ANTIBACTERIAL EFFECT OF STRAWBERRY (*Fragaria x ananassa*) EXTRACT ON PATHOGENIC SEROTYPE 1-11 *Escherichia coli* REVEALED USING DILUTION METHOD

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ABSTRAK

Diare adalah gejala klinis pencernaan (usus) gangguan yang ditandai dengan berulang-ulang dan peningkatan frekuensi buang air besar lebih dari biasanya, disertai dengan perubahan bentuk tinja dan konsistensi, yang menjadi lunak atau cair. Salah satu cara untuk mengatasi diare adalah untuk membunuh bakteri yang menyebabkan diare. Escherichia coli adalah salah satu contoh dari kuman yang menyebabkan diare. Escherichia coli adalah bakteri gram negatif berbentuk batang. Meskipun dalam tubuh kita Escherichia coli diperoleh sebagai flora normal, tetapi ada beberapa strain Escherichia coli yang bersifat patogen bagi manusia dan mampu menyebabkan diare. Salah satu jenis yang dapat menyebabkan diare pada manusia adalah patogen E. coli serotipe 1-11. Stroberi (Fragaria x ananassa) atau disebut juga strawberry kebun buah dibudidayakan di seluruh dunia karena aroma, warna merah cerah dan manis khas. Selain itu, stroberi juga terbukti memiliki banyak konten, termasuk potasium, magnesium, fosfor, kalsium, zat besi, dan vitamin A, E, K, C. Beberapa penelitian juga menunjukkan bahwa stroberi juga memiliki senyawa flavonoid, salah satunya adalah antibakteri. Tujuan dari penelitian ini adalah untuk mengetahui efek antibakteri stroberi (Fragaria x ananassa) ekstrak terhadap bakteri patogen Escherichia coli serotipe 1-11 in vitro. Penelitian ini dilakukan di Laboratorium Mikrobiologi Medis, Fakultas Kedokteran, Universitas Airlangga pada Desember 2011. Persiapan bahan yang digunakan metode ekstrak tanpa pelarut, yang kemudian menghasilkan strawberry (Fragaria x ananassa) ekstrak konsentrasi 100%. Kemudian, kami menguji ekstrak terhadap bakteri patogen Escherichia coli serotipe 1-11 dilakukan dengan metode dilusi untuk mencari efek bakteriostatik, kemudian mulai penanaman pada media padat (plate) untuk membuktikan efek bakterisida. Hasil penelitian menunjukkan bahwa efek bakteriostatik (konsentrasi bakteri-hambat minimum) dan efek bacteriocidal (minimum membunuh bakteri konsentrasi) tidak ditemukan dalam stroberi (Fragaria x ananassa) ekstrak setiap konsentrasi (100%, 50%, 25%, 12,5%, 6,25 %, 3,125%, 1,5625%) terhadap bakteri patogen Escherchia coli serotipe 1-11. ***(FMI 2012;48:167-173)***

Kata kunci: *stroberi, Fragaria x ananassa, Escherichia coli serotipe 1-11 patogenik, uji sensitivitas metode dilusi*

ABSTRACT

Diarrhea is a clinical symptom of digestive (intestinal) disorders characterized by repeated and increased frequency of defecation more than usual, accompanied by a change in fecal form and consistency, which becomes soft or liquid. One way to deal with diarrhea is to kill the bacteria that cause diarrhea. Escherichia coli is one example of germs that cause diarrhea. Escherichia coli is a rod-shaped gram-negative bacteria. Although in our body Escherichia coli is obtained as normal flora, but there are some strains of Escherichia coli that are pathogenic to humans and able to cause diarrhea. One of the strains that can cause diarrhea in humans is pathogenic E. coli serotypes 1-11. Strawberries (Fragaria x ananassa) or also called strawberry orchard fruit is cultivated worldwide because of its aroma, bright red color and distinctive sweetness. Besides, strawberries are also shown to have a lot of content, including potassium, magnesium, phosphorus, calcium, iron, and vitamins A, E, K, C. Some studies also demonstrated that strawberries also have a flavonoid compound, one of which is antibacterial. The purpose of this study was to determine the antibacterial effects of strawberries (Fragaria x ananassa) extracts against pathogenic bacteria Escherichia coli serotype 1-11 in vitro. The research was conducted at the Laboratory of Medical Microbiology, Faculty of Medicine, University Airlangga on December 2011. Preparation of materials used extract methods without solvent, which then produced strawberry (Fragaria x ananassa) extracts in concentration of 100%. Then, we tested the extract against pathogenic bacteria Escherichia coli serotype 1-11 conducted with dilution method to search for bacteriostatic effects, then proceeded to the planting on solid media (plate) to prove the bactericidal effect. Results showed that bacteriostatic effect (minimum bacteria-inhibitory concentration) and bacteriocidal effect (minimum bacteria-killing concentration) were not found in strawberries (Fragaria x ananassa) extracts at all concentrations (100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5625%) against pathogenic bacteria Escherchia coli serotype 1-11. ***(FMI 2012;48:167-173)***

