 2019. There were six common carps used in the study which bought from three different local markets. The material used in the study was carp, andaliman, Total Plate Count (TPC) agar, and sterile water. Blenders, aluminium foil, surgical blade, tweezers, Erlenmeyer flasks, scales, and biosafety cabinet were utilized to equip the study.

In the study, there were 10 grams of carp meat obtained from the local market in each group, pretest and posttest, followed by milling the carp using a blender and combine it with 100 ml of sterile water. The solution was then separated into eight reaction tubes, which consist of 9 ml of sterile water. Subsequently, it was mixed with the carp solution and it formed eight different concentrations of carp. Nevertheless, based on the microbiologist consideration, there were only three different concentration of carp solution applied onto six Petri dishes, 10^{-3}, 10^{-4}, and 10^{-5}. Incubation was performed at 45°C for 24 hours while two microbiologists conduct manual total plate counting to determine the quantitative number of bacterial colonies which contaminated the carp. The posttest group was applied using 10 grams of andaliman solution treated similarly as carp solution.

Counting measurement was performed by the microbiologist using established method “manual counting of bacterial colonies” which uses total plate count principle in colony forming unit per milliliter (cfu formula = number of bacterial colonies x dilution factor divided with volume of culture plate) in duplex fashion (first and second, table 1). The data of total plate count results were analyzed using SPSS (Statistical Package for The Social Sciences) ver 22. A similar group of the test was then determined its significance using T-paired whether the normal distribution of the data was then established using the Shapiro-Wilk normality test, it is plausible by the use of samples under 50 [10].

3. Results and Discussions
The normality test of Shapiro-Wilk proved its p-value over 0.05, and the normal distribution was evident. Subsequently, the data analysis of mean difference test of bacterial colonies used T-paired test preceded by the correlation test. The observation of descriptive data demonstrated a considerable reduction of bacterial colonies in both pretest-posttest groups. Baseline bacterial colonies in six
different samples were over $3 \times 10^5$; the application of andaliman using three different concentrations has diverse results, yet producing consistent results (Table 1). Statistical analysis of T-paired test failed to show the significant of mean difference of the bacterial colonies in pre- and post-test groups ($p = 0.51$) (Table 2).

Bacterial colonies in a carp categorized as high contaminant, which then ineligible to be consumed based on the SNI regulations 01-2729.1-2006 ($5.0 \times 10^5$) [11]. These findings are consistent in previous studies testing the large number of bacterial colonies in the carp of gills and intestines which found that in three samples contained considerable amount of bacterial colonies, $4.3 \pm 2.9 \times 10^6$, $1.6 \pm 3.9 \times 10^7$, $6.5 \pm 0.8 \times 10^9$ in gills while in its intestine $8.7 \pm 4.1 \times 10^9$, $5.4 \pm 3.2 \times 10^{10}$, and $1.1 \pm 2.4 \times 10^{10}$ [6].

### Table 1. Total plate count results using three different concentrations of carp and andaliman solution.

| Sample | Dilution | Pretest | Posttest | Mean Pretest | Mean Posttest |
|--------|----------|---------|----------|--------------|---------------|
| A      | $10^{-3}$| $>3$    | $>3$     | $>3$         | $>3$          |
|        | $10^{-4}$| 2.46    | 2.62     | 2.54         | 1.43          |
|        | $10^{-5}$| 0.46    | 0.3      | 0.38         | 0.35          |
| B      | $10^{-3}$| $>3$    | $>3$     | $>3$         | $>3$          |
|        | $10^{-4}$| $>3$    | 2.09     | 0.48         | 0.54          |
|        | $10^{-5}$| 0.49    | 0.21     | 0.35         | 0.07          |
| C      | $10^{-3}$| $>3$    | $>3$     | $>3$         | $>3$          |
|        | $10^{-4}$| $>3$    | $>3$     | $>3$         | $>3$          |
|        | $10^{-5}$| 1.86    | 2.66     | 2.26         | 0.56          |
| D      | $10^{-3}$| $>3$    | $>3$     | $>3$         | $>3$          |
|        | $10^{-4}$| 2.48    | 2.76     | 2.62         | 1.67          |
|        | $10^{-5}$| 2.15    | 1.57     | 1.86         | 0.75          |
| E      | $10^{-3}$| $>3$    | $>3$     | $>3$         | $>3$          |
|        | $10^{-4}$| 1.17    | 0.89     | 1.03         | 0.72          |
|        | $10^{-5}$| 0.07    | 0.13     | 0.1          | 0             |
| F      | $10^{-3}$| $>3$    | $>3$     | $>3$         | $>3$          |
|        | $10^{-4}$| $>3$    | 2.72     | $>3$         | 0.68          |
|        | $10^{-5}$| 0.64    | 0.80     | 0.72         | 0.04          |

