**KAJIAN PENGENDALIAN CEMARAN *Salmonella sp*. PADA UDANG PUTIH (*Litopenaeusvannamei*) MENGGUNAKAN ANTIMIKROBA ALAMI DARI BUAH TOMAT CHERRY (*Lycopersicumcerasiformae Mill.)***

**(***STUDY CONTROL OF Salmonella Sp.CONTAMINATION.ON WHITE SHRIMP (Litopenaeusvannamei) USING NATURAL ANTIMICROBIAL FROM EXTRACT OF CHERRY TOMATOES FRUITS (Lycopersicumcerasiformae Mill.)*

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**ABSTRACT**

***STUDY CONTROL OF Salmonella Sp. CONTAMINATION. ON WHITE SHRIMP (Litopenaeusvannamei) USING NATURAL ANTIMICROBIAL FROM EXTRACT OF CHERRY TOMATOES FRUITS (Lycopersicumcerasiformae Mill.***

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White shrimp (*Litopenaeusvannamei*) is one of the most widely produced of fishery commodities in Indonesia. The white shrimp’s production in Indonesia is increasing every year, but the exports of it face obstacles and rejection, it is caused by the contamination of *Salmonella sp*.One of the alternative ingredients which is safe and natural used to reduce contamination of *Salmonella sp.* on white shrimp besides using antibiotics is extracts of cherry tomatoes fruit and leaves. This study aims (1) to find out the inhibitory power extracts of cherry tomato fruits on the contamination of*Salmonella sp*. on white shrimp (*Litopenaeusvannamei*), (2) to determine the best concentration for the extract of cherry tomatoes fruits in the inhibition of the contamination of *Salmonella sp*. on white shrimp (*Litopenaeusvannamei*).

The research design was RAKL using single factor and six repetitions. The data were analyzed by variance analyzed and the Smallest Differential Test (BNT) at the level α 5%. The results showed that extracts of cherry tomatoes fruits has inhibitory effect against the contamination of *Salmonella sp.* on white shirmp. Tomato fruits extract is able to inhibit the growth of *Salmonella sp*. with the diameter of the inhibitory area 17.29 mm with strong antimicrobial activity. The best extract of cherry tomatoes fruits concentration on decreasing the contamination *Salmonella sp*. on white shrimp is 100% for each. Extracts of cherry tomatoes fruits could reduce the contamination of *Salmonella sp*. on white shrimp, with total decrease by cherry tomatoes extract is 2,66 x 107 CFU/ml (97,06%).

Keywords: Antimicrobial, Salmonella sp, Antibiotic, Inhibitory, Extract of Cherry

Tomatoes Fruits, White Shrimp, Contamination, Smallest

 Differential Test

1. **INTRODUCTION**

**1.1. Background**

White shrimp (*Litopenaeusvannamei*) is one of the most widely produced of fishery commodities Indonesia. According to the Data Center of Statistical and Information (2014), national shrimp production has increased an average of 23% per year. White shrimps or vannamei shrimps have increased by 20% per year. This is because Indonesia has the potential of marine aquaculture of 2 million ha and brackish cultivation (pond) reaches 913,000 ha, one of which is the potential of white shrimp farming (Lasabuda, 2013). According to SlametSoebjakto, Director General of Aquaculture Ministry of Marine Affairs and Fisheries (KKP), national shrimp production in 2016 amounted to 535,237 tons. This amount includes 392,513 tons of white shrimp, 127,908 tons of windu shrimp and 14,816 tons of other shrimps (Agrina, 2016).

Increased production of shrimp is not offset by an increase in shrimp exports to export destination countries. Export destination countries include the United States, Japan, China and Australia. According to the Data Center of Statistical and Information (2016), demand for shrimp exports in Indonesia to various export destinations has decreased. This is seen in the export demand to China in 2013 of 5,600.1 tons fell to 5,531 tons in 2014. Demand for shrimp exports to Japan in 2013 amounted to 32943.7 tons fell to 27,597.8 tons in 2014. The United States in 2009 Imports of 45,213.6 tons fell to 43,560.9 tons in 2010. Australia did the same, namely demand in 2013 of 895.8 tons fell to 780.7 tons in 2014.

