

Antibacterial Effectiveness Test of Potato Peel Ethanol Extract (*Solanum tuberosum* L.) against *Lactobacillus acidophilus*: An In Vitro Study

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ABSTRACT

Lactobacillus acidophilus is one of the bacteria that play a major role in the caries process. It is recommended to use cavity cleanser to clean and disinfect caries cavities post-preparation. However, the currently used cavity cleansers, 2% chlorhexidine digluconate and sodium hypochlorite, have limitations. Thus, exploring natural alternatives, like potato peel, as a cavity cleanser material is necessary. This study aims to determine the antibacterial effectiveness of the ethanol extract of potato peel (*S. tuberosum* L.) on the growth of *L. acidophilus*. The method used was true experimental laboratories with a post-test-only control group design and broth dilution. The samples were divided into 8 groups, including 6 treatment groups with varying concentrations of extract (100%; 50%; 25%; 12.5%; 6.25%; and 3.125%), positive control, and negative control. Each group was repeated 4 times. The Minimum Inhibitory Concentration (MIC) was determined by observing solution turbidity in the tubes which began to appear clear after 24 hours of incubation, while the Minimum Bactericidal Concentration (MBC) was determined by the absence of bacteria that grew on media after being given the test solution and incubated for 24 hours. The MIC of potato peel ethanol extract on the growth of *L. acidophilus* could not be determined, while the MBC was at a concentration of 50%. The *Kruskal-Wallis* test showed that concentration levels had a significant difference in the average number of *L. acidophilus* colonies. The ethanol extract of potato peel (*S. tuberosum* L.) has antibacterial effectiveness against the growth of *L. acidophilus*.

Keywords: potato peel (*S. tuberosum* L.); *L. acidophilus*; MIC; MBC; dilution

INTRODUCTION

The prevalence of dental and oral problems in Indonesia can be categorized as quite high. Based on Riset Kesehatan Dasar (Riskesdas) 2018 data, the proportion of the Indonesian population experiencing dental and oral health problems is 57.6%. Among these problems, dental caries is the most common disease. According to Riskesdas data for 2018, the prevalence is 88.8% (Tim Riskesdas, 2018). This number has increased from data in 2013 which was only 53.2% (Tim Riskesdas, 2013).

Dental caries is a disease of the hard tissue of the teeth which is characterized by damage to the enamel or dentin. Caries are formed due to the interaction of four important factors: microorganisms, host, food, and time (Ramayanti & Purnakarya, 2013). The process of caries begins with the attachment of bacteria to the enamel surface. These bacteria metabolize food residue and ferment sucrose into lactic acid which causes a decrease in the pH of the oral cavity to less than 5.5.

The pH reduction prompts the calcium phosphate within hydroxyapatite to dissolve, causing demineralization. If this continues, it can result in caries formation. (Karpiński & Szkaradkiewicz, 2013).

In the caries process, *Lactobacillus acidophilus* is one of the bacteria that play a dominant role. These bacteria are isolated more frequently in deep carious lesions than in the early stages of caries. Studies show that *L. acidophilus* makes an important contribution to the stages of caries development, especially in dentin. *L. acidophilus* can grow in an acidic environment and metabolize sugar to lactic acid very quickly (Karpiński & Szkaradkiewicz, 2013).

Currently, the use of cavity cleansers that have antibacterial properties is becoming popular (Kimyai et al., 2017). Cavity cleanser is a material used to clean, wet, and disinfect cavities after preparation (Setianingrum et al., 2017). Cavity cleaning is done to remove debris, bacteria, and microbes that have settled on the prepared cavity walls, such as in the formed smear layer, dentinoenamel junction (DEJ), or the dentinal tubules (Setianingrum et al., 2017; Siwinata et al.,

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2020). An ideal cavity cleanser must have a low level of toxicity to the pulp cells without interfering with the adhesion of the restorative material (Setianingrum et al., 2017).

The material commonly used for cavity cleansers is 2% chlorhexidine digluconate. Chlorhexidine digluconate has been widely used as a dentine disinfectant to reduce the number of bacteria. Chlorhexidine digluconate is considered effective as an antibacterial agent because it binds to the amino acids present in dentine and can kill bacteria within hours. The use of chlorhexidine digluconate as a cavity cleanser can reduce the risk of secondary caries and increase tooth sensitivity (Siwinata et al., 2020). Nonetheless, chlorhexidine digluconate has several limitations, including its incapability to dissolve organic tissue and its potential for causing allergies when exposed continuously for extended periods. (Mohammadi & Abbott, 2009).

