Antimicrobial Purple Okra

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Antimicrobial inhibition zone test of purple okra (Abelmoschus esculentus) extract on the growth of gram-negative bacteria

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ABSTRACT
Okra (*Abelmoschus esculentus*) contains secondary metabolite compounds that can function as an antibacterial which can inhibit the growth of gram-negative bacteria. This study aims to determine the antimicrobial inhibition zone of purple varieties okra extract on the growth of gram-negative bacteria (*Escherichia coli*). This research is an experimental study using a Completely Randomized Design (CRD) with a disk diffusion testing method. The object used was purple okra fruit extract at concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% with negative control of DMSO and positive control of chloramphenicol. Inhibition zone diameter measurements are indicated by clear areas, ie areas that are not overgrown with bacteria. The results showed that the most optimum inhibition zone of purple varieties of okra fruit extract was at a concentration of 40%, 50% and 60% with an average value of 6.67 mm.

INTRODUCTION

Indonesia is a tropical country whose people are often infected with various infectious diseases caused by microbes such as itching, colds or diarrhoea which are often caused by bacteria. One of the agents that cause bacterial infection is *Escherichia coli* which is a bacterium in the lower digestive tract and can turn into a pathogen if its development in the body exceeds normal limits.[1] *Escherichia coli* is a gram-negative, straight rod-shaped bacterium with periostracum or non-motile flagellum, which grows easily on a simple nutrient medium. The cell wall has little peptidoglycan layer and does not contain acids, but contains several polysaccharides and is more susceptible to mechanical and chemical damage.[2] This bacterium is resistant to beta-lactam and its prevalence in China is 14%, Hong Kong 13%, Philippines 6.2%, Singapore 4%, Taiwan 13.8%, and Japan 1.4%.[3, 4]

E. coli is the main species that can cause health problems in humans (infection) so it must be handled properly. The high incidence of infection, as well as the resistance to antibiotics, is a major problem in treatment so that new strategies are needed in establishing alternative therapies for the treatment of infections.[5, 6] Treatment of diseases that continues to increase demands the role of various fields to find various types of new medicines. Lifestyle back to nature is becoming a trend at this time so that the people of Indonesia in particular re-use various natural ingredients, including treatment with medicinal plants (herbs) .[7]

The use of natural ingredients as a traditional medicine in Indonesia has been carried out by our ancestors for centuries ago. The trend in the use of traditional medicines in the world is caused by the increasing side effects of the use of chemical

drugs. The presence of bioactive components in plants is known to have an antibacterial effect. So that at this time many tested for antibacterial effects using natural ingredients. Natural ingredients have lower side effects compared to chemical drugs, besides they are cheap and easy to obtain. One of the natural ingredients that can be used as an antibacterial is okra fruit (*Abelmoschus esculentus*).

Okra (*Abelmoschus esculentus*) is commonly used as a vegetable, infused water and often used as a pickle. The okra plant is an annual plant that can grow to a tree height of about 2 meters, can grow well in soils under sunlight with a pH ranging from 6 to 6.7 but can tolerate various types of soil from pH 5.5 to 8.[8] Okra fruit shows low calories, has a good source of fibre that can be eaten, contains important bioactive compounds such as carotene, folic acid, thiamine, riboflavin, niacin, vitamin C, oxalic acid and amino acids.[9] Okra can also be used as a source of protein, carbohydrates, minerals, vitamins, and dietary fibre as well as alternative oil sources.[10]

Okra has mucus that can be applied as a drug, which is used as a replacement for plasma or blood volume.[11] Okra fruit is known to be used as a remedy for several chronic diseases, such as for recovery of dysentery, gastric irritation, irritation of the large intestine, laryngitis and gonorrhea.[12] The consumption of okra is highly recommended because it has nutritional value and antibacterial activity against pathogens, which are most often associated with diseases such as diarrhoea and salmonellosis.[13]

The importance of nutrition and the benefits contained in the okra fruit makes the plant widely produced commercially. Testing the antimicrobial efficacy of medicinal plants against test microbes by in vitro needs to be done so that the concentration of medicinal plants that is most effective in inhibiting the growth of test microbes is needed.[14] So, to obtain scientific evidence of the use of okra as a natural antibiotic in medicine, it is necessary to research by testing various concentrations of purple okra extract on the growth of gram-negative bacteria (*Escherichia coli*). This research is quite important because it can be used as an alternative material for natural medicine to overcome various health problems that can be caused by *Escherichia coli* bacteria.