The decline in the number of shrimp exports to various destination countries such as China, Japan, the US and Australia because the shrimp do not meet the quality standards of consumer countries such as the requirement for the negative *Salmonella sp*. terms of quality and safety of fresh shrimp by the Indonesian National Standard (SNI) 01-2728.1-2006 namely Escherichia coli bacterial contamination maximum <2 APM / g, Vibrio cholerae is negative, and *Salmonella* is negative in units APM / 25 gram. The shrimp were exported allegedly still contain pathogens, antibiotics and preservatives. Shrimp should be free of pathogenic bacteria such as *Salmonella sp*. and Vibrio cholera (National Standardization Body, 2006). The decline in shrimp exports has an impact on the country's economy and an obstacle to the marketing of Indonesian shrimp export destination country. Noviani (2013), states that the decline in shrimp exports affect marine and fisheries ministry targets to increase in shrimp production and most will be devoted to export products to various countries.

Shrimp export from Indonesia a lot of rejection because in general is still contaminated with Salmonella sp. In 2012, the United States refused 181 fishery products from Indonesia due to polluted Salmonella sp. (Supriadi, 2012). The Food and Drug Administration (FDA) in July 2013 rejected 5 white shrimp lots from Indonesia because export shrimp was contaminated with *Salmonella sp*. (Maas, 2013). *Salmonella* contamination sp. in *vannamei* shrimp may cause a reduction in the quality of shrimp. *Salmonella* contamination sp. in food can cause Salmonellosis which can cause serious infections in humans and weaken the immune system of children, elderly and pregnant women (Anjung, 2016). According to Sorrels et al. (1970) in Isyana (2012), *Salmonella sp.*is causing disease of digestive organs. People with salmonellosis may show symptoms such as diarrhea, nausea, headache, and fever.

*Salmonella sp.* contamination in white shrimp can be derived using antibiotics such as chloramphenicol and nitrofuran. The Food Drug and Administration has determined that imported commodities, including shrimp, are prohibited from foreign bodies and the use of prohibited or exceeding maximum limits such as antibiotics. It is also stipulated in SNI 01-2728.1-2006 that chemical contaminants such as chloramphenicol and nitrofuran are the maximum 0 in units of μg / kg. The use of antibiotics can have a serious impact because the problem of residual antibiotic ingredients in shrimp can lead to the emergence of bacterial resistance to antibiotics (Muliani and Atmomarsono, 2010). Residues of antibiotics found in shrimp consumed can adversely affect consumer conditions such as causing allergic reactions or retention, physiological disorders and poisoning (Wibowo et al., 2010).

Natural ingredients are needed to reduce contamination of *Salmonella sp.* as a natural antimicrobial. Natural antimicrobials are thought to be extracted from cherry tomato fruit. According to research Kartikasari (2008) showed that tomatoes contain compounds alkaloids and saponins. National tomato production in 2015 was 915,987 tons, while tomato production in Lampung province was 244,900 tons (BadanPusatStatistik, 2017). The research of the utilization of cherry tomato fruit and leaves (Lycopersicumcerasiforme Mill.) as an antimicrobial to decrease contamination of Salmonella sp. is not much done yet. This research needs to be done because to know the existence of antimicrobial from tomato on *Salmonella sp.* on white shrimp that can be used instead of antibiotics. This research needs to be done because to get the best concentration that can be used as the natural antimicrobial to decrease contamination of *Salmonella sp.* on white shrimp.

**1.2. Research Purposes**

The purposes of this research are:

1. to find out the inhibitory power extracts of cherry tomato fruits on the contamination of Salmonella sp. on white shrimp (Litopenaeusvannamei)

2. to determine the best concentration for the extract of cherry tomatoes fruits in the inhibition of the contamination of Salmonella sp. on white shrimp (Litopenaeusvannamei).