Keywords: *Strawberry, Fragaria x ananassa, pathogenic Escherichia coli serotype 1-11, dilution method sensitivity test*

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INTRODUCTION

The types of food consumed by humans are increasingly diverse in line with the development of lifestyle. One is the increased availability of fruits in the community. Often we do not realize that many pieces around us who have hidden properties. One of the widely consumed fruits and has the benefit of covert is strawberry. Strawberries (*Fragaria x ananassa*) or also called strawberry fruit orchard is cultivated worldwide for its aroma, bright red color, and the typical sweetness. The fruit is consumed on a large scale, either in the form of fruit or in processed form such as ice cream, fruit juices, yoghurts and milk shakes. Garden strawberry was first bred in Brittany, France in 1740 via a cross of *Fragaria virginiana* and *Fragaria chiloensis* from Chile and Argentina brought by Amedee-Francois Freizer. Based on data collected, in the range of 2005 to 2008, the number of strawberries harvested reached 3.5 million tons worldwide each year, with a peak of 4 million tons of strawberry fruit production in 2008 (United States Department of Agriculture nd).

The content in strawberries, among others, potassium, magnesium, phosphorus, calcium, iron, vitamin A, E, K, C. Vitamin C plays an important role in reducing the risk of cancer of the gastrointestinal tract. In addition, the fruit is also a good source of antioxidants, and are able to lower high blood pressure that is associated with heart disease and blood vessels. Some studies also show that strawberries help reduce the ability of memory and rheumatoid arthritis. But that is not widely known by the public is that strawberries also contain flavonoids (United States Department of Agriculture 2007).

In the collection of data released by the United States Department of Agriculture (nd), strawberries reported to contain 40 mg of flavonoids per 100 grams of her which was a large group of antioxidants called polyphenols. Polyphenols contained in strawberries that ellagic acid, catechins and anthocyanins. Over the last decade, scientists have become increasingly interested in the potential of various dietary flavonoids to explain some of the health benefits associated with fruit and vegetable-rich diet (Higdon 2005). Based on the studies that have been conducted by Erycesar (2007), found the minimum inhibitory concentration of strawberry extract against Streptococcus mutants by 12.5%.

Antibacterial effects of flavonoids found in strawberries can be used to cure infections. Infectious diseases are a state entry of microorganisms into the body, then multiply and eventually lead to disease. The so-called micro-organisms are bacteria, fungi and viruses. Microorganisms that can cause infection include bacteria. The bacteria can cause infections both locally and systemically. In general, infectious diseases can be cured with antibiotics. The use of antibiotics for local infection has been reduced due to the tendency of causing hypersensitivity locally on the skin or mucous membranes (Ganiswara 1995).