### Table 2. Statistical analysis of bacterial analysis

| Descriptive data          | Pretest  | Posttest | $p$-value |
|---------------------------|----------|----------|-----------|
| N                         | 6        | 6        | 0.51      |
| Mean                      | 9450000.00 | 3433333.33 |           |
| Median                    | 5500000.00 | 18500000.00 |           |
| Standard Deviation        | 8949134.036 | 3724871.362 |           |

Post test group of the andaliman application in carp specimen descriptively proved fewer bacterial colonies compared to pretest group. The antibacterial properties of andaliman have been postulated caused by the active ingredients, such essential oils, as well as secondary metabolite including saponin, alkaloid, tannin, flavonoids. Essential oils are mentioned as aromatic oil, alcoholic soluble, and consisted of the combination among esters, aldehydes, ketones, and terpenes. The substances are responsible for the odor features of andaliman fruit. The mechanism of antimicrobial properties is highly dependable on the active component which has a distinct pathway to inhibit bacterial growth.
In general, the antimicrobial mechanism of essential oils is divided into three phases. First, essential oils will spread to the bacterial cell wall to increase membrane permeability; therefore, it triggers the release of cellular components. Second, it creates an acidic atmosphere in the cell by blocking the cellular energy production (ATP) due to ion loss, the failure of the proton pump, and decreased membrane potential. Third, the destruction of genetic material will initiate a cell death cascade pathway [13].

Essential oils also have the ability to agglomerate and damage the lipid structure, wall proteins, and cell membranes, causing leakage of macromolecular components and bacterial lysis [14]. Besides, essential oils have antibacterial effects on some bacteria including gram positive (Arthrobacter sp, Bacillus sp, Brevibacterium ammoniagenes, B. linens, Clostridium botulinum, C. perfringens, Corynebacterium sp, Enterococcus faecalis, Lactobacillus sp, Listeria monocytogenes, Listeria innocua, Propionibacterium acnes, Staphylococcus aureus, and Staphylococcus simulans) and negative (Acetobacter sp, Acinetobacter sp, Campylobacter jejuni, Enterobacter aerogenes, Escherichia coli O157:H7, Klebsiella pneumonia, Moraxella sp, Neisseria sp, Pseudomonas sp, P. aeruginosa, Proteus vulgaris, Salmonella sp, Vibrio parahaemolyticus, and Yersinia enterocolitica) [12].

There have been a few studies extensively unfold the secondary metabolites in andaliman fruit, for instance, alkaloids, flavonoids, saponins, and tannins. Alkaloids are a group of organic compounds that are widely produced by plants and contain nitrogenic atoms [15]. The mechanism of action of alkaloids as antibacterial via inducing the destruction of bacterial cell wall components that will finally impair the cell wall it will be killed more efficiently [16]. Additionally, saponins are one of the glycoside groups composed of sugar and non-sugar components that are produced by plants and foam-producer when it is shaken with water as solvent [15]. Saponins also have antibacterial effects through alleviating the surface tension of bacterial cell walls and disrupting membrane permeability [16].

Flavonoids contain aromatic heterocyclic rings and glycosidic, while it is commonly found in the form of pigments of various plants. Antibacterial effects of flavonoids work in three ways, the inhibition of nucleic acids synthesis, cytoplasmic membranes, and energy metabolism [17]. Tannins are a complex class of polyphenolic biomolecules produced in various types of plants that are frequently used as pesticides [18]. Inhibition of the reverse transcriptase and DNA topoisomerase enzymes so that they can suppress the process of bacterial replication is one of the antibacterial properties in tannins [19]. Tannins could also induce the inactivation of adhesin, protein transport, and cell envelope. The strong binding effect of tannins to iron also acts as an antibacterial feature which makes the availability of iron reduced, consequently, tannins will impede the availability of iron that essentially engaged in multiple metabolism pathways disrupting the life processes of bacteria [20].

4. Conclusion
Andaliman fruit has various bioactive active substances, including essential oils, alkaloids, flavonoids, saponins, and tannins. These compounds have an antimicrobial effect that finally leads to the use of andaliman in raw carp could reduce the number of bacterial colonies. Nevertheless, further research is needed to determine the accurate levels of andaliman could inhibit bacterial growth. The government could also endorse the antimicrobial effect concurrent with the tradition preservation of the andaliman use not only among bataknese people but also the general population since the delicacy could reduce bacterial colonies in the raw material.

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