1. **RESEARCH METHODS**

**3.1. Place and time**

This research was conducted in Laboratory of Microbiology of Agricultural Products and Agricultural Processing Farming Laboratory, Department of Agricultural Product Technology Faculty of Agriculture, University of Lampung in January - March 2017.

**3.2. Materials and tools**

The materials used in this research are cherry tomato fruit from Kemiling, Bandar Lampung, white shrimp obtained from RawajituTimur pond, TulangBawang, 96% ethanol, filter paper, aquades, aluminum foil, label paper , Paper disc (Whatman no.42), cotton, 70% alcohol, Salmonella sp., NaCl, Peptone Water Buffer (BPW), Sodium Agar (NA), and Xylose LisineDeoxycholate (XLD) agar.

The tools used include digital scales, blenders, vortex, colony counters, petri dishes, bunsen lamps, Erlenmeyer, Beaker glasses, test tubes, drop pipettes, measuring pipettes, micropipets, tip pipettes, spatulas, ose needles, incubators, vacuum Rotary evaporator, funnel, blade, scooter and waterbath shaker.

**3.3. Method**

The study looked for the best concentration of antimicrobial extracts on tomatoes. The experiment used a Completely Randomized Block Design (RAKL) with a single factor and six replications. In the first study using tomato-ethanol extract 96% with five levels of concentration 0%, 25%, 50%, 75% and 100%.

Tomato fruit extract used was 10 ml for 100% extract concentration, 7.5 ml extract plus 2.5 ml of distilled water for 75% concentration treatment. At a concentration of 50%, 5 ml of the extract was added with 5 ml of distilled water, then for 25% concentration was used 2.5 ml of extract plus 7.5 ml of distilled water at the level of 0% using aquades. The data obtained were tested for their similarity by using Bartlet test. Data were analyzed by fingerprint to obtain the error estimator. Data analysis was continued by using the Smallest Differential Differential (BNT) test at α = 5%.

1. **RESULTS AND DISCUSSION**

**3.1. Results of Antimicrobial Activity Testing by Using ExtractsCherry Tomato Fruit**

The result of observation of inhibitory diameter diameter by using cherry tomato extract obtained by average of diameter which were vary from each treatment of extract concentration given. The results of these observations are presented in Table 1.

Table 1. The results of observation of inhibitory power of cherry tomato extract on *Salmonella sp.*

|  |  |
| --- | --- |
| Extract Concentration (%) | Average resistor diameter (mm) |
| 0 | 0,00 |
| 25 | 3,80 |
| 50 | 11,45 |
| 75 | 14,85 |
| 100 | 17,29 |
| Control (Etanol 96%) | 0,8 |
| Control (Cakram Paper) | 0 |

The results of the antimicrobial testing using paper disc with different concentration treatments (Table 1) showed that at 100% concentration formed the largest obstacle zone with the mean diameter of 17.29 mm. The concentration of cherry tomato extract is 75%, 50%, 25% respectively resulted 14.85 mm, 11.45 mm, and 3.80 mm per each. Treatment at 0% concentration (using aquades) which is a negative control does not form a clear zone (0 mm). A drag zone test trial was also conducted to determine the antimicrobial activity in 96% ethanol solvent. Resistor zone that is formed that is equal to 0,8 mm. This suggests that ethanol, presumably still present in the extract, contributes to the formation of a 0.8 mm drag zone, but the activity of the active compound in the extract shows a larger drag zone compared to the ethanol solvent.