MATERIALS AND METHODS

This research is an experimental study using a completely randomized design (CRD) which describes the antimicrobial inhibition zone test of okra extract (*Abelmoschus esculentus*) of purple varieties on the growth of gram-negative bacteria. The population in this study were all okra plants and all pathogenic bacteria that cause disease in humans. The samples in this study were purple varieties of okra fruit extracts with concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% as well as *Escherichia coli* bacteria using negative control DMSO and positive control of chloramphenicol.

The research was carried out at the Microbiology Laboratory of the State University of Malang in July-August 2019. The independent variable was the concentration of purple okra extract (*Abelmoschus esculentus*) and the dependent variable was the inhibitory zone of gram-negative bacteria (*Escherichia coli*). The tools used in the form of blenders, knives, petri dishes, tweezers, incubators, spirits lamps, laminar airflow (LAF), callipers, needle inoculation berries, analytical balance, and test tubes, while the material used is a pure culture of Escherichia coli, fruit purple okra, NA medium, distilled water, sterile cotton bud, and filter paper.

The test method uses disc diffusion by measuring the diameter of the inhibitory zone of *E. coli* bacteria growth at each concentration of purple okra fruit. Bacterial growth inhibition zone diameter = clear zone diameter - disc diameter. The data obtained were analyzed by ANOVA one way analysis with SPSS. If the analysis results F count> F table 0.05, then further tests with Post Hoc Analysis Least Significant Difference (LSD) with a significance level of 5% to determine differences in antimicrobial inhibition zones of purple okra fruit extract (*Abelmoschus esculentus*) on the growth of *Escherichia coli* bacteria.

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RESULTS AND DISCUSSION

Results of research on antimicrobial inhibition zones of purple okra (*Abelmoschus esculentus*) on the growth of *Escherichia coli* bacteria using the disc diffusion method and the results of measuring the average diameter of inhibition zones can be seen in Figure 1.

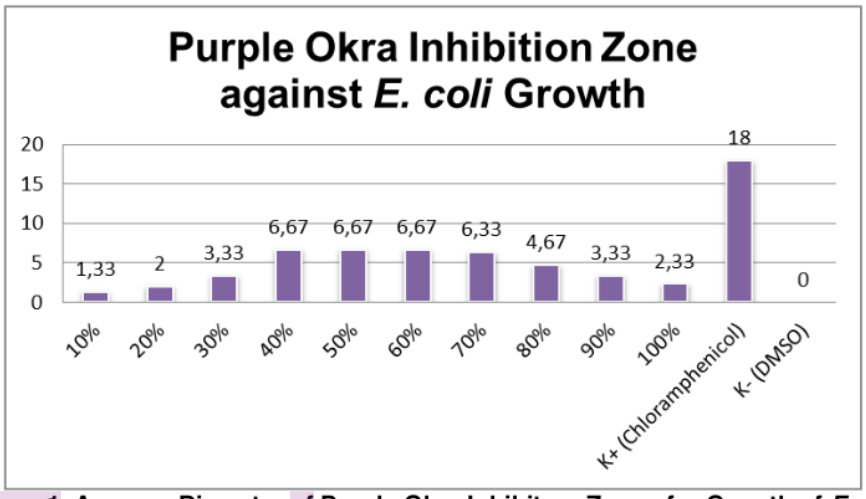

Figure 1. Average Diameter of Purple Okra Inhibitory Zones for Growth of *E. coli*

Figure 1 above shows that the purple okra can inhibit the growth of *Escherichia coli* bacteria, this is evidenced by the inhibition zone that can be found in testing with agar medium. The most optimum concentration to inhibit the growth of test bacteria is a concentration of 40%, 50%, and 60%, with 6.67 mm. Okra fruit can inhibit the growth of *E. coli* bacteria even though it is not as good as the positive control (chloramphenicol). The diameter of the growth inhibition zone of *E. coli* bacteria formed at extract concentrations of 40%, 50% and 60% is greater than that of other extract concentrations. This proves that okra fruit has the optimum concentration to suppress the growth of *E. coli* bacteria which can be seen from the diameter inhibition zone formed around the disc paper.

