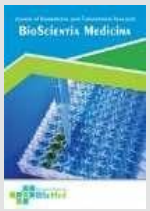
eISSN (Online): 2598-0580



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

The Effect of Red Okra Fruit Extract (*Abelmoschus esculentus*) on *Lactobacillus acidophilus* Growth

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ARTICLE INFO

Keywords:
Effectiveness
Antibacterial
Lactobacillus acidophilus
Red okra

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/bsm.v6i17.728

A B S T R A C T

Background: Dental caries occupies the top rank of dental and oral diseases that many Indonesians complain about. Dental caries is caused by *Lactobacillus acidophilus*. One of the latest treatments for caries disorders is by giving mouthwash. Mouthwash currently circulating, almost all contain chlorhexidine as the main ingredient. However, there are side effects from using chlorhexidine for a long period of time, namely discoloration of the teeth, which cannot be removed simply by brushing the teeth. Red okra fruit contains flavonoids which are useful as antibacterial. This study aimed to determine the potential of red okra fruit extract (*Abelmoschus esculentus*) as an antibacterial against *Lactobacillus acidophilus* in vitro. **Methods:** In vitro experimental studies. A total of 28 petri dishes contained bacterial cultures of *Lactobacillus acidophilus* grouped into 7 groups consisting of 2 control groups and 5 treatment groups of red okra fruit extract concentration of 10% -50%. Inhibition zone diameter analysis was carried out with the help of SPSS software using univariate and bivariate methods. **Results:** The group that received chlorhexidine showed the highest ability to produce the largest diameter of the inhibition zone compared to all treatment groups. Along with increasing the dose of red okra fruit extract, the ability of okra fruit extract to increase in producing a larger diameter of the inhibition zone. **Conclusion:** Red okra fruit extract shows effectiveness as an antibacterial *Lactobacillus acidophilus* and increases the extract's concentration.

1. Introduction

Dental and oral disease is a health problem that is quite dominantly complained about by the people of Indonesia. The results of the study show that around 57.6% of Indonesia's population have complaints about dental and oral health. Dental caries occupies the top rank of dental and oral diseases that many Indonesians complain about. Dental caries is caused by two main bacteria, *Streptococcus mutans,* and *Lactobacillus acidophilus.* Bacteria *Streptococcus mutans* has a role in caries, namely when the process of caries formation begins, and then it is continued by *Lactobacillus* bacteria which becomes a continuation of caries and secondary infection. *Lactobacillus acidophilus* is the dominant species among other *Lactobacillus* bacteria. These bacteria have expertise in metabolizing carbohydrates into acid and lowering the pH of plaque which causes the demineralization process to occur, causing caries. *Lactobacillus acidophilus*, namely gram-positive bacteria, can grow in anaerobic conditions and cause secondary caries. These bacteria are often found in saliva, which

contributes to caries. One of the latest treatments for caries disorders is by giving mouthwash.[1-5]

Mouthwash currently circulating, almost all contain chlorhexidine as the main ingredient. Chlorhexidine is able to destroy bacterial cell walls and is able to inhibit bacterial growth and inhibit the growth of dental plaque. However, there are side effects from using chlorhexidine for a long period of time, namely discoloration of the teeth, which cannot be removed simply by brushing the teeth. This encourages efforts to explore mouthwash ingredients with minimal side effects and the same efficacy as chlorhexidine. Indonesia is a country that has a variety of natural resources. Fruits grow in various ways, one of which is red okra. Red okra (*Abelmoschus esculentus*) is a fruit that grows in tropical countries such as Asia and Africa. In addition, this fruit is known to have many benefits for the body. Usually, people use the fruit or boil it as a vegetable. In Indonesia, this fruit is not very popular, but according to previous studies, red okra contains higher levels of flavonoids than green okra. Flavonoids are secondary metabolite compounds that are useful as antibacterials. Several studies have shown the benefits of flavonoid content as an antibacterial against various gram-positive and gram-negative bacteria. This study aimed to determine the potential of red okra fruit extract (*Abelmoschus esculentus*) as an antibacterial against *Lactobacillus acidophilus* in vitro.[6-10]

2. Methods

This study was an in vitro experimental study and used bacterial culture *Lactobacillus acidophilus* on petri dishes obtained from the laboratory of microbiology, Faculty of Pharmacy, Universitas Sumatra Utara, Medan, Indonesia. Red okra (*Abelmoschus esculentus*) was the test material, and the extraction process was carried out using 96% ethanol solvent by maceration for 1x24 hours. The macerate resulting from maceration is thickened into an extract using a rotary evaporator. This study was approved by the medical and health research ethics committee, Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia (Number: 013/KEPK/UNPRI/XI/2022).