In the treatment of cherry tomato extract showed that the more concentrated the extract of cherry tomato fruit then form the diameter of the greater obstacles. This is in accordance with the statement Ajizah (2004) that the concentration of antibacterial compounds greatly affected the ability of these compounds in inhibiting the growth of microorganisms. According to Noer(2011) one of the factors affecting the drag zone diameter is concentration. The higher the antimicrobial concentration used, the more microorganisms can be inhibited. This shows that the antimicrobial active substance in cherry tomatoes will be lower in potency as the concentration of extract

According to Suryawiria (1978) in Saraswati (2015) based on the inhibition zone formed the antimicrobial activity can be classified into several classes, namely antimicrobials are classified as weak (inhibition zone <5 mm), medium (inhibition zone between 5-10 mm), strong (zone Resistor between 10-20 mm), and is very strong (inhibition zone> 20 mm). Cherry tomato extract at 25% concentration is relatively weak, while concentrations of 50%, 75%, and 100% are strong. The results of this study showed that all treatments of cherry tomato extract at concentrations of 25%, 50%, 75%, and 100% had antibacterial activity with resistance diameters ranging from 3.80 mm - 17.29 mm. Negative control test results in the form of sterile distilled water in this study showed that there was no inhibit zone or 0 mm (Table 1). The absence of inhibition zone in negative control was used as an indicator of Salmonella sp. Normally at various treatments. The analysis results of various inhibit zone data are presented in Table 2

Table 2. Test analysis of antimicrobial assays of cherry tomato extract on *Salmonella sp* bacteria.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sources of Diversity | db | JK | KT | F hitung | F tabel |
| 0.05 | 0.01 |
| Group | 5 | 7.414 | 1.483 | 1.546 | tn | 2.711 | 4.103 |
| Treatment | 4 | 1295.236 | 323.809 | 337.694 | \*\* | 2.866 | 4.431 |

Statistical results (Table 2) showed that between treatments (concentrations of 100%, 75%, 50%, 25% and 0%) had a very significant difference to inhibit *Salmonella sp.* growth. Seen on Ftest>Ftable (5%) = 337,694> 2,866. Significance for the source of group diversity shows an unreal result, this is because Ftest<Ftabel (5%) = 1.546 <2.711, this means that the group does not affect the research data. A further test using BNT at the α = 5% level is obtained as shown in Table 3.

Table 3. BNT test result α 5% to know the concentration of cherry tomato extract effective in inhibiting bacteria *Salmonella sp.*

|  |  |
| --- | --- |
| Concentrations of Extracts (%) | Middle value |
|  100 | 17,29a |
| 75 | 14,85b |
| 50 | 11,45c |
| 25 | 3,80d |
| 0 | 0,00e |

Description: The numbers followed by the same letter show no significant difference at the α 5% level.

The result of BNT test (Table 3) showed that the average of negative control zone control zone diameter (0%) was significantly different with all treatments of 25%, 50%, 75%, and 100% cherry tomato extracts. The concentration of 0% was significantly different with concentrations of 25%, 50%, 75% and 100%. The 25% concentration was significantly different with concentrations of 50%, 75% and 100%. Concentrations of 50% differ significantly with concentrations of 75% and 100%. Concentrations of 75% of differ obviously with 100% concentration. The concentration of cherry tomato extract is effective as an antimicrobial against*Salmonella sp.* is 100%, because it has a drag diameter of 17.29 mm and is significantly different from the drag diameter at lower extract concentrations. The resulting inhibit zone can be seen in Figure 1.



100%

75%

50%

25%

0%

Figure 1. Clear zone area formed around disc paper by cherry tomato extract on *Salmonella sp.*bacteria.

Treatment with 100% concentration has the most inhibitory or transparent zone in inhibiting the growth of Salmonella sp bacteria. It can be seen in Figure 12 that the area around the disc paper is not overgrown by a test bacterial colony called a radical zone. The radical zone is an area around the disc where there is no bacterial growth (Pelczar and Chan, 1986).

The difference is apparent between the lowest concentration (25%) and the highest concentration (100%). There is a tendency to increase the diameter of the growth inhibition area of test bacteria along with the increase of extract concentration. The difference in the diameter of the inhibitory area of each concentration can be caused by the difference in the amount of active compound contained in the extract. According to Katno*et al*. (2009), the factors that influence the inhibitory test are the concentration of active compounds, the susceptibility of microbial growth test, the thickness and viscosity of the medium and the reaction between the active substance to the medium and the incubation temperature.

