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Reduction of Escherichia coli Contamination in Vannameii Shrimp (*Litopenaeus vannamei*) using Cassava Leaf Extract (*Manihot glaziovii*) and Noni Fruit (*Morinda citrifolia* L.)

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ABSTRACT

This research is to study the inhibition power of rubber cassava (Manihot glaziovii) and noni (Morinda citrifolia L.) leaves extract in reducing Escherichia coli contamination in vannamei shrimps (Litopenaeus vannamei). The research uses a completely randomized block design experimental design with three replications. The treatments in this study were M0S0 (control), M1S1 (25:0), M2S2 (20:5), M3S3 (15:10), M4S4 (10:15), M5S5 (5:20), M6S6 (0:25). The observations included amount Escherichia Coli, antimicrobial inhibition, Escherichia coli total decrease, antimicrobial activity application, pH, and sensory testing (color and appearance). Data were tested with the Barlett test and the Tuckey test to see the homogeneity. The data analysis was carried out using ANOVA to see the effect of treatment on the parameters being observed and followed with an LSD test. The results of this research showed that the extract of noni was more effective in inhibiting the contamination of Escherichia coli in vannamei shrimps with an inhibition zone of 7.23 mm compared to cassava leaves with an inhibition zone of 0.43 mm. The best formulation of cassava and noni leaves was 25:0 with microbial activity of 7.23 mm (medium categorize).

1. INTRODUCTION

Lysine, histidine, arginine, tyrosine, tryptophan, and cysteine are examples of essential amino acids found in shrimp meat. Shrimp have high demand and selling power, not only delicious, but the nutrition contained in shrimp is also high. Vannamei shrimp is a shrimp that is mostly produced for export. At present, the demands from consumers regarding the quality of food ingredients are increasing so it is necessary to prioritize food quality and safety. Apart from the use of harvesting machines (Maulaya & Herodian, 2017; Arista *et al*., 2013) processing management also determines the quality of the shrimp. One of the causes of decreased shrimp quality is the result of poor shrimp handling during harvest (Munadi & Datulinggi, 2017).

Shrimp cannot be stored for a long time because they are easily damaged and easily experience a decrease in quality. Decreased quality is associated with black spots or melanosis and microbial decay. This happens due to bacterial activity, chemical reactions, and enzyme activity which is commonly known as an autolysis reaction (Suwetja, 2011).

This decrease in the quality of vanameii shrimp is related to shrimp damage caused by totally contaminating microbes and bacteria. Escherichia coli is a bacterium that is known to often infect the digestive tract. Microbial contamination in food can cause infection in the digestive tract, resulting in the death of those who consume it (Rohmah *et al.,* 2018). Based on surveys, many ingredients that contain high protein are easily exposed to microbes such as *Salmonella sp.* contamination in broiler chickens (Sartika *et al*., 2016a), *E. coli* in tuna (Puri *et al*., 2016); *E. coli* in chicken meat (Sartika *et al*., 2016b); and contamination of pathogenic bacteria in processed fish products (Sartika *et al*., 2020). Shrimp has a high protein content so it is suspected that it is also easily exposed to microbes.

Decreasing the quality of shrimp can be prevented by inhibiting the development of spoilage microbes. A storage environment that is not optimal will accelerate the growth of spoilage microbes in shrimp. The treatment of adding spices is one effort that can be done to prevent the growth of spoilage bacteria (Tirtaningrum *et al*., 2014). Reducing food contaminating microbes can be done in natural ways such as using phages to reduce *Salmonella sp.* (Sartika *et al*., 2012), banana peel and flower extracts in reducing *E. coli* (Sartika *et al*., 2019a), extract of red dragon fruit peel in reducing *E. coli* and *Salmonella* contamination (Sartika *et al*., 2019b), and cassava peel extract in reducing *E. coli* and *Salmonella* contamination (Sartika *et al*., 2021).

Flavonoids, saponins, and tannins are compounds found in cassava leaves and have been shown to have antimicrobial activity (Syaifullah, 2019). Meanwhile, anthraquinones and scopoletin are compounds from noni roots and fruits that also have antimicrobial or antibacterial activity (Sindora *et al*., 2017). This study aimed to determine the antimicrobial activity of rubber cassava leaves and noni fruit in reducing *E. coli* contamination in shrimp.

