CHAPTER 5

RESULT

5.1 Data of Research Result

5.1.1 *Escherichia coli* Identification Result

Escherichia coli isolation that was used in this experiment was obtained from Microbiology Laboratory, Faculty of Medicine, Universitas Brawijaya. In order to determine the real and pure *Escherichia coli*, few test were done. The first foremost identification method used to identify this bacterium was Gram Staining. In this identification method, a pink colored (gram negative) rod shape bacteria can be seen under microscope of 1000x magnification (Figure 5.1). Secondly, the bacterium was inoculated on EMB and hence a green metallic sheen can be observed (Figure 5.2). The third test was the MacConkey lactose fermentation test. The bacterium fermented the medium and hence a red colonies appeared as in figure 5.3

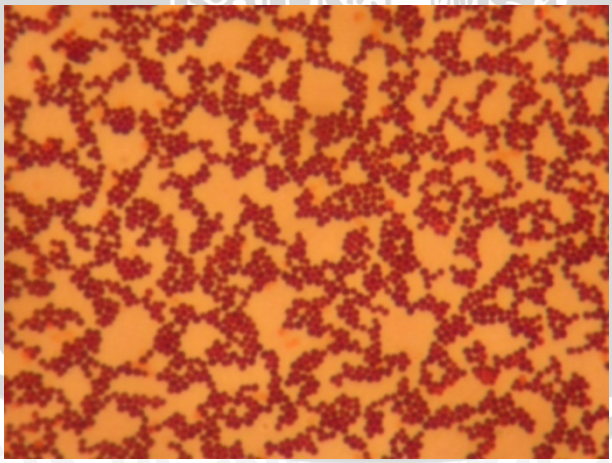

Figure 5.1 Escherichia coli identification using Gram Staining

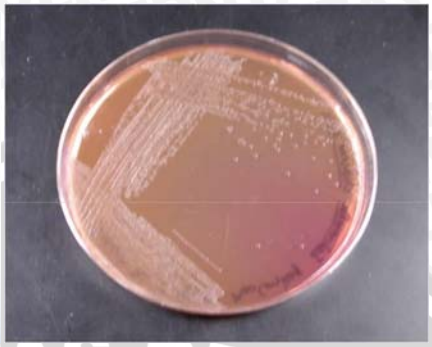

Figure 5.2 **Figure 5.3**

Figure 5.2 are *Escherichia coli* metallic sheen on EMB.

Figure 5.3 are Escherichia coli on MacConkey agar

5.1.2 The Result of Tube Dilution Method to detect Minimal Inhibition Concentration (MIC) and Streaking Method to detect Minimum Bactericidal Concentration (MBC)

The research was done using 5 different concentrations of *oregano* leaves ethanolic extract, 9%, 10%, 11%, 12% and 0%. The *oregano* leaves ethanolic extract was in liquid form and soluble in water. 1ml of bacteria was added to test tube 9%, 10%, 11% and 12% then the final concentration becomes 4.5%, 5%, 5.5%, and 6%. The test tubes were incubated overnight in an incubator. Meanwhile in test tube 0%, only 2ml of bacteria suspension was added and incubated. The following day the difference in clarity was observed. The result will be observed carefully as this determined the Minimal Inhibition Concentration (MIC).

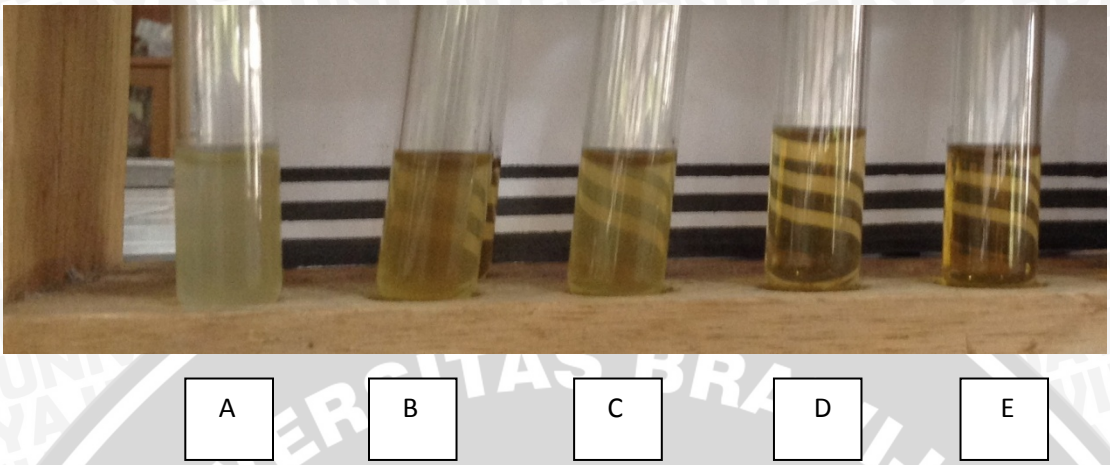

Figure 5.4 Level of Clarity for Different *Oregano* leaves Ethanolic Concentration in MH Broth (Tube Dilution Test)