Bacterial culture *Lactobacillus acidophilus* standardization of bacterial concentrations was carried out using McFarland 0.5 solution. The similarity of turbidity levels showed the same concentration of bacteria between test groups. A total of 28 petri dishes that had been added 1-2 ose of bacterial culture *Lactobacillus acidophilus* and MHA (Mueller Hinton Agar) were prepared for this study. There are 7 test groups, chlorhexidine 0,2% as a positive control (K1), negative control, DMSO (K2), red okra fruit extract treatment group 10%, 20%, 30%, 40%, and 50% respectively as K3-K7. A total of 4 test petri dishes were used in each group. Furthermore, the inhibition of bacteria was measured by measuring the diameter of the inhibition zone of each treatment group. Data analysis was performed with the help of SPSS software version 25. Univariate analysis was performed to present the data distribution for each test variable. Bivariate analysis was carried out to see statistical differences in each test variable, where $p<0.05$.

3. Results

Table 1 shows the average diameter of the inhibition zone for each treatment group. K1, the group that received chlorhexidine, showed the highest ability to produce the largest diameter of the inhibition zone compared to all treatment groups. Along with increasing the dose of red okra fruit extract, the ability of okra fruit extract to increase in producing a larger diameter of the inhibition zone. Although it was not able to beat the diameter of the inhibition zone in group 1, red okra fruit extract at a dose of 50% showed considerable potential in producing the diameter of the inhibition zone. The diameter of the inhibition zone produced by a 50% dose of okra fruit extract was only 2.9 mm, different from the diameter of the inhibition zone produced by chlorhexidine.

Table 1. The average diameter of the inhibition zone for each treatment group.

Group	The average diameter of the inhibition zone (mm) ± SD	p-value
K1	15,50±0,27	0,000*
K2	0	-
K3	9,73±0,10	0,000*
K4	10,53±0,31	0,000*
K5	11,43±0,59	0,000*
K6	11,83±0,13	0,000*
K7	12,60±0,42	0,000*

* Pos Hoc LSD VS K2, p<0,05.

4. Discussion

Increasing the concentration will affect the increase in the active substance so that the effectiveness of the antibacterial is higher. Based on the results of phytochemical screening from previous studies, it was stated that the ethanol extract of okra fruit was positive for flavonoids, tannins, saponins, triterpenoids, steroids, alkaloids, and quercetin. As well as carbohydrates. Quercetin, including types of flavonoid compounds amounting to 60-75%, has many benefits, including antioxidant, neuroprotective, antiviral, anticancer, cardiovascular, antimicrobial, anti-inflammatory, and anti-obesity. These active compounds have been widely used in herbal medicine as traditional medicine for hundreds of years. In addition, quercetin also has phenol bonds, is able to coagulate proteins, inhibit cell walls, and is bactericidal in nature. If the bacterial cell wall is not formed, it can cause cell leakage, resulting in bacterial lysis.[11-15]

The triterpenoid compounds contained in the effectiveness of okra fruit extract function as an antibacterial by reducing the membrane permeability of the bacterial cell so that the content can react with the transmembrane protein (porin) in the outer membrane of the bacterial cell wall and form strong polymeric bonds that also cause damage to the porin. In addition, flavonoids can play an antibacterial role by forming a complete content in extracellular proteins so that they can block the integrity of the bacterial cell membrane. Another study stated that the highest levels of flavonoids in okra fruit were 1.9%. Saponins can reduce surface tension and increase permeability, causing the release of intracellular compounds that can cause cell lysis. Alkaloid substances can inhibit the peptidoglycan component of bacterial cells, causing the cell wall layer not to be perfectly formed.[16-20]

5. Conclusion

Red okra fruit extract shows effectiveness as an antibacterial *Lactobacillus acidophilus* and increases the extract's concentration.

6. References

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