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Test the effectiveness of antibacterial effect of the skin of the pomegranate fruit (*Punica granatum*) extract against the growth of *Escherichia coli in vitro*

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ABSTRACT

Background: Pomegranate (*Punica granatum*) is one of the traditional medicines that contains antibacterial compounds that are effective against bacterial growth. Its chemical content includes saponins, tannins and flavonoids. Escherichia coli is a gram-negative bacterium which is a normal flora germ found in the human large intestine. These bacteria are pathogenic when they are outside the intestine and produce enterotoxins in epithelial cells which cause diarrhea. The purpose of this study was to determine the antibacterial effect of pomegranate skin extract on the growth of *Escherichia coli*.

Methods: This study used an experimental design study with a complete randomized design study divided into 5 groups namely groups 1 (15 ug/ml), 2 (20 ug/ml), 3 (25 ug/ml), positive control (ciprofloxatin), negative control. Making pomegranate peel extract was done by maceration method then rotary, after that the effectiveness of pomegranate extract extracted by the diffusion method was tested using Anova one-way test.

Results: The results showed that the extract of pomegranate peel showed that it was able to inhibit the growth of *Escherichia coli* with a ratio of constants (15 ug/ml, 20 ug/ml, 25 ug/ml with inhibition diameters of 6.7 mm, 6.7 mm, while for positive control with ciprofloxatin showed bacterial resistance to antibiotics.

Conclusions: Statistically, pomegranate skin extract has an antibacterial power which is meaningful with p 0.005. Pomegranate rind extract has antibacterial activity against *Escherichia coli* medium because inhibition zone is 5-10 mm.

Keywords: Antibacterial, Escherichia coli, Pomegranate

INTRODUCTION

Diarrhea is a major cause of morbidity and mortality in children under five, estimated at about 1.3 million deaths of children under five years of age occur all over the world. The prevalence of high incidence of diarrhea in Indonesia in the year 2018 occurs in young toddlers (age 1-5 years), namely by 6.7%. Morbidity and mortality of diarrhea in Indonesia is still high.

Based on a survey of morbidity carried out by the Ministry of Health of the year 2010 s/d 2018 noticeable

trend of increased incidence of diarrhea. Percentage of the morbidity of diarrhea in the year 2010 is 37.4%, then increased to 42.3% in 2012, and decreased by 41.1% in year 2013.² The percentage of cases of diarrhea in children under five in the Province of West Sumatra in the year 2018 by 31.400 cases (department of health of West Sumatra Province, 2018). Diarrheal diseases still occupy the order of 10 common diseases in the City Padang.³

Escherichia coli is often the cause of infection of the urinary tract, biliary tract and other places in the

abdominal cavity. *Escherichia coli* is the cause of diarrhea and tract infections kemih. ⁴ *E. coli* belongs to the bacteria heterotrophs that obtain food in the form of substances organic from the environment because cannot draw up its own organic substances that it needs. These bacteria are pathogenic if they are outside the intestines and produce enterotoxins which cause diarrhea and are associated with enteropathogenic produce enterotoxins on the cell epitel. ⁵

Infectious diarrhea caused by bacteria can actually be done with natural ingredients, namely, insufficient fluid intake of the body. However, if diarrhea cases are settled more than two days it is recommended to do a medical consultation. Usually the doctor will give you antibiotics if the diarrhea is caused by bacteria.⁶ Administration of antibiotics that are not rational can trigger the occurrence of bacterial resistance. Bacteria that are resistant to antibiotics also caused due to the use of antibiotics which is increasingly extends.⁷ Therefore, with the presence of bacteria that are resistant to antibacterial will encourage the importance of utilization of herbal remedies in natural.⁸

Plants that can be used as a herbal medicine d acts as an antibacterial is the pomegranate (Punica granatum L.).9 The content of antibacterial namely alkaloids, saponins, and flavonoids are potential as kemoprotektif and is able to inhibit the lipid peroxide is non-enzymatic. The higher the levels of flavonoids, the potential antioxidant will be higher. Mechanism of action of flavonoids as antibacterial compounds are divided into three, namely inhibiting the synthesis of nucleic acids, inhibits the function of cell membranes, and inhibit the metabolism energy. 10 Alkaloids are nitrogen compounds heterocyclic containing at least one nitrogen atom and is alkaline. The cluster bases will react with the acidic compounds that exist in bacterial cells such as DNA, which is the main constituent of the cell nucleus. With the disruption of the DNA, then the synthesis of proteins and nucleic acids in the cells will disturbed.¹¹

Because of the usefulness of this, pomegranate has a lot of be used as one of the alternative treatment traditional.¹²

Each active substance has a different mechanism as an antibacterial. Previous research has shown that the methanol extract of pomegranate peel has been shown to have activity against the bacteria *Shigella dysenteriae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli*. ¹³ The purpose of this study was to determine the antibacterial effect of pomegranate skin extract on the growth of *E. coli*.

METHODS

This study used an experimental design study with a complete randomized design study divided into 5 groups, group 1 (25 ug/ml), 2 (20 ug/ml), 3 (15 ug/ml), positive control (Ciprofloxatin 500 mg), negative control (aquades). Ciprofloxatin 500 mg dosage form was suspension powder that was liquefied into 1 cc of sterile aquades. After that the antibiotic disk was dissolved. Making pomegranate peel extract was done by maceration method then rotary, after that the testing of the effectiveness of pomegranate peel extract was done by diffusion method by using ANOVA one-way test.

This research was conducted at the regional health laboratory of West Sumatra Province. Held on 4th November - 7th December 2019. With inclusion criteria ripe pomegranate skin. Making pomegranate rind extract was done by maceration method then rotary, after that the testing of the effectiveness of pomegranate extract was carried out by diffusion method.