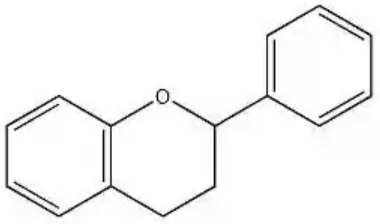

Figure 1. The basic structure of flavonoids

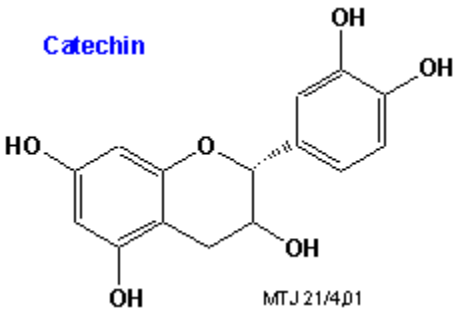

Figure 2. Structure of catechins

One particular strain of bacteria that often cause disease in humans is a bacterial infection of *Escherichia coli* (abbreviated *E. coli*). *E. coli* is a large and diverse group of bacteria. Although most strains of *E. coli* are harmless, certain types can make sick. Some types of *E. coli* can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other diseases (Centers for Disease Control and Prevention, 2011). As we know, lately there has been a large outbreak of *E. coli* in Europe, which has resulted in a number of deaths. Most cases were identified in the northern part of Germany, while some have been reported in other European countries. Cases associated with travel to Germany have also been reported in the United States and Canada. Most cases occur in adults and especially women. Strains of *Escherichia coli* outbreak still have not been identified yet suspected cause is a new strain that is more infectious and toxic.

In addition to the cases of epidemic in Western countries these days is, in Indonesia itself *Escherichia coli* has resulted in a high incidence of diarrhea. Approximately 162 thousand children die each year, or

about 460 children each day (Drug Information Center, Gadjah Mada University, 2011). From the results of Household Health Survey (Survey) in Indonesia, diarrhea is the second leading cause of death in infants and number 3 for infants and 5 for all ages.

Escherichia coli is a large and diverse group of bacteria. Although most strains of *E. coli* are harmless, Namu particular strain can make people sick. Some types of *E. coli* can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other diseases. Other types of *E. coli* is used as a marker for water contamination as is often heard about the *E. coli* found in drinking water, which may itself is not dangerous, but it is a sign of contaminated water.

One strain of *E. coli* is a common cause of diarrhea is *E. coli* O111. Enteropathogenic *E. coli* (EPEC) O111 is a classic cause of diarrhea in children, especially in developing countries (Durso et al 2007). Enterohemorrhagic *E. coli* (EHEC) O111, which usually carry at least one toxin gene Shiga-, is one of the non-O157 causes bloody diarrhea and hemolytic uremic syndrome-(HUS) which is most common in the United States (Brooks et al 2004), and also cause disease in Europe (Karch et al 1999), Asia (Jeon et al 2006, Kato et al 2005), and Australia (Paton et al 1996).

Symptoms of *E. coli* infection include abdominal cramps and diarrhea, which can lead to bloody diarrhea and hemolytic uremic syndrome (HUS). *E. coli* infection is transmitted from person to person by the fecal-oral route. Symptoms usually develop within two to 10 days after exposure (Public Health Agency of Canada, 2011). Therapy Effective treatment of diarrhea is still undiscovered. In the mid-20th century found a glucose-electrolyte solution for the treatment of acute diarrhea via oral, especially for infants and children. However there is a lack of ORS solution is commonly called, is issued by the patient stool volume is not reduced or even increased. Various attempts have been made to repair them by reducing the osmolality ORS standard with polymers such as starch-rich materials ("rich starch"), but the diarrhea persists until the infection is resolved.

Other than an unsuccessful attempt repairs maximum, the presence of bacterial resistance to antibiotics used is a different matter again. With the development of resistant bacterial populations, the antibiotics that were once effective in treating certain diseases reduced the value of its effectiveness. On the other hand, modern antibiotics also cause some side effects. This requires the development of new drugs to replace the different drugs that have not effectively and with minimal side effects.

Based on the data and facts above, the researcher intends to prove the effect of extracts of strawberry (*Fragaria x ananassa*) in inhibiting the growth of *Escherichia coli* in vitro by using the method of dilution and diffusion. This study aims to determine the antibacterial activity and inhibition of the extracts of strawberry (*Fragaria x ananassa*) against *Escherichia coli* and examine the ability of extracts of strawberry (*Fragaria x ananassa*) in several concentrations to inhibit the growth of *Escherichia coli* which is often a source of infectious diseases, particularly diarrhea, in Indonesia. The purpose of this study was to analyze the effect of strawberry extract (*Fragaria x ananassa*) in inhibiting the growth of pathogenic *Escherichia coli* serotypes 1-11.

