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EFFECTIVITY TEST OF MORINGA LEAF EXTRACT ON *Bacillus cereus* AS FISH SPOILAGE BACTERIA AND *Staphylococcus aureus* AS COMPARISON

Winly Felicia[1], Fatimah Azzhara Lubis[1], BoykeMarthin Simbolon[1]

[1]Medicine Study Program, Faculty of Medicine, Prima Indonesia University, Medan

Corresponding Author: winlyfelicia@yahoo.co.id

ABSTRACT

This study was aimed to determine whether moringa leaf extract showed an effectivity on 25%, 50%, 75%, and 100% concentrations as an antibacterial on *Bacillus cereus* as fish spoilage and on *Staphylococcus aureus* as a comparison. *Bacillus cereus*are rod-shaped gram-positive bacteria with harmful effects, and *Staphylococcus aureus*are infectious round-shaped gram-positive bacteria. This study was conducted using an experimental method, and the samples were collected through purposive sampling. The effectivity test of moringa leaf extract on *Bacillus cereus* and *Staphylococcus aureus* was performed by diffusion using disk papers and measurement of bacterial inhibition zone on disk papers stained by leaf extract. Afterward, data analysis was performed using the Post-hoc test, which explained that there were significant differences between each group with a 95% confidence interval. One hundred percent Moringa leaf extract had higher effectivity on *Bacillus cereus* compared to *Staphylococcus aureus*. This was caused by a higher inhibition zone of 100% Moringa leaf extract on *Bacillus cereus*, which consisted of 8 mm, compared to *Staphylococcus aureus*, with 6.2 mm.

Keywords: Moringa leaf extract, *Bacillus cereus*, and *Staphylococcus aureus*.

INTRODUCTION

As an archipelagic state, Indonesia had vast storage areas for fisheries. Unfortunately, not all Indonesian people took advantage of this and consume fishes available in Indonesia due to a lack of knowledge regarding the importance of the nutrition contained in these fishes. The impact of the lack of protein consumption in Indonesia causes Indonesian people to become vulnerable to malnutrition. Fishes in Indonesia are often found fresh and very healthy. However, many fish bones and fishy smell prevents people from consuming them (1).

The rate of fish consumption in Indonesia is considered low compared to other ASEAN countries. The increase of fish consumption in Indonesia occurred from 2000 to 2010, with an increase of 29.08 kg/year to 30.48 kg/year and occurred again in 2012 to 33.89 kg/year. Meanwhile, the fish consumption rate in 2013 was 35 kg/year (1).

Fish is a high protein source. It contains many proteins, especially short fiber protein, which is better than those contained in chicken, beef, and other protein sources. Fish also contains omega 3, which has many benefits compared to other animals, due to high unsaturated fatty acid composition, easily oxidized and causes unpleasant aroma in fish. Other contents in fishes include omega, iodine, selenium, fluoride, iron, magnesium, zinc, taurine, and coenzyme Q10. The pH of fish is close to neutral; thus, spoilage bacteria easily grows in fishes (2)(3).

There are many spoilage organisms in fish, which include *Bacillus cereus*. They are gram-positive bacteria, with large cell sizes. These bacteriagrow in facultative aerobic. The bacteria often found in contaminated foods and can infect humans are called *Bacillus cereus.* They are often known as pathogenic bacteria. The findings of *Bacillus cereus* are heat-resistance spores and dehydration process (4)(5).

Moringa leaf is a wild plant that can grow anywhere, even in extreme conditions. During the dry season, moringa leaves can still grow and survive and will grow well in

high rainfall. Moringa leaf is also a plant that has been widely investigated for its nutritional content and benefits, such as anti-malignancy, anti-bacteria, and stopping the growth of fungi and bacteria. Moringa leaf also has several active substances which have benefit as anti-bacteria, including saponin, triterpenoid, and tannin, which act in arresting and inhibiting the growth and development of bacteria (2)(6).

Moringa leaf had been investigated in 2012, in which it was effective in arresting wound in the gastrointestinal tract. It can inhibit the process of lipid peroxidation due to flavonoid and essential oil (2). Moringa leaf can also be used as a storage for raw food materials and a natural preservative. This was due to the chemical substance of phenolic in Moringa leaf which inhibits oxidation of fresh meat. High bioactive components, such as ascorbic acid, carotenoid, and phenolic substance had good effectivity and mechanism in lengthening the storage duration of raw food materials (7).