A: Bacteria Suspension, no *oregano* leaves ethanolic solution (Control Bacteria)

B: Bacteria suspension + 9% *oregano* leaves ethanolic concentration = Final concentration is 4.5%.

C: Bacteria suspension + 10% *oregano* leaves ethanolic concentration = Final concentration is 5%.

D: Bacteria suspension + 11% *oregano* leaves ethanolic concentration = Final concentration is 5.5%.

E: Bacteria suspension + 12% *oregano* leaves ethanolic concentration = Final concentration is 6%.

Different level of cloudiness of every reaction tube after one day incubation can be seen in figure 5.4. In test tube A, all of the three lines cannot be seen at all (cloudy). It is because there are abundance of bacteria growth. Meanwhile, in test tube B, there is slight cloudiness where almost all of the three lines can still be seen. This shows that there are bacteria still growing inside the tube. The C test tube,

begins to show some clarity where all the 3 lines starting to be seen a little bit clearer. The clarity becomes more apparent in test tube D and E.

From the observation, it can be concluded that the clarity of each test tube is greatly influenced by the *oregano* leaves ethanolic extract. The Minimal Inhibition Concentration (MIC) of *oregano* leaves ethanolic extract towards *Escherichia coli* is 5.5%. This was obtained by observing the clarity level in each concentrated tubes.

After the test tubes were incubated at 37^0C for 24 hours, the clarity was observed to determine the Minimal Inhibition Concentration (MIC). Each concentration was then streaked on NAP (Nutrient Agar Plate) and then incubated at the temperature of 37^0C for 24 hours. After incubation, the colony growths on each NAP were counted by using colony counter. This was done to determine the Minimal Bactericidal Concentration (MBC) for the *oregano* leaves ethanolic extract. The minimum bactericidal concentration is the lowest concentration of antimicrobes which can kill bacteria (showed by the absence of growth on NAP).The colony growth on each concentration is shown in fig 5.5.