MATERIALS AND METHODS

This research is observational research laboratory is testing the antimicrobial activity of fruit extracts of strawberry (*Fragaria x ananassa*) on the growth of pathogenic *Escherichia coli* serotypes 1-11 in vitro using dilution method. This study was designed using a randomized controlled design post test. The sample in this study is the fruit of the strawberry (*Fragaria x ananassa*) obtained in the market or shopping in the city of Surabaya. In this study the number of treatment groups were studied as much as 7 tube, so that the amount of replication that must be done at least 4 times. Research will be conducted at the Laboratory of Microbiology, Faculty of Medicine, University of Airlangga. The study was conducted for 7 months in July 2011-February 2012.

Test of antimicrobial activity using dilution method. With this method it can be seen minimal inhibitory concentration (MIC) is the smallest concentration that was able to inhibit the growth of bacteria and kill the minimum concentration (MBC) is the smallest concentration that can kill germs. Extract strawberries (*Fragaria x ananassa*) prepared with 100% concentration which will be diluted serially. Then we made the suspension of germs. Existing stocks were cultured in advance to obtain a healthy growth (thrives and the logarithmic growth phase or not mutated or phase lag or dead). Suspension initial test is made equivalent to 0.5 Mc Farland turbidity (cloudiness mixture of barium sulfate and HCl) or proportional to the number of bacteria 1. 108 CFU / ml (CFU: Colony Forming Unit) or 250-300 colonies on solid media. Taken a few colonies of bacteria then thinned or diluted with isotonic solution (PBS or PZ) so that the concentration in accordance with Mc Farland concentration of 0.5.

In the dilution test is provided as much as 8 vitro with the label T1-T7, and K1-K2. T1 tube filled with extracts of strawberry (*Fragaria x ananassa*) concentration of 100 to obtain the concentration of extracts of strawberry (*Fragaria x ananassa*) on T1 tube at 100%. T2 tube filled with strawberry fruit extract concentration of 100% and a liquid medium with a ratio of 1: 1. Between the liquid medium and the extract was mixed to obtain the concentration of strawberry fruit extract on the tube T2 by 50%. Then take half of the mixture between the liquid medium and extracts from this tube to be inserted into the tube T3.

T3 tube containing a mixture taken from a tube inserted into the tube T2 and T3 is added to the sterile liquid medium volume ratio mixture of T2 tube with sterile liquid medium at 1: 1. Mix well so that the concentration of the extract obtained strawberries (*Fragaria x ananassa*) on T3 tube by 25%. Then take half to put the tube T4. The above steps followed in succession by tube T7, after concentration of the extract obtained is mixed and strawberries (*Fragaria x ananassa*) on T7 tube of 1.5625%.Then taken half to be discarded so that the volume of the same T7 tube with another tube.

Then the bacteria suspension was added into each tube. Tubes K-1 is a control, only filled with fruit extracts strawberry (*Fragaria x ananassa*) up to the same volume with the other tube. Tubes K-2 is control, filled with a liquid medium which has been added a suspension of germs. All the tubes were incubated for 24 hours in a 37ºC temperature. Then we determine the minimum inhibitory concentration (MIC) is to see where the tube is still clear. In regard with concentration of strawberry fruit extract (*Fragaria x ananassa*), the smaller in the clear tube, the minimum the inhibitory concentration (MIC).

This observation is done visually. Each tube is still clear of each dilution was taken, and then replanted in order Nutrient plate and incubated for 24 hours in a temperature of 37ºC. Concentration of strawberry fruit extract (*Fragaria x ananassa*), the smallest on the tube, which after dilution replanted in order Nutrient plate showed no growth of germs, a minimum kill concentration (MBC).

This observation was also carried out visually. Data growth of pathogenic *Escherichia coli* serotypes 1-11 of the control group and the treatment group tested with descriptive statistics and Analysis of Variance (ANOVA), which if found significant differences followed by LSD (Least Significant Difference) with significance level of p <0, 05.