Apart from chlorhexidine digluconate, a cavity cleanser that is also frequently used is sodium hypochlorite (NaOCl). NaOCl can dissolve organic tissue well and has strong antibacterial properties against bacteria. However, the disadvantages are that it can be toxic, irritate the surrounding tissue, cause allergic reactions, give off an unpleasant odor, and is corrosive (Bin-Shuwaish, 2016).

Therefore, natural ingredients that fulfill the requirements of cavity cleansers need to be developed. The use of traditional medicines, including medicines made from natural ingredients, is increasing rapidly in developed and developing countries. The World Health Organization (WHO) recommends the use of traditional medicine for public health care and disease prevention and treatment (Kepmenkes, 2007). When compared to conventional medicines, traditional medicines are generally safer and have fewer side effects (Putri, 2015).

Potato peel is one of the natural ingredients that can be developed. Potato (*Solanum tuberosum* L.) is an annual crop that ranks fourth in the world's most important food crops, after rice, wheat, and maize. Potatoes can be consumed as vegetables or used for industrial raw materials, for example, processed into potato chips (Hidayah et al., 2017). Currently, the use of potatoes is mostly focused on the tubers only. The peel is not used much leading to its eventual disposal as waste. Nonetheless, potato peel can be used. An example of the use of potato peel in the field of dentistry is the potential to be used as an alternative material for cavity cleansers. This is because potato peel contains several antibacterial compounds.

Based on the description above, research on the antibacterial activity of potato peel extract against *L. acidophilus* was conducted.

METHODOLOGY

This research received ethical clearance (No. 115/ECH/FK-UNDIP/X/2022) from Komite Etik Penelitian Kesehatan (KEPK) of the Faculty of Medicine, Diponegoro University. The type of research used was true experimental laboratories with a post-test-only control group design.

Materials

Potato plants were obtained from Kopeng Village, Getasan District, Semarang Regency. Before use, a determination test was carried out on potato plants at the Biology Laboratory, Faculty of Science and Mathematics, Diponegoro University to prove that the plant species was indeed *S. tuberosum* L.

Extraction of potato peel ethanol extract

Potatoes were washed and peeled. The potato peel was put in the oven at 50-60°C for 48 hours to dry. The dried potato peel is mashed using a blender. Then, 500 grams of the potato peel was put into a jar and soaked in 5 liters of 70% ethanol while stirring for 6 hours. The mixture is then left for eighteen hours with occasional stirring. After that, the mixture was put into a percolation tube and filtered. The process was repeated once again with the same solvent but with half the volume of the first process. After filtering, an evaporation process is carried out to evaporate the ethanol in the solution. The liquid extract was evaporated at 40°C with a rotary evaporator until it became thick. The condensed extract was then diluted with 10% DMSO to obtain a concentration of 100%; 50%; 25%; 12.5%; 6.25%; 3.125%.

Phytochemical screening of potato peel ethanol extract

Potato peel ethanol extract was undergone qualitative phytochemical screening for the presence of flavonoids, anthocyanins, alkaloids, tannins, terpenoids, and steroids. This screening was conducted by Cendekia Nanotech Hutama Laboratory Semarang.

Preparation of the bacterial suspension

The *L. acidophilus* bacterial strain which was obtained from the Microbiology Laboratory, Faculty of Medicine, Diponegoro University and had been previously cultured was taken using a sterile inoculation loop and dissolved in 0.9% NaCl. The suspension was then incubated at a temperature of 37°C for 24 hours.

Determination of MIC and MBC value

Antibacterial effectiveness testing was carried out using the dilution method. Eight test tubes containing 1 ml of deMan Rogosa Sharpe Broth (MRSB) were provided. Each tube was then added 1 ml of potato peel ethanol extract at various concentrations; CHX 2% for positive control; and DMSO for negative control. After that, 1 ml of bacterial suspension was added to each tube. All tubes were incubated at 37°C for 24 hours, then MIC was observed by visually examining the solution turbidity. The solution that started to appear clear was set as MIC. Next, 1 µl of the test material in each tube was inoculated into a petri dish containing deMan Rogosa Sharpe Agar (MRSA). All dishes were incubated at 37°C for 24 hours, then MBC was observed by counting the growing bacterial colonies. The concentration that did not grow bacteria at all was set as MBC.

RESULT AND DISCUSSION

Potato peel ethanol extract result

The results of the determination test on potato plants proved that the plants used were *S. tuberosum* L. Samples of 5 kg wet potato peel were dried using