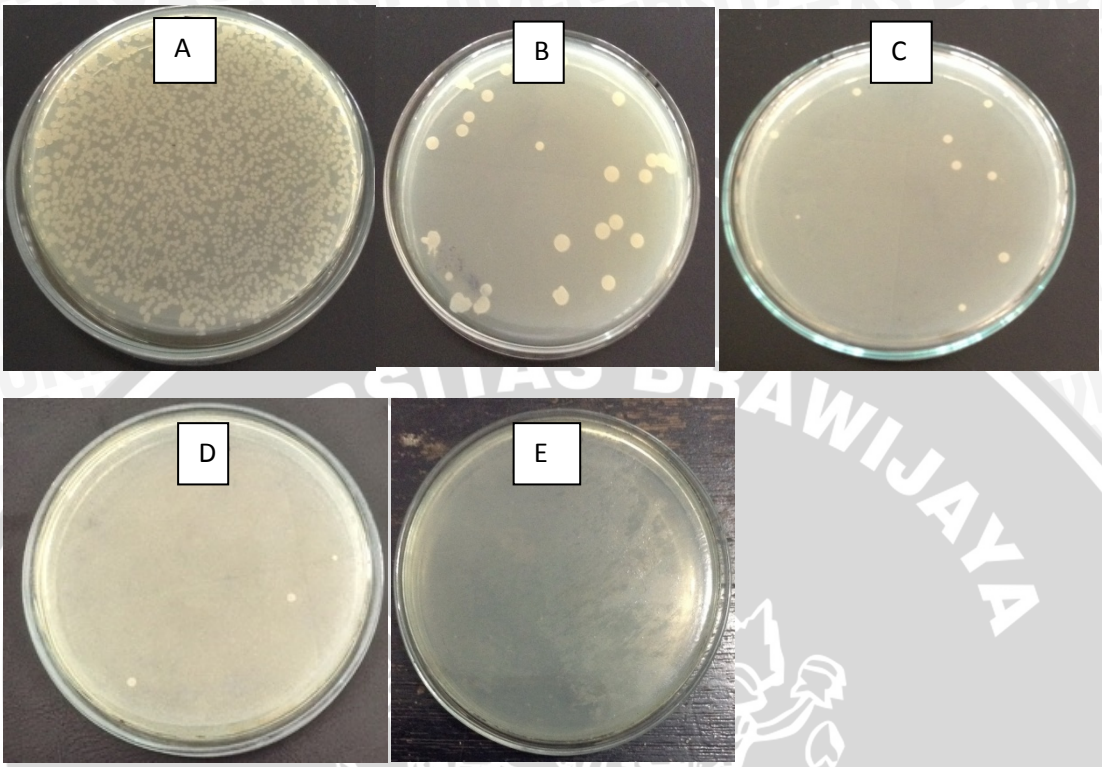

Figure 5.5 *Escherichia coli* colony growths on NAP

Figure explanation:

A: colony growth in 0% *oregano* leaves ethanolic concentration (control bacteria)

B: colony growth in concentration 4.5%.

C: colony growth in concentration 5%.

D: colony growth in concentration 5.5%.

E: colony growth in concentration 6%.

Concentration	Total colony (Repetition)				(mean ± SD)
	I	II	III	IV	
0% (CB)	1628	1908	1539	1424	1624.75 ± 206.47
4.5%	70	50	100	50	67.50 ± 23.63
5%	20	20	30	40	27.50 ± 9.57
5.5%	10	10	0	10	7.50 ± 5.00
6%	0	0	0	0	0

Table 5.1 Result from *Escherichia coli* colony counted on NAP by using colony counter.

The amount of total *Escherichia coli* colony growth on NAP in various *oregano* leaves ethanolic concentration is shown in table 5.6. Differences between *oregano* leaves ethanolic extract concentration can produce various effects in terms of an antimicrobial towards *Escherichia coli* growth on NAP. From figure 5.4, it was found that the minimum inhibitory concentration (MIC) is at 5.5% while from table 5.1, the minimum bactericidal concentration (MBC) is found to be at 6%.

The effect of increasing *oregano* leaves ethanolic extract concentration is a significant decrease in the number of *Escherichia coli* colony growth for an example at concentration of 4.5%, the total colony growth is by far much less than the total growth colony in 0% concentration (control bacteria). So it can be said that, as the *oregano* leaves ethanolic extract concentration increases, the total colony growth on NAP decreases. Furthermore, it can also be deduced that there is no more colony growth on NAP starting from the concentration of 6%.

From the descriptive calculation, there is an average in the number of *Escherichia coli* colony on NAP in each concentration. Therefore we can deduce that *oregano* leaves ethanolic extract concentration has antimicrobial effect towards *Escherichia coli* when compared to the control bacteria (0%). The average number of *Escherichia coli* colony at each concentration was used to plot a graph as can be seen in figure 5.6.