In addition to the concentration factor, the type of antimicrobial material produced also determines the ability to inhibit bacterial growth.[15] In this study, the antibacterial activity of okra fruit was due to the presence of nutritious compounds, such as flavonoids, tannins, saponins, triterpenoids, steroids, and alkaloids.[16] Flavonoids cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes as a result of interactions between flavonoids and bacterial DNA.[17] Flavonoids have lipophilic properties so it is possible to damage bacterial cell membranes.[18] Besides, flavonoids are also useful as a powerful antioxidant in reducing the risk of chronic disease, prevention of several cardiovascular diseases, the process of cancer, anti-inflammatory, antibacterial, anticoagulant, and antiallergic. The antibacterial mechanism of flavonoid compounds is thought to denaturation bacterial cell proteins and damage cell membranes beyond repair.[4]

Tests using one-way Anova showed the calculated F value (41.199)> F table 0.05 (3.98) so that the purple varieties of okra extract can inhibit the growth of gram-negative bacteria (*Escherichia coli*). The results of further tests with Post Hoc Analysis Least Significant Difference (LSD) can be seen that the inhibition zone diameter is significantly different in the positive control of chloramphenicol (Figure 2), while the

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treatment group at a concentration of 40%, 50% and 60% produces the best inhibition zone compared to all treatments, but not significantly different with concentrations of 70% and 80% but significantly different from concentrations of 10%, 20%, 30%, 90% and 100%. Inhibition zone diameters for negative control DMSO showed significant differences in positive control and various extract concentrations. The negative control shows the absence of inhibitory zones (Figure 2), this indicates that the control used does not affect the antibacterial test. Positive control showed antibacterial activity against test bacteria compared with the negative control.

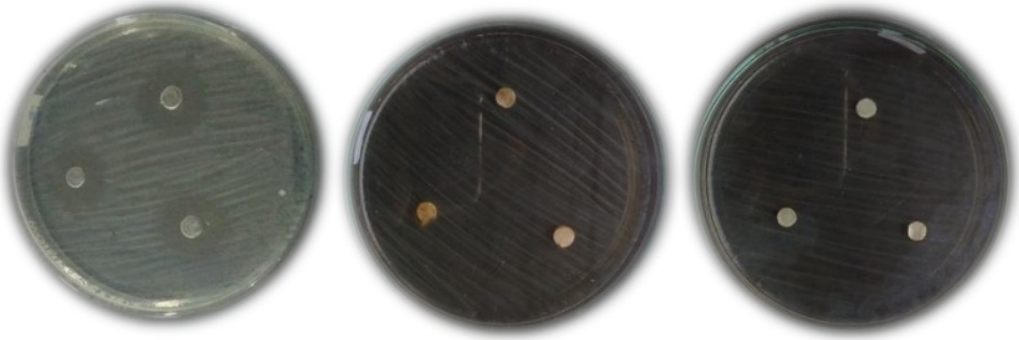

Figure 2. Disk diffusion in positive control of chloramphenicol (left), negative control of DMSO (middle), and concentration of 50% (right)

Okra can be can inhibit the growth of *Escherichia coli* bacteria because it has a lot of chemical contents. The profile of the bioactive components in different parts of okra is well documented: for okra pod polyphenolic compounds, carotene, folic acid, thiamine, riboflavin, niacin, vitamin C, oxalic acid, and amino acids.[7, 9, 19] Utilization of "Okra" is not only as food (vegetables), but also has the potential as a traditional medicinal ingredient. Many of components (flavonoids, polysaccharides, and vitamins) of okra have been proved to possess significant biological activities.[20, 21, 22, 23] Okra is commonly used for several diseases such as anticancer, antibacterial, hypoglycemic, and antiulkus.[24] Other benefits of okra are cholesterol-lowering, hypertension, antidiabetic, dysentery, hemorrhoids, and anti-inflammatory.[12]

CONCLUSIONS

Purple Okra fruit has the effectiveness to inhibit the growth of *Escherichia coli* bacteria, where the antimicrobial inhibition zone at the concentration of 40%, 50% and 60% yields the best inhibition zone compared to all treatments, but not significantly different from 70% and 80% concentration. Inhibition that occurs in *Escherichia coli* bacteria proves that purple okra fruit extract can be used as an alternative to natural medicine (back to nature).

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