RESULTS

Table 1. Results of dilution MIC testing with extracts of strawberry (*Fragaria x ananassa*) in various concentrations in the first replication

Tube Name	Growth of *Escherichia coli*	
	In liquid media	In solid media (Plate)
T1 (100%)	+	+
T2 (50%)	+	+
T3 (25%)	+	+
T4 (12.5%)	+	+
T5 (6.25%)	+	+
T6 (3.125%)	+	Not performed
T7 (1.5625%)	+	Not performed
K+	+	+
K-	-	-

- Description:
T1-T7: a tube containing extracts of strawberry (*Fragaria x ananassa*) with a concentration of 100%; 50%; 25%; 12.5%; 6, 25%; 3.125%; and 1, 5625%
K +: control tube (+) containing germs + media
K -: tube control (-) contains extracts of strawberry (*Fragaria x ananassa*) concentration of 100% + liquid medium

Table 2. Results of dilution MIC testing with extracts of strawberry (*Fragaria x ananassa*) in various concentrations on the second replication

Tube Name	Growth of *Escherichia coli*	
	In liquid media	In solid media (Plate)
T1 (100%)	+	+
T2 (50%)	+	+
T3 (25%)	+	+
T4 (12.5%)	+	+
T5 (6.25%)	+	+
T6 (3.125%)	+	Not performed
T7 (1.5625%)	+	Not performed
K+	+	+
K-	-	-

- Description:
T1-T7: a tube containing extracts of strawberry (*Fragaria x ananassa*) with a concentration of 100%; 50%; 25%; 12.5%; 6. 25%; 3.125%; and 1. 5625%
K +: control tube (+) containing germs + media
K -: tube control (-) contains extracts of strawberry (*Fragaria x ananassa*) concentration of 100% + liquid medium

The first experiment showed that extracts of strawberry (*Fragaria x ananassa*) do not have antimicrobial activity against pathogenic bacteria *Escherichia coli* serotypes 1-11. This can be seen from the results of dilution test to determine the bacteriostatic effect (minimum inhibitory concentration) which shows the change in turbidity starts from the first tube (T1) which has a concentration of fruit extracts strawberry (*Fragaria x ananassa*) of 100% up to a tube-7 which has a concentration respectively 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.5625%. While the control variables obtained turbidity change in control (+) which

is the control of the growth of germs in the media and not found changes in turbidity in the control (-) control material containing strawberry extract.

Table 3. Results of dilution MIC testing with extracts of strawberry (*Fragaria x ananassa*) in various concentrations in the third replication

Tube Name	Growth of *Escherichia coli*	
	In liquid media	In solid media (Plate)
T1 (100%)	+	+
T2 (50%)	+	+
T3 (25%)	+	+
T4 (12.5%)	+	+
T5 (6.25%)	+	+
T6 (3.125%)	+	Not performed
T7 (1.5625%)	+	Not performed
K+	+	+
K-	-	-

- Description:
T1-T7: a tube containing extracts of strawberry (*Fragaria x ananassa*) with a concentration of 100%; 50%; 25%; 12.5%; 6. 25%; 3.125%; and 1. 5625%
K +: control tube (+) containing germs + media
K -: tube control (-) contains extracts of strawberry (*Fragaria x ananassa*) concentration of 100% + liquid medium

Table 4. Results of dilution MIC testing with extracts of strawberry (*Fragaria x ananassa*) in various concentrations in the fourth replication

Tube Name	Growth of *Escherichia coli*	
	In liquid media	In solid media (Plate)
T1 (100%)	+	+
T2 (50%)	+	+
T3 (25%)	+	+
T4 (12,5%)	+	+
T5 (6,25%)	+	+
T6 (3,125%)	+	Not performed
T7 (1,5625%)	+	Not performed
K+	+	+
K-	-	-

- Description:
T1-T7: a tube containing extracts of strawberry (*Fragaria x ananassa*) with a concentration of 100%; 50%; 25%; 12.5%; 6, 25%; 3.125%; and 1, 5625%
K +: control tube (+) containing germs + media
K -: tube control (-) contains extracts of strawberry (*Fragaria x ananassa*) concentration of 100% + liquid medium