Extracts of cherry tomatoes used as antimicrobial compounds can be used to inhibit gram-positive or negative bacteria. According to Al-Oqaili*et. al*., (2014) tomato extract proved effective to inhibit *E. coli* bacteria with 75% concentration and inhibition zone of 35-50 mm. According to Nasser (2012) chloroform extract and tomato ethanol showed antimmicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli* and *Klebsiellapneumoniae.* This study shows that cherry tomato extract has the same ability to inhibit the growth of Salmonella sp bacteria. This suggests that tomato extract can inhibit the growth of bacteria that have gram negative cell walls other than E. coli, the Salmonella sp bacteria. (Test bacteria). There is a difference in cell wall structure between gram positive and gram negative. Gram positive bacteria wall structures consist of several layers of peptidoglycan forming a thick and rigid structure and contain a cell wall substance called teicoic acid. Gram-negative bacteria have a thinner peptidoglycan layer, only one to two percent of their dry weight. This is because the gram-negative bacteria contains a small layer of peptidoglycan and does not contain the te acidic acid. Gram-negative bacteria have two or three layers of peptidoglycan. While a gram-positive cell, it can contain peptidoglycan 20 times, enough for 40 layers or more (Brock and Madigan, 1991). This is what makes the walls of gram-negative bacteria such as *Salmonella sp.*More susceptible to physical shocks, such as antibiotics or other antibacterial agents (Radji, 2011).

The results showed that cherry tomato extract can be used as an antimicrobial in the control of bacterial contamination of *Salmonella sp.* According to Kartikasari (2008), the content of alkaloids and saponins in tomato extract can be an antimicrobial compound. According to Gunawan in Saputra and Lilis (2012), alkaloid compounds utilize the reactive properties of basic groups (-NH) to react with amino acid groups in bacterial cells. The presence of such a base group, when in contact with the bacteria will react with amino acid compounds and bacterial DNA. This reaction occurs because chemically an alkaline compound will react with the acid compound in this case is an amino acid. This reaction results in changes in the structure and structure of amino acids since most of the amino acids have reacted with the basic groups of the alkaloid compounds. This amino acid sequence change will obviously alter the arrangement of DNA chains in the nucleus of cells that originally have an interlocking array of acids and bases. This will cause a change in the genetic balance of the DNA acid so that the bacterial DNA will be damaged.

Damage to cells that occur in bacteria will make the bacterial cells are not able to perform metabolism so it will become inactive and destroyed. According Fitrianti (2011) states that the working mechanism of the alkaloids is associated with the ability to intercalate (put themselves in between the DNA). The presence of substances that exist between the DNA will inhibit DNA replication itself. Cell death will occur due to DNA replication. Thus the bacteria *Salmonella sp.* will become inactive and destroyed (lysis).

Saponins are compounds that naturally contain glycosides, widely present in plants (Naidu, 2000). Gruiz (1996) stated that 76% of Asian plant species contain Saponins.Saponins are like soap. The presence of saponins can be detected by observing its ability to form foams. Saponins inhibit growth or kill microbes by interacting with sterol membrane. The main effect of saponins on bacteria is the release of proteins and enzymes from within cells (Zablotowicz et al., 1996). Saponins can be antimicrobial by damaging cell membranes. Damage to the membrane causes an important substance out of the cell and also can prevent the entry of important ingredients into the cell. If cell membrane function is destroyed it will lead to cell death (Monalisa et al., 2011)

Test of total decrease of *Salmonella sp.* on white shrimp performed to determine the ability of antimicrobial extract of fruit and tomato leaf in controlling contamination of *Salmonella sp*. on white shrimp (*Litopenaeusvannamei*). Test result of total decrease of Salmonella sp. on white shrimps using cherry tomato extract is presented in Table 4.