2. RESEARCH METHOD

2.1. Materials and Tools

The materials used in this study were rubber cassava leaves (*Manihot glaziovii*) and noni fruit (*M. citrifolia* L.), vannamei shrimp (*Litopenaeus vannameii*), 70% alcohol, distilled water, aluminum foil, cotton, H_2SO_4, $BaCl_2$, ethanol 96%, Na_2CO_3, folin-ciocalteu reagent, physiological NaCl, *Escherichia coli* culture, Eosin Methylene Blue (EMB), Nutrient Agar (Oxoid), Buffer Pepton Water (Oxoid), filter paper, disc paper, and Nutrient Broth (Merck).

In this study, the tools used included pH meters, analytical balances, ovens, calipers, Petri dishes, spectrophotometers, vortexes, autoclaves, incubators, Erlenmeyer, and other laboratory equipment.

This research was started by extracting rubber cassava leaves and noni fruit separately. The extraction procedure was carried out based on the following steps: 1) selection of raw materials for cassava and noni fruit; 2) drying; 3) manufacture of cassava powder and noni fruit; 4) extraction of cassava and noni fruit using 96% ethanol for 24 hours; 5) Evaporation of cassava and noni fruit extracts; 6) Cassava leaf extract and noni fruit are ready to use.

2.2. Design of Experiment

The experimental design was a non-factorial Completely Randomized Block Design which was carried out with three replications. The formulation for each treatment was 25% (w/v) of the solvent. The data obtained was analyzed with the Barlett test to determine the homogeneity of the data. Data were analyzed using ANOVA to determine the effect of treatment on the observed parameters. The data was tested with the Tuckey test at α = 5%.

Table 1. Experimental formulation design

Treatment Code	Noni Fruit (M)	Rubber Cassava Leave (S)
M0S0	0 (control)	0 (control)
M1S1	25	0
M2S2	20	5
M3S3	15	10
M4S4	10	15
M5S5	5	20
M6S6	0	25

2.2. Oservation

Observations made in this study include:

a) Total *Escherichia coli*

This test was carried out by counting the number of colonies that grew on media that had previously been sterilized and then inoculated with bacteria. First, prepare 5 g of vanameii shrimp samples and put them in an Erlenmeyer containing 45 ml of Buffer Pepton Water (BPW). Then homogenized and diluted up to 10^{-5}. After dilution, 1 ml of sample was taken and poured into a sterile petri dish. Then poured Eosin Methylene Blue (EMB) media and incubated at 37 °C for 24 h. After the incubation was complete, the *Escherichia coli* growth was calculated on the media.

b) Antimicrobial Inhibitory (AI)

This test was carried out using the paper disc diffusion method (Ainurrochmah *et al.*, 2013). The inhibition test was carried out to determine whether there was inhibition of microbial growth activity from noni fruit extract and rubber cassava leaves. The first stage of this test is to make nutrien agar (NA) media. The NA media that has been sterilized is used as a place to culture of *Escherichia coli* bacteria. After that, 0.1 ml of *Eschericia coli* suspension was inoculated on the surface of the Nutrient Agar medium using the spread plate method and leveled using a loop (ose) L needle. Then the disc paper was placed which had been soaked in each extract (according to treatment) for ±30 min on the surface. media. Then incubated for 24 h in an incubator at 37 °C, then the inhibition area formed was measured using a caliper.

c) Total Reduction of *Escherichia coli*

Five grams of vannamei shrimp was added with 1 ml of *Eschericia coli* and 1 ml of extract (according to treatment), then put in 100 ml Erlenmeyer. Then it was homogenized and added 45 ml of BPW solution (a). Take 1 ml of solution (a), then

add 45 ml of BWP, do it up to 6 times to get a 10^{-6} dilution. Take 1 ml and poured into a sterile petri dish. Then poured Eosin Methylene Blue (EMB) media and incubated in an incubator at 37 °C for 24 h. After that, the total *Escherichia coli* bacteria that grew was counted.