The same dilution test results are also obtained in the replication of the 2nd and 3rd, and 4th in which there is a change in turbidity which starts from T1 to T7 tube, control (+) and no change in the turbidity of the control (-). In a study conducted there is difficulty in distinguishing turbidity obtained from the extract and from the growth of bacteria. Therefore, planting in order to plate, from planting to-1 replication in order this plate, obtained growth of germs on the tube to 1, 2, 3, 4, and 5. Tube 6 and 7 do not participate planted with consideration savings agar plate used. And the planting of control (+) obtained the growth of some bacteria in the agar plate. While the cultivation of the control plate (-) not found colonies of germs. In each subsequent replication with replication to-1, only the tube 1, 2, 3, 4, 5, and control (+) and (-), followed by planting in the agar medium. In replication tube 2, 3, and 4 results in accordance with replication to-1, ie, the tube plate 1 to 5 obtained growth of bacterial colonies.

DISCUSSION

Research on the content of the antimicrobial of extracts of strawberry (*Fragaria x ananassa*) on the growth of pathogenic *Escherichia coli* serotypes 1-11 was conducted in the laboratory of Microbiology, Faculty of Medicine, Airlangga University in December 2011. The study was conducted in an experimental test using the dilution method. A method that can be used to measure the Minimal Inhibitory Concentration an antimicrobial material by observing the change in the turbidity of the medium mixture, extract materials, and bacteria grown in the tube. In the research, the tube that contained changes in turbidity compared to the negative control (-) is defined as there is still growth of germs in it.

Materials made from crude drug extracts of strawberry (*Fragaria x ananassa*). Making extract conducted at the Research Institute and Consulting Industry (Laboratory Research and Consulting Industry) Surabaya. Strawberries (*Fragaria x ananassa*) have been demonstrated in relation to the content of flavonoids which is antibacterial in it (Erycesar 2007).

Flavonoids are phenolic that present in abundance in plants (Amelia 2005). Phenol is a potent antimicrobial when in direct contact with microorganisms (Walton & Torabinejad 2008). Flavonoids have antibacterial power that can kill or inhibit the growth of microorganisms. Mechanism of action of flavonoids as antibacterial is to denaturate molecules with protein and nucleic acid bacteria. Besides antibacterial flavonoids also works by damaging bacterial cytoplasmic membrane of cells (Pelczar & Chan 1988). Denaturation of proteins and nucleic acids occurs because flavonoids bacteria capable of forming a complex compound with the protein through hydrogen bonding (Harborne 1987). These conditions will cause permanent damage to the bacterial cell and subsequently cause the death of bacteria (Pelczar & Chan 1988).

Mechanism of action of antibacterial substances, including flavonoids, influenced by factors, among others, the type of substance, the type of bacteria, the speed of dissociation and diffusion of a substance, the

pH of the substance, viscosity, concentration, temperature, amount of bacteria, and the level hydrosolubility (Sugiyono 2005). Selection of *Escherichia coli* bacteria was done because it has been widely apart as normal flora in the human body, as well as pathogen capable of causing disease. Virulent strain of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rare cases, virulent strains are also responsible for the hemolytic-uremic syndrome, peritonitis, mastitis, septicemia and Gram-negative pneumonia. How to distinguish between *Escherichia coli* bacteria in the body's normal flora and pathogenic *Escherichia coli* is through a series of tests which, among others, consists of the use of selective and differential media, catalase test, oxidase, Gram staining, carbohydrate fermentation test, motility, gelatinase, and urease.

In the current work, the tube 1 extracts of strawberry (*Fragaria x ananassa*) directly mixed with the bacteria on the tube dilution to obtain a bacterial condition which is mixed with strawberry extract concentrate 100% homogeneous. As for the second tube 100% strawberry extract is mixed with the planting medium with a ratio of 1: 1 in order to get the condition of germs mixed with a liquid medium and extract homogeneous. At the tubes then performed the addition of a mixture of extracts of growing media + previous growing media (serially). Research carried out for 4 times. Determination of the amount of replication is based on the formula of Freeder (Steel & Torrie 1989).

According to the results presented in Table 5.1, of the present study found changes in turbidity at all tube that has been planted *Escherichia coli* except in the negative control tube (K), which can be interpreted that the extracts of strawberry (*Fragaria x ananassa*) do not have antimicrobial activity to the level of 100% and the negative control, the absence of changes in turbidity indicates that extracts of strawberry (*Fragaria x ananassa*) no contamination.