**3.2. Total*Salmonella sp.* on White Shrimp**

Test of total decrease of *Salmonella sp*. on white shrimp performed to determine the ability of antimicrobial extract of cherry tomato fruit in controlling contamination of *Salmonella sp.* on white shrimp (Litopenaeusvannamei). Decrease in the amount of *Salmonella sp*. produced, indicating that the active compound on fruit extract and cherry tomato leaves can kill *Salmonella sp.* test result of total decrease of *Salmonella sp.* on white shrimps using cherry tomato extract is presented in Table 7.

Table 7. Test results of total decrease of Salmonella sp. On white shrimp using fruit extract and cherry tomato leaves

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment | Amount of*Salmonella sp.* (CFU/ml) | Total decrease (CFU / ml) ± Standard Deviation | Total decrease (%) |
| Without theextract | With extract |
| Cherry Tomato Fruit Extract | 2,74 x 107 | 8,07 x 105 | 2,66 x 107±2516611,5 | 97,06 |

Test results of total decrease of Salmonella sp. (Table 7) shows that the extract of cherry tomato fruit has the potential to decrease contamination of Salmonella sp bacteria on white shrimp. White shrimp used is shrimp that comes from farming pond RawajituTimur, TulangBawang. The amount of bacteria Salmonella sp. is calculated on shrimp that has not been given treatment on XLD media. The results showed negative or no *Salmonella sp* bacteria. that grows. This is according to Anjung (2016) which states that testing of Salmonella sp. on white shrimp from RawajituTimur is negative. Shrimp that have been testedis then given the culture of *Salmonella sp.* which comes from the veterinary hall, Lampung, 1 ml (McFarland standard 108 CFU / ml).

Tests showed that total bacteria on shrimp before being given an average extract of 2.74 x 107 CFU / ml. Provision of tomato extract as much as 5 ml into 5 grams of white shrimp showed a decrease in the number of bacteria. The average number of Salmonella sp. after being given cherry tomato extract of 8.07 x 104 CFU / ml. This shows that there is a decrease in the number of *Salmonella sp.* of 2.66 x 107 CFU / ml, which, when suppressed by 97.06%. This proves that the extract of cherry tomato fruit is able to control the contamination of Salmonella sp bacteria which is in white.

The content of compounds present in extracts of cherry tomatoes such as alkaloids and saponins are able to control contamination of Salmonella sp. bacteria on white shrimp. Salmonella bacteria sp. that existed in the shrimp will interact with antimicrobial compounds. The alkaloid compounds present in the tomato extract will react with the amino acid group in the bacterial cell. This will make the structure and structure of amino acids changed and can lead to changes in the genetic makeup of DNA acids that are damaged. Damage that occurs will result in metabolic disturbance in the body of bacteria that will become inactive and destroyed and resulted in dead bacteria (Gunawan, 2004). Saponins present in tomato extracts can cause damage to bacterial cell membranes resulting in cell death (Gruiz, 1996).

Cherry tomato extract showed a more acidic pH (3,4). *Salmonella sp.* can generally live at an optimum pH of 6-8 (Hiramatsu et al., 2005). The degree of acidity or acid pH can generally affect the growth of *Salmonella sp.* Because it can cause membrane damage, interfere with protein or genetic synthesis systems (DNA synthesis / RNA), and microbial deaths due to run out of ATP due to the use of ATP to run proton pumps in order to remove H + from within cells to maintain pH equilibrium in cells (Ray, 2005 ).

1. **CONCLUSIONS AND RECOMMENDATIONS**

**4.1. Conclusion**

Based on the results of research that has been done then it can be concluded as follows:

1. Extracts ofcherry tomatoes fruits has an inhibitory effect on contamination of *Salmonella sp.* bacteria on white shrimp. Tomato extract is able to inhibit the growth of *Salmonella sp* bacteria with the inhibitory diameter diameter of 17.29 mm with strong antibacterial activity.

2. The best concentration of cherry tomato fruit in the decrease of contamination *Salmonlla sp*. on white shrimp that is each concentration of extract 100%.

**4.1. Suggestion**

Further research is needed by using fruit extract and cherry tomato leaves on other microbial species, gram-negative bacteria and gram-positive bacteria.