d) Antimicrobial activity

As much as 75 g of vannamei shrimp were cleaned, then soaked with extracts from the best treatment in the total Eschericia coli reduction test. Soaking was carried out for 20 min with a ratio of vannamei shrimp and filtrate, namely 1:1.25 (w/v). From 75 g of vannamei shrimp, 5 g were taken and inoculated with 1 ml of Eschericia coli on the surface of the carcass and allowed to stand for 10 min for the absorption of the test bacteria into the carcass. Shrimp carcasses soaked in distilled water and inoculated with Escherichia coli were used as controls. The number of Eschericia coli bacteria was calculated based on Lay (1994). The natural antimicrobial activity of the extract in the best treatment was classified as effective if it reduced total Escherichia coli by more than 50% (Ragine *et al.,* 2010).

e) Degree of Acidity (pH)

The degree of acidity (pH) was measured by soaking 5 g of vannamei shrimp samples in 12.5 ml of the extract according to the treatment for 10 min. The vanameii shrimp sample was then mashed using a mortar. After that, 25 ml of distilled water was added as a dilution. The pH value is determined using a pH meter

f) Sensory Testing (Color and Appearance).

Sensory testing which includes color and appearance was carried out only on the treatment which showed the highest reduction in total Eschericia coli. The sensory test used was a scoring test involving 15 panelists consisting of students from the Department of Agricultural Product Technology, University of Lampung. The initial stage in this sensory test was to soak the vannamei shrimp in the best treatment extract for 10 minutes, then drain for 5 min and make observations. The vanamei shrimp scoring test questionnaire can be seen in Figure 1.

Quisioner Scoring Test for Vannamei Shrimp

Panelist:..... Date :.....

In front of you, there are 2 samples of vannamei shrimp. You are asked to provide an assessment of color and appearance by giving a score on a scale of 1-5 for each of these samples.

Parameter	Sample Code	
	347	589
Color		
Appearance		

Color score :
1. Very dark
2. Dark
3. Slightly bright
4. Bright
5. Very bright

Appearance score:
1. Very bad
2. Less Good
3. Fairly Good
4. Good
5. Very good

Figure 1. Questionaire of scoring test for vannamei shrimp

3. RESULTS AND DISCUSSION

3.1. Extract Characteristics

The results showed that noni fruit extract has a blackish brown color, coarse powder texture (2-5 mm in size), and has a very distinctive aroma. Meanwhile, cassava leaf powder has a brownish-green color, a rather fine texture, and a slightly distinctive aroma. The discoloration produced by the noni fruit is due to the loss of water in the noni fruit. The color change in rubber cassava leaf extract occurs due to heat during oven which causes degradation of green leaf chlorophyll to brown pheophytin (Fitria *et al*., 2017). Noni fruit extract has a distinctive noni aroma and dark brown color, while cassava leaf extract has a characteristic aroma of cassava leaves and a dark brown color.

3.2. Total *Escherichia coli* on Vannamei Shrimp

The results of total Escherichia coli testing on vannamei shrimp showed that there was Escherichia coli contamination. Salmonella and E. coli are commonly found in aquatic environments (Jang *et al*., 2017). According to Rohmah *et al*. (2018) the cause of the presence of pathogenic bacteria in foodstuffs shows a lack of sanitation and hygiene in handling and processing. The results of the survey for *Escherichia coli* on vannamei shrimp revealed a total colony of 1.5×10^7 CFU/g. The limit of microbial contamination in fresh fish, the content of *Escherichia coli* in fresh fish should be < 3/g (BSN, 2009). *Escherichia coli* is a bacteria commonly found in the intestines that can cause diarrhea.

3.3. Effect of Extract on *Escherichia coli*

Table 2 summarizes the effect of extract addition on the *E. coli* found in the vannamei shrimp. The extract performance is discussed in the following sections.

Table 2. The effect of adding noni fruit and rubber cassava leaves extract on *Escherichia coli*.