METHODS

This study used laboratory experimental method followed by post-test only design. The duration needed for this study was 1 month, whereas this study began in November to December 2019. It was performed in the Microbiology Department of the University of North Sumatera. The samples were Moringa leaves obtained from Pajak 4, Amplas. The sampling technique used in this study was purposive sampling. The concentration of Moringa leaves (*Moringa oleifera*) were 25%, 50%, 75%, and 100%.

Instruments

The instruments in this study were autoclave, oven, spectrophotometer, bacteria incubator, petri dish, Erlenmeyer flask, reaction tube, hotplate, vortex, serological pipette, micropipette, and digital caliper. Meanwhile, the ingredients used in this study were NA media, MHA media, distilled water, disk papers, *Staphylococcus aureus* and *Bacillus cereus* isolates, and Moringa leaf extract.

Calculation of Solvent

The measurement of Moringa leaf solvent before drying was 6000 g. Moringa leaf powder was 500 g. The 96% alcohol volume needed was:

V = B/ (96% alcoholρ) = (6000 g)/(0.884 g/ml) = 6787 ml
75 parts solvent: 75/100 x 6787 ml = 5090 ml
25 parts solvent: 25/100 x 6787 ml = 1696 ml

The Making of Moringa Leaf Extract

The making of Moringa leaf extract in this study was as follows:

1. The Moringa leaves were measured to 500 g and poured into a vessel. 75 parts solvent (5090 ml) was poured into the vessel.
2. Close the vessel, wait until the total became 5 days and avoid sunlight while stirred several times.
3. After 5 days, the residue was rinsed with the rest 25 parts solvent to obtain 1696 ml.
4. Afterward, the macerate was placed for 2 days and poured to a vessel. The macerate was then evaporated using a rotary evaporator (60^oC – 65^oC)until a thick Moringa leaf extract was obtained (8).

Working Procedure

Instrument Sterilization

Before the procedure, the instruments were cleaned and sterilized to obtain pathogen-free and sterile instruments for an anti-bacterial test. The instruments were wrapped in paper and sterilized at 121^oC in an autoclave with 15 psi pressure and 15 minutes duration (9).

The Making of Agar Media

1. Nutrient Agar media (NA) acted as a growth medium for bacterial isolates. 28 g of media powder was mixed with 1 L distilled water.
2. Mueller-Hinton agar media (MHA) acted as a medium for antimicrobial ability test. 38 g media powder was dissolved in 1 L distilled water.

3. The media were mixed in an Erlenmeyer flask and heated on a hotplate to obtain a homogenous solution.
4. A total of 5 mL NA media was obtained using a serological pipette and moved to a reaction tube.
5. NA media, NHA media, disk paper, distilled water, and cotton swabs were sterilized in an autoclave at 121^{o}C, 15 psi pressure, and 15 minutes duration. Meanwhile, the sterilization of the petri dish was performed in the oven at 180^{o}C and 2 hours duration.

Antimicrobial Test of Moringa Leaf Extract

1. Sterile NA media in the reaction tube, which was still in liquid form, was tilted.
2. Pure isolates of *Staphylococcus aureus* and *Bacillus cereus* were inoculated in solid form tilted sterile NA media in the reaction tube using an aseptic method on a round loop.
3. Media were incubated less than 24 hours in an incubator.
4. Twenty-four hours of *Staphylococcus aureus* and *Bacillus cereus* culture were swabbed using inoculation loop and inoculated to sterile distilled water.
5. The suspension was homogenized using a vortex and the absorbance was measured until OD = 0.5 at 600 nm wavelength.
6. Sterile MHA media was heated on a hotplate to melt and mixed into a sterile petri dish using an aseptic method.
7. The instrument used for the bacterial suspension was a sterile cotton swab, which was smeared on the surface of solidified sterile MHA media.
8. A 6 mm sterile disk paper was moved using an aseptic method on the media surface and Moringa leaf extract was dropped onto it using a micropipette. The media were incubated for 24 hours, and the diameter of the clear zone found around the disk paper was calculated using a digital caliper.

RESULTS AND DISCUSSION

Results

The data in this study were collected and recorded in the Microbiology Department of the University of North Sumatera. Moringa leaf extract was divided into 4 concentrations (25%, 50%, 75%, and 100%). Then, its effectivity on *Bacillus cereus* was tested with *Staphylococcus aureus* as a comparison.

Inhibition Zone Diameter of Moringa Leaf Extract (*Moringa oleifera*) on *Bacillus cereus.*

The inhibitory power obtained was the appearance of a clear zone on disk paper filled with several concentrations of Moringa leaf extract. Afterward, inhibition zone power was conducted using digital caliper vertically and horizontally.