RESULTS

The results of the research can be seen in Table 1 based on the calculation that has been done. Based on the calculation that has been done, the average value of the diameter of the inhibition zones in group 1 at 6.7 mm, group 2 of 6.7 mm, group 3 by 7.7 mm, and the negative control group and positive control do not have the power resistor because the average value of the diameter of the inhibitory zone of 0.00 mm.

Table 1: Inhibition zone diameter of pomegranate rind extract (*Punica granatum*) against *Escherichia coli* bacteria by diffusion method.

Treatment	Deuteronomy			A 21020 (D*)
	1	2	3	Average (D*)
Positive control	0	0	0	0
Negative control	0	0	0	0
1 (15 ug/ml)	6 mm	7 mm	7 mm	6.7 mm
2 (20 ug/ml)	6 mm	7 mm	7 mm	6.7 mm
3 (25 ug/ml)	7mm	8 mm	8 mm	7.7 mm

DISCUSSION

The results of the study showed that the average value of the diameter of the inhibition zone of one group of 6.7 mm, a group of two 6.7 mm, a group of three 7.7 mm, negative control group and positive not have a zone of inhibition. In the positive control group was given treatment with the antibiotic ciprofloxacin, the zone of

inhibition is not formed. It is shown that ciprofloxacin is not likely to provide the effect of the inhibition at low concentrations. So that confirms that the results of the antibiotic ciprofloxacin resistance to bacteria E. coli. Antibiotics group florokuinolon the most widely used for the treatment of infections is ciprofloxacin, especially that caused by gram-negative bacteria, especially E. coli.14 Resistance E. coli to the antibiotic ciprofloxacin is generally caused by chromosomal mutations in the genes gyr A and par C. However, recent research shows that resistance to a low level can also be mediated by plasmids through the acquisition hen gnr-mediated by plasmids pMG252.¹⁵ Increased resistance of *E. coli* to the antibiotic ciprofloxacin has been widely reported. Other studies reported as much as a 20,65% isolates of E. coli resistant against ciprofloxatin among the 155 isolates clinic E. coli in Pakistan. A specimen of urine patients with UTI in RSUD Abdoel Moeloek (RSUDAM) Lampung Province and obtained 30 isolates positive for E. coli. 16 Research in Makassar found 48% isolates of E. coli resistant ciprofloxatin among the 39 isolates positive for E. coli. 17

According to the WHO (2012), inaccuracy as well as not rational the use of antibiotics is the cause of most of the main spread of resistant microorganisms. So, a drug effective for the treatment, then it should reach the place of its activity in the body with accuracy and a sufficient amount to produce a concentration of effective. 18 Antibiotics will experience transportation is dependent with the power process to plasma proteins. The form that not bound by such a protein which is pharmacologically active, have the ability as antibakteri. 19 Other mechanisms that cause resistance is decreased accumulation of the drug in the cells by the increase in the pump efflux to native and decreased membrane outer porins.²⁰ Some species of *Enterobacteriaceae*, including bacteria E. coli have a chromosomal native pump efflux AcrAB-To1C which belongs to the families RND (resistance-nodulasi division).²¹

The criterion of strength antibacterial power is divided into negative control group did not have a zone of inhibition from the results, the look the higher the concentration the inhibition pomegranate the larger the diameter of the inhibition zone formed. The inhibition zone diameter of 5 mm or less is categorized weak, the diameter of the inhibition zone 5-10 mm categorized as moderate, the diameter of zone of inhibition of 10-20 mm are categorized powerful and the inhibition zone 20 mm or more are considered very strong. This means that the bacteria *E. coli* sensitive to extracts of pomegranate peel because the results of inhibition the greater the in accordance with the high concentration given.

The inhibition zone formed around the disk that has spilled fruit extract pomegranate red shows that the extract contains active compounds that are as antibacterial.²³ The content of the antibacterial contained in pomegranates, namely alkaloids, saponins, and flavoid potential as kemoprotektif and is able to inhibit the lipid

peroxide is non-enzymatic. The higher the levels of flavonoids, the potential antioxidant will be higher.²⁴ Because of the usefulness of this, pomegranate has a lot of to uses one of the alternative treatments traditional each active substance has a different mechanism as an antibacterial. Mechanism of action of flavonoids as antibacterial compounds are divided into three, namely inhibiting the synthesis of nucleic acids, inhibits the function of cell membranes, and inhibit the metabolism energy.²⁵

The mechanism in inhibiting the synthesis of nucleic acids is to inhibit the formation of DNA and RNA through the ring A and B which play a role in hydrogen bonding. This leads to the buildup of bases of nucleic acid, and the occurrence of damage to the permeability of the bacterial cell wall, lysosomes, as well as mikrosom.²⁶ The mechanism in inhibiting the function of cell membranes is by forming complex compounds with proteins of the extracellular and dissolved which cause damage to bacterial cell membranes and followed by a discharge of compound is intracellular. While the mechanism of flavonoids in inhibiting energy metabolism is to inhibit the cytochrome C reductase and inhibits the use of oxygen on the bacteria. Whereas the energy needed bacteria in the conduct of the biosynthesis of macromolecules.²⁷ Alkaloids are nitrogen compounds heterocyclic containing at least one nitrogen atom and alkaline Cluster bases will react with the acidic compounds that exist in bacterial cells such as DNA, which is the main constituent of the cell nucleus. With the disruption of the DNA, then the synthesis of proteins and nucleic acids in the cells will disturbed.²⁸

CONCLUSION

Statistically the ethanol extract of pomegranate peel has antibacterial power that meaningful with p 0.005. The ethanol extract of pomegranate peel has antibacterial activity against the bacteria *Escherichia coli* was due to the inhibitory zone of 5-10 mm.

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