Similar results were also obtained in the replication of the 2nd, 3rd, and 4th are presented in tables 5.2, 5.3, and 5.4. In these tables it can be seen that persists turbidity change starts from the first tube to tube-7 with the exception of the tube with the K mark. After all observed changes in turbidity observation tube, then we planted bacteria into Nutrient Agar. The purpose of planting germs on nutrient this order is to ensure that changes in turbidity on the observation tube is really due to the growth of bacteria. From planting germs in order to obtain that result Nutrient planting of tubes T1 to T5 obtained colony growth of germs. So it can be concluded that the turbidity changes that occur in the tube T1 to T7 due to the growth of pathogenic *Escherichia coli* bacteria colonies serotypes 1-11.

Although the research made by Erycesar (2007) explains that the strawberries are flavonoid-containing antibacterial activity, this study proves the content of strawberry fruit extract (*Fragaria x ananassa*) does not have an effective antibacterial activity against *Escherichia coli* pathogenic serotypes 1-11. There are several factors that can affect the results of this study, among others, is the selection of the active substance means that the separation of strawberries (*Fragaria x ananassa*) is less precise. Factors that influence are the nature of the crude drug, the stability of the crude drug (thermolabile or not), the price of crude drug, solvent, product concentration, recovery of solvent (Handa et al 2008). The next factor is the procedure of making extracts that do not conform to the standard, as is exemplified in the study by Agustin (2007) includes washing with water, cutting into small, aerated to dry (without sunlight), immersion in 96% ethanol for 24 hours, filtering with a Buchner funnel and suction flask; The next result is processed with rotary immersion (Department of Health 2000). Then, when the atmosphere is less awake research can lead to contamination when conducting research. Although antibacterial substances (flavonoids, tannins, anthocyanins, ascorbic acid, salicylic acid) present in strawberry fruit extract, possible active substances this implies little in strawberries (*Fragaria x ananassa*). The active substance in the form of salicylic acid and flavonoids and other active substance is antibacterial in strawberry (*Fragaria x ananassa*) may be damaged during manufacture extracts.

In addition, the bacteria in this study were classified as *Escherichia coli* gram-negative bacteria that have a sheath cells are very complex and multi-layered structure, and has a special component of the cell wall, namely lipoprotein, outer membrane and lipopolysaccharide. Besides *E. coli* pathogen also has a system of protection against the bactericidal substances in the form of K antigen and LPS (lipopolysaccharide). Antibacterial substances in strawberry (*Fragaria x ananassa*) that may dissolve in the manufacture of extracts can not penetrate the cell protection components pathogenic *Escherichia coli* serotypes 1-11. In addition, the concentration of the extract strawberries (*Fragaria x ananassa*) still has not reached the Minimum Inhibitory Concentration (MIC) to inhibit the growth of pathogenic *Escherichia coli* serotypes 1-11. The results of this study showed no antibacterial properties on strawberries against pathogenic *Escherichia coli* serotypes 1-11, but there is still the possibility of strawberries with vitamin C it is able to reduce the vulnerability of diarrhea by *E. coli* pathogens

by increasing the body's immune system or by role as a cofactor in the repair of damaged cells, including cell regeneration.

CONCLUSION

There are no antimicrobial activity in extracts of strawberry (*Fragaria x ananassa*) on the growth of pathogenic *Escherichia coli* serotypes 1-11.

REFERENCES

Agustin ET (2007). Daya antibakteri ekstrak buah makutadewa (Phaleria macrocarpa (Scheff) Boerl) terhadap bakteri Streptococcus alpha haemolyticus: penelitian laboratoris. Bachelor Thesis. Universitas Airlangga, Surabaya

Amelia A (2005). Mahkota Dewa sebagai Bahan Obat Ditinjau dari Segi Kedokteran dan Islam. Available from http://www.bicaramuslim.com/bicara7/viewtopic.php?p=143977&sid=882bac2f3fa1f32d390d557ef94e5305. Accessed January 15, 2012

