CHAPTER 6

DISCUSSION

This experiment was done to prove that different concentration of *oregano* leaves ethanolic extract has antibacterial effects on the growth of *Escherichia coli* in vitro. *Oregano,* one of the world's most common herbs, was chosen because not many people know about its hidden values especially its health benefits. It is more popularly and exclusively known as a herb used in cooking Western and Mediterranean cuisine. Although, it has been used as a medicine since the 15th century. However, its medicinal properties has been forgotten as time goes by. On the other hand, *Escherichia coli* was chosen because it is common and yet harmful. One of the most common infection caused by Escherichia coli is diarrhea. Tube dilution method was used in this research to determine Minimum Inhibitory Concentration (MIC). The result of the tube dilution was used in inoculation on NAP to determine Minimum Bactericidal Concentration (MBC).An extraction method called 'Soxhlet method' with 96% ethanol was used to perform the extraction process. A total of 200 grams of dried oregano leaves was used.

Escherichia coli bacteria used in this experiment were obtained from stock culture belonging to Laboratory of Microbiology, Faculty of Medicine, Universitas Brawijaya. Various tests were undertaken to identify this bacteria before carrying out the experiment. Gram staining was one of the test done to identify the bacteria. The result was the colony of red rod bacteria which concludes it is gram negative bacteria. The bacteria were then inoculated on EMB (eosin methylene blue) medium

and a green metallic sheen will appear. The third test done was the MacConkey lactose fermenting test. In this test, the *Escherichia coli* colony gave a red coloured colony as it ferment lactose.

The exploration experiment was done using serial dilution method with concentrations of 100%, 50%, 25%, 12.5%, 6.125% and 3.125%. The result of exploration gave a range of concentration at which bacteria lived and died. It was from 6.25% till 12.5%. But further narrowing of the concentrations were needed to determine the real effective concentrations and so the experiment was further carried out by using a range of concentration starting from 0%, 2%, 4%, 6%, 8%, 10%, 12% and 14%. In this particular concentration range, all the bacteria died and hence it was deduced that human error might have occured. The following week, another range of concentration was chosen, ranging from 0.5%, 1%, 2%, 3%, 4% and 5%. The bacteria at this concentrations lived and hence this range was invalid. Another range of concentrations were chosen such as 7%, 7.5%, 8%, 8.5%, 9%, 9.5% and 10%. At this concentrations again all the bacteria lived but there was decrease in *Escherichia coli* growth from concentration 9% to 10%.

According to a research done in Brazil in 2013, on the effectiveness of antimicrobial activity against 2 types of bacteria *Escherichia coli* and *Salmonella enteriditis* and 1 type of fungi such as *Penicillium* on sliced bread using *oregano* essential oil, the effective concentration are from 5% to 15%. In the research, concentration higher than 15%, effectively killed the bacteria and fungi. (Passarinho, 2013).

This research provided an insight to determine the concentrations which are suitable for the current research. Thus, the concentrations were narrowed down to the final concentrations of 4.5% to 6.0% of *oregano* leaves ethanolic extract. Therefore this experiment used 0%, 4.5%, 5.0%, 5.5% and 6.0%.. In this experiment, control bacteria (CB) 0% were used as a comparison towards the bacteria that have been given different concentration *oregano* leaves ethanolic extract. Control extract was not used because the 100% *oregano* leaves ethanolic extract has a very dark colour and hence unable to be used as a comparison.

The MIC (minimum inhibitory concentration) was determined by observing the clarity of each test tubes after being incubated for 24 hours. The MIC for this experiment was at 5.5%. The control bacteria and other *oregano* leaves ethanolic concentrations were streaked on NAP and incubated for 18-24 hours. The total bacteria colony growth was then calculated using colony counter. The MBC (minimum bactericidal concentration) was determined through this calculation and was at 6%.

The result of *Escherichia coli* colony counted using colony counter was then analyzed by means of parametric statistics, One Way ANOVA, Post Hoc test, and Pearson Correlation test. The Normality test done showed that the data had normal distribution and the Homogeneity of Variances test showed that the data were homogenous and hence these fulfills the ANOVA criteria.

One Way ANOVA test showed a significance value of 0.000 ($p<0.05$) and hence it can be deduced that there is significant difference on the effect of *oregano*

leaves ethanolic extract on the growth of *Escherichia coli*. The Post Hoc showed significant value of 0.000 which means there are antimicrobial differences between each concentration towards the growth of *Escherichia coli.* The Correlation test also showed a significant value and the coefficient was -0.973 which means that the *oregano* leaves ethanolic extract has significant effect on *Escherichia coli* growth with a negative direction where the higher the concentration of *oregano* leaves ethanolic extract, the lesser the growth of *Escherichia coli* on NAP.

The antibacterial effect took place because *oregano* leaves ethanolic extract contains active substances such as *flavonoids, monoterpenes* and *phenolic acid.* These substances started to control and inhibit a large number of bacteria. The exact mechanism of action of each active substances are as following, *flavonoids* inhibit the synthesis of nucleic acid, inhibit the synthesis of cytoplasmic membrane and also inhibit the process of energy metabolism. All this mode of action were believed to caused the death of the bacteria. On the other hand, active substance such as *monoterpene*, increases the permeability of cytoplasmic membrane and hence causes intracellular leakage. Lastly, the active substance known as *phenolic acid* increases the permeability of outer membrane and hence it is vulnerable to dangers. All these components work together to inhibit the growth of *Escherichia coli* and eventually causing the death of these bacteria.

The research has few weaknesses that have to be given quite an attention. Firstly, the bacteria used and observed were only *Escherichia coli*, a species in the *Escherichia* genus. It is an opportunistic gram negative bacterium that lives in our body specifically in our gastrointestinal tract. These bacteria, which are non-

pathogenic by nature, cause disease once it becomes pathogenic. The changes mainly occur due to weak immunological status. No other gram negative or gram positive bacteria are tested in this research. The second weakness is the *oregano* leaves ethanolic extract. This is because it was extracted in a place where many extraction processes were done and was kept in a fridge with inconsistent temperature. This caused writer's concern whether there is any consequence of the act towards *oregano* leaves ethanolic extract effectiveness as antimicrobial.

By observing the result of the research, it is concluded and proved that as *oregano* leaves ethanolic extract concentration increases, the total colony of *Escherichia coli* decreases. Finally, the research showed that *oregano* leaves ethanolic extract is an active material and has the ability as an antimicrobial and hence the hypothesis is proven.