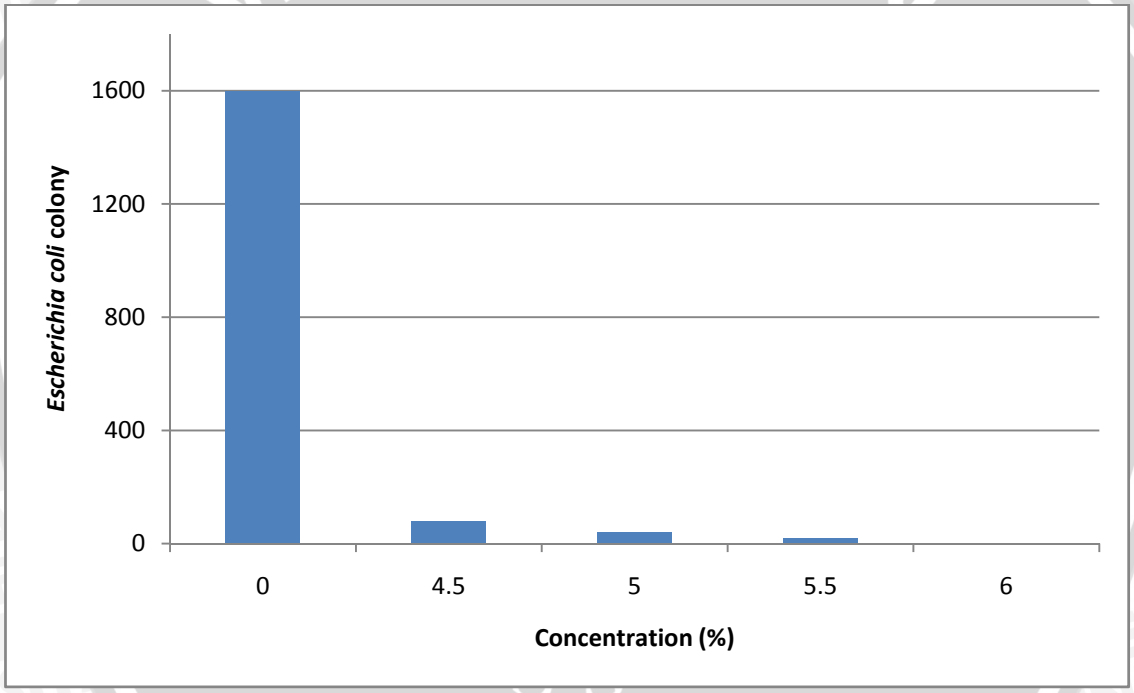

Figure 5.6 Average Total *Escherichia coli* Colonies in Different *Oregano* Leaves Ethanolic Concentration.

5.2 Data Analysis

5.2.1 Assumption Test Data

The use of One Way ANOVA statistic requires that the relevant data are normal and homogenous. Therefore the distribution and variant data of total colony of *Escherichia coli* on NAP at different concentrations should be tested using Normality Test and Variant Test as they are the criteria to fulfill the ANOVA requirement. The data are only accepted by The ANOVA Statistic test if the distribution data is normal which is ($p>0.05$) and the variant data also the same with ($p>0.05$) signification (homogeny). The independent variable was the concentrations of *oregano* leaves ethanolic extract whereas the number of colonies in each NAP was the dependent variable. The Statistical Product of Service Solution (SPSS) was used to carry out the relevant statistical test.

The first test done was the descriptive test which shows the mean, standard deviation, standard error, mean interval, minimum and maximum value. In order to minimize error each concentration was repeated 4 times. The mean for concentrations 0%, 4.5%, 5%, 5.5%, 6% are as follows 1624, 67, 27, 7.5 and 0. The Descriptive Test result is attached in appendix 2 (1).

1. Normality Test (Kolmogorov-Smirnov)

This normality test was done to determine whether the data has normal distribution or not. The result of data analyzed using the normality test was, a numerical value of $p = 0.877$ ($p>0.05$) which means that the data of bacterial colony

after treatment were normally distributed. The Normality Test is attached in appendix 2 (2).

2. Homogeneity of Variances Test (Levene's Test)

This particular test was performed to assess the equality of variances in different samples. Based on this homogeneity test, the data variance of bacterial colony after treatment was $p = 0.083$ ($p>0.05$) and this means it's homogenous. Since the data were normal and the variance data was homogenous, it was appropriate to perform one way ANOVA to investigate whether different concentration of extract has different effects on colony growth. The Levene's test result is attached in appendix 2 (3).

5.2.2 One Way ANOVA Analysis

The experiment was carried out using numerical variable and the corresponding result which is the difference of *Escherichia coli* colony grown on NAP at 5 different concentration of *oregano* leaves ethanolic extract. The ANOVA value is examined by the hypothesis. H_0 is accepted when $p>0.05$ and rejected when $p<0.05$. One way ANOVA test showed the p value of 0.000 and it means that H_0 is rejected. This indicates that different dose of concentration would result in different number of colony. The One Way ANOVA Analysis Test is attached in appendix 2 (4).

5.2.3 Post Hoc Test (Tukey Test)

Multi comparison Post Hoc Test is the multiple comparison between the total *Escherichia coli* colony which was grown on NAP medium and different concentrations of *oregano* leaves ethanolic extract. This helps to determine which concentration in the intervention caused significant differences towards the total *Escherichia coli* colony. Based on the result, it can be concluded that from concentration 4.5%-6% the differences are not significant while from concentration 0%-6% the differences showed significant values. Post Hoc Test (Tukey Test) is attached in appendix 2 (5).

5.2.4 Correlation Test

Correlation test is a test done to determine how big or significant the relationship between using *oregano* leaves ethanolic extract as an antimicrobial and the growth of *Escherichia coli* bacteria colony on NAP. The result from this test shows that the usage of *oregano* leaves ethanolic extract as an antimicrobial had a very strong significant correlation (0.973) with a negative correlation direction. This negative direction shows that as the concentration of *oregano* leaves ethanolic extract increases, the growth of *Escherichia coli* bacteria colony decreases. This test result is attached in appendix 2 (6).