Treatment	AI (mm)	Total colony (CFU/g)	pH	Color score	Appearance score
M1S1	7.263±0.027 ^{a}	0	6.533±0.153 ^{a}	3.467±0.291 ^{ab}	3.311±0.168 ^{ab}
M2S2	7.000±0.018 ^{b}	0	6.800±0.265 ^{a}	3.356±0.234 ^{abc}	3.267±0.353 ^{ab}
M3S3	2.640±0.003 ^{c}	0	7.267±0.208 ^{a}	3.044±0.379 ^{bc}	3.044±0.252 ^{bc}
M4S4	1.387±0.019 ^{e}	0	7.470±0.100 ^{a}	3.044±0.336 ^{bc}	3.000±0.267 ^{bc}
M5S5	1.150±0.006 ^{d}	0	7.567±0.208 ^{a}	3.000±0.176 ^{c}	2.867±0.115 ^{c}
M6S6	0.440±0.005 ^{f}	0	7.630±0.100 ^{a}	2.267±0.267 ^{d}	2.733±0.115 ^{c}
M0S0	0.000±0.000 ^{g}	3.1×10^7	7.653±0.025 ^{a}	3.600±0.067 ^{a}	3.622±0.038 ^{a}

Note: Numbers followed by different letters in the same column means significantly different ($P<0.05$)

3.3.1. Antimicrobial Inhibitory

The results of testing the inhibition of *Escherichia coli* bacteria with the addition of a mixture of noni fruit and rubber cassava leaves can be seen in Table 3. It can be seen that the zone of inhibition of *E. coli* bacteria began to form when the mixture of noni fruit and rubber cassava leaves was added. The results of the analysis of variance showed that the combination of extracts had a very significant effect on the inhibition of *E. coli* bacteria. The largest inhibition zone for *E. coli* bacteria was M1S1 (7.23 mm), then M2S2 (7.00 mm), M3S3 (2.63 mm), M5S5 (1.37 mm), M4S4 (1.10 mm), and the smallest was M6S6 (0.43 mm).

The Tukey test at the 5% level showed that the addition of a mixture of noni fruit extract and rubber cassava leaves was significantly different in each treatment. In the dishes that were given vannamei shrimp extract and *E. coli* bacteria, it was seen that the M1S1 treatment formed a clear zone that was larger than the other 6 treatments, namely M0S0, M2S2, M3S3, M4S4, M5S5, and M6S6. In the M1S1 treatment, namely only water without combined extracts, there was no inhibition. Water is a compound composed of H_2O molecules. Physically, water is clear, odorless and tasteless. Chemically, water has a neutral pH and does not contain chemicals. This shows that the combination of extracts has the opportunity to become an antimicrobial that can reduce *E. coli* bacteria in vannamei shrimp.

3.3.2. Reduction in Total *Escherichia coli*

The results showed that the combination of noni fruit extract and rubber cassava leaves could reduce the total *Escherichia coli* bacteria in vannamei shrimp. The results showed that all treatment combinations reduced *Escherichia coli* bacteria in shrimp (Table 2). Meanwhile vannamei shrimp soaked with distilled water alone could not experience a decrease in total *Escherichia coli* bacteria. The results of this study indicate that giving a combination of noni fruit extract and rubber cassava leaves can reduce total microbes, which means that there is a synergy between noni fruit extract and rubber cassava leaf extract in inhibiting the growth of *Escherichia coli* bacteria. Based on research conducted by Hasyim *et al*. (2016) showed that the simplicia extract of cassava leaves (*Manihot esculenta* Crantz) contained 30.70 mg Gallic Acid eq/g phenolic in the methanol extract, and showed the presence of a total flavonoid of 881.33 mg Retinol eq/gram in the extract using methanol.

3.3.3. Degree of Acidity (pH) of Vannamei Shrimp

The addition of a combination of noni fruit extract and rubber cassava leaves did not affect the pH of the vannamei shrimp. The degree of acidity (pH) of vannamei shrimp in this study ranged from 6.5 to 7.63, which tended to be neutral (Table 2). The results of the analysis of variance of the combination of extracts had no significant effect on the pH of vannamei shrimp. The Tukey test (a = 0.05) regarding the degree of acidity of the vannamei shrimp showed that the pH of the vannamei shrimp without treatment (M0S0) was not significantly different from the shrimp that had been treated. The results of this study indicate that even though it is not significant, the addition of a combination of extracts with more and more formulations of rubber cassava leaf extract tends to cause an increase in pH. The M4S4, M5S5 and M6S6 treatments had the same pH as M0S0 (aquadest), which tended to be neutral. The insignificant change in pH of the vannamei shrimp was probably due to soaking in the extract for only 10 min.

3.4. Sensory Test (Color and Appearance)

3.4.1. Vannamei Shrimp Color

Factors that indicate the quality of shrimp are color, aroma, texture and appearance. According to Gustina *et al*. (2015) fresh shrimp have a bright white color. The results of this study showed that the color of the vannamei shrimp ranged from 2.27 – 3.6 (dark to slightly light) which can be seen in Table 2. ANOVA analysis of the combination of extracts on the vannamei shrimp had a very significant effect on the color of the vannamei shrimp. The results of the 5% BNT test of vannamei shrimp color given a combination of extracts showed that the M0S0 treatment was significantly different

from M3S3, M4S4, M5S5 and M6S6 but not significantly different from M1S1, M2S2. The treatment of M6S6 was significantly different from M1S1, M2S2, M3S3, M4S4, M5S5 and M6S6. Treatment of M0S0 (without the addition of combined extracts) and M1S1, M2S2, M3S3, M4S4, and M5S5 (with the addition of combined extracts) produced the color of vannamei shrimp respectively 3.6; 3.47; 3.35; 3.04; 3.04; 3.00 (rather bright) while M6S6 (only rubber cassava leaf extract) produces a color of 2.27 (dark). This is due to the shrimp that are obtained not from the pond directly so that the color is not as bright as fresh shrimp after being harvested. The addition of a combination extract from noni fruit and rubber cassava leaves changed the color of the vannamei shrimp. According to research by Syaifullah (2019) the color of tuna meat has changed to slightly brown due to the color of the ethanol extract of cassava leaves. Meanwhile, noni fruit does not affect the color of the product.

3.4.2. Vannameii Shrimp Appearance Score

Appearance is important because it is the characteristic that is assessed first when using a product. A good appearance will direct the panelists to assess other things such as taste, smell and texture. Indeed, Appearance does not absolutely determine the sensory level but is related to acceptance from consumers. According to Sartika *et al.* (2020) the first impression felt by consumers when they see a product is usually through the appearance or appearance of the product and usually products that have an attractive appearance will attract more consumers' attention. The appearance of vannamei shrimp in this study ranged from 2.73 to 3.62 (slightly good to good). The results of the sighting of vannamei shrimp can be seen in Table 2.

The results of the analysis of variance showed that the addition of the combined extract had a significant effect on the appearance of vannamei shrimp. The results of this study showed that the appearance of vannamei shrimp treated M0S0 was not significantly different from M1S1 and M2S2 but significantly different from M3S3, M4S4, M5S5 and M6S6. The treatment of M1S1 was significantly different from M5S5 and M6S6 in the appearance of vannamei shrimp. The appearance assessment of the vannamei shrimp is the same as the color assessment of the vannamei shrimp. This is because appearance and color are one of the parameters to determine consumer acceptance. In general, panelists assess the appearance based on the color of the product (Capule & Barcelon, 2014). The color of the vannamei shrimp that had been given the combination extract M1S1 to M4S4 had a bright yellow color compared to the color of the original combination extract which was yellow to brown. This is because the color of the combined extract is absorbed by the white vannamei shrimp, thus fading the original color of the extract itself. The five sensory senses will affect the sensory assessment of the other parameters.

The appearance with the lowest score was vannamei shrimp with the M6S6 treatment with a value of 2.73 (slightly good) and the highest score with the M4S4 treatment with a value of 3.62 (good). This is also the same as the color parameter, namely the lowest value is the vannamei shrimp with the M6S6 treatment and the highest value is with the M4S4 treatment.

4. CONCLUSION AND SUGGESTION

Based on the results and discussion, the conclusions drawn from this research are that noni fruit extract (*Morinda citrifolia* L.) is more effective in inhibiting *Escherichia coli* bacterial contamination in vannamei shrimp with an inhibition zone of 7.23 mm compared to rubber cassava leaves (*Manihot glaziovii*) with an inhibition zone of 0.43

mm. The best formulation of rubber cassava leaf and noni fruit extracts is noni fruit (25%) and rubber cassava leaves (0%) that can inhibit *Escherichia coli* contamination in vannamei shrimp with an inhibition zone of 7.23 mm. Therefore, it is suggested to use natural antimicrobials from noni fruit without a mixture of cassava leaves, rubber is more recommended because it can inhibit *Escherichia coli* contamination with a greater percentage or by replacing other types of cassava leaves. In addition, further research is needed regarding rubber cassava leaves which are less effective in their use.

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