Brooks JT, Bergmire-Sweat D, Kennedy M, Hendricks K, Garcia M, Marengo L, Wells J, Ying M, Bibb W, Griffin PM, Hoekstra RM, Friedman CR (2004). Outbreak of Shiga toxin-producing *Escherichia coli* O111:H8 infections among attendees of a high school cheerleading camp. Clin Infect Dis 38, 190-198

Centers for Disease Control and Prevention (2011). National Center for Zoonotic, Vector-Borne, and Enteric Diseases. Available from http://www.cdc.gov/nczved/divisions/dfbmd/diseases/ecoli_o157h7/. Accessed June 5, 2011

Departemen Kesehatan RI (2000). Parameter Standar Umum Ekstrak Tumbuhan Obat, Jakarta, Dirjen Pengawasan Obat dan Makanan Direktorat Pengawasan Obat Tradisional, p 147-148

Durso LM, Bono JL, Keen JE (2007). Molecular serotyping of *Escherichia coli* O111:H8. J Microbiol Methods 69, 381-383

Erycesar GY (2007). Perbandingan efek antibakteri jus stroberi (Fragaria vesca L) pada berbagai konsentrasi terhadap Streptococcus mutans. Bachelor Thesis. Universitas Diponegoro, Semarang

Ganiswara SG (1995). Farmakologi dan Terapi, 4th ed, Jakarta, Bagian Farmakologi FKUI

Handa SS, Khanuja SPS, Longo G, Rakesh DD (2008). Extraction Technologies for Medical and Aromatic Plants, Trieste, United Nations Industrial Development Organization and the International Centre for Science and High Technology

Harborne JB (1987). Metode Fitokimia Penuntun Cara Modern Menganalisis Tumbuhan, 2nd ed, Bandung, Penerbit ITB, p 48

Higdon J (2005). Micronutrient Information Center. Available from http://lpi.oregonstate.edu/infocenter/phytochemicals/flavonoids/#intro. Accessed June 5, 2011

Jeon BW, Jeong JM, Won GY, Park H, Eo SK, Kang HY, Hur J, Lee JH (2006). Prevalence and characteristics of *Escherichia coli* O26 and O111 from cattle in Korea. Int J Food Microbiol 110, 123-126

Karch H, Bielaszewska M, Bitzan M, Schmidt H (1999). Epidemiology and diagnosis of Shiga toxin-producing *Escherichia coli* infections. Diagn Microbiol Infect Dis 34, 229-243

Kato K, Shimoura R, Nashimura K, Yoshifuzi K, Shiroshita K, Sakurai N, Kodama H, Kuramoto S (2005). Outbreak of enterohemorrhagic *Escherichia coli* O111 among high school participants in excursion to Korea. Jpn J Infect Dis 58, 332-333

Paton AW, Ratcliff RM, Doyle RM, Seymour-Murray J, Davos D, Lanser JA, Paton JC (1996). Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin-producing *Escherichia coli*. J Clin Microbiol 34, 1622-1627

Pelczar MJ and Chan ECS (1988). Dasar-dasar Mikrobiologi 2, Jakarta, UI Press, p 465-459

Public Health Agency of Canada (2011). E coli Outbreak in Europe. Available from http://www.phac-aspc.gc.ca/tmp-pmv/thn-csv/ecoli-eng.php. Accessed Juni 7, 2011

Pusat Informasi Obat Universitas Gadjah Mada (2011). Kasus Diare di Indonesia. Available from http://piogama.ugm.ac.id/index.php/2009/01/epidemiologi-kasus-diare/. Accessed June 7, 2011

Steel RGD and Torrie JH (1989). Prinsip dan Prosedur Statistika, Jakarta, Gramedia Pustaka

Sugiyono (2005). Statistika untuk Penelitian, Bandung, CV Alfabeta, p 68-70

United States Department of Agriculture (2007). USDA Database for the Flavonoid Content of Selected Foods Release 2.1. Available from http://www.scribd.com/doc/43839695/USDA-Database-for-the-Flavonoid-Content. Accessed Juni 3, 2011

United States Department of Agriculture (nd). US Strawberry Industry. Available from http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1381. Accessed Juni 3, 2011

Walton RE and Torabinejad M (2008). Prinsip dan Praktik Ilmu Endodonsia, 3rd ed, Jakarta, Penerbit Buku Kedokteran EGC, p 295