Figure and Table 1 revealed different results. EDK 25% revealed that the largest zone was in the second treatment, with a 6.8 mm diameter and an average of 6.6 mm diameter. EDK 50% showed that the largest zone was in the third treatment, with 6.8 mm and an average of 6.6 mm. EDK 75% had the largest zone in the second treatment, with 7.4 mm and an average of 6.9 mm. EDK 100% had the largest zone in the second treatment, with an 8 mm diameter and an average of 7.5 mm.

This showed that the higher the concentration of Moringa leaf extract, the higher the anti-bacterial effectivity, which was proven by the inhibition zone diameter.

Inhibition Zone Diameter of Moringa Leaf Extract (*Moringa oleifera*) on *Staphylococcus aureus as a comparison.*

The inhibitory power obtained was the appearance of a clear zone on disk paper filled with several concentrations of Moringa leaf extract.Afterward, inhibition zone power was conducted using digital caliper vertically and horizontally.

According to Figure and Table 2, EDK 25%, 50%, and 75% showed no effectivity in inhibiting the growth of *Staphylococcus aureus*. However, EDK 100% had the largest zone in the first treatment with a 6.5 mm diameter and an average of 6.2 mm. An analysis should be conducted regarding why Moringa leaf extract was less effective in inhibiting *Staphylococcus aureus.*

Discussion

This study concludes that there was an effectivity of Moringa leaf extract on *Bacillus cereus* as fish spoilage bacteria. The higher the concentration of Moringa leaf extract, the higher the inhibitory power on *Bacillus cereus*.

This result may be caused by the anti-bacterial substances contained in Moringa leaf, which include saponin, triterpenoid, and tannin, which damaged the bacterial cell membrane. The essential oil contained in the Moringa leaf contains a phenol derivative, which was the hydroxyl group and carbonyl group, which can be used as anti-bacterial. It can interact with the bacterial cell wall structure, which was absorbed and penetrated to bacterial cells and caused protein precipitation and denaturation, which lead to bacterial cell membrane lysis (10).

According to the inhibition zone classification by Greenwood, Table 3.7 showed that the effectivity of Moringa leaf extract on *Bacillus cereus* was considered less effective because the strongest inhibition zone at 100% concentration was 8 mm in the second treatment, with < 10 mm. Meanwhile, Moringa leaf extract with 25%, 50%, and 75% did not show effectivity on *Staphylococcus aureus* (0 mm) and considered less effective due to < 10 mm inhibition zone.

This study was not in line with Mukriani *et al*., 2015 (12), who investigated Moringa leaf ethanol extract with 20%, 40%, 60%, and 80% concentrations on Mueller Hinton Agar (MHA) media, which stated that Moringa leaf ethanol extract had inhibitory power on *Staphylococcus aureus* with 14.02 mm at 80% concentration, 12.03 mm at 60%, 9.00 mm at 40%, and 7.98 mm at 20%. All these results were considered weak according to inhibitory zone classification by Greenwood. This study showed that the effectivity of Moringa leaf extract on *Bacillus cereus* and *Staphylococcus aureus* was considered less effective.

Contrary to Maulidi *et al*., 2020 (13), in his study on 25%, 50%, 75%, and 100% concentrations on Nutrient Agar (NA) media, the extract of turmeric rhizome had better inhibitory power on *Bacillus cereus* compared to the Moringa leaf extract used in this study, in which 25% concentration produced 9.4 mm zone, 50% concentration produced 9.8 mm, 75% produced 10.6 mm, and 100% produced 11.1 mm. All these results were considered weak according to the inhibitory power classification by Greenwood.

The possible cause of ineffective inhibitory power of Moringa leaf extract in inhibiting *Bacillus cereus*could be that the sample was too watery, thus the concentration obtained was not exactly as expected (14).

CONCLUSION

Moringa leaf extract had ineffective inhibitory power on *Bacillus cereus*, which can be seen from the inhibition zone diameter produced at 8 mm, whereas considered less effective according to Greenwood's classification. Moringa leaf extract also showed ineffective inhibitory power on *Staphylococcus aureus* as a comparison, which can be seen from the resulting inhibition zone of 6.5 mm, which was considered less effective by Greenwood's classification. The analysis results using One-way ANOVA followed by the Post-hoc test showed P-value = 0.023, which means that $P < \alpha$, where $\alpha < 0.05$. Thus, Ha was supported, and Ho was rejected. There was an effectivity of Moringa leaf extract on *Bacillus cereus* as fish spoilage bacteria and *Staphylococcus aureus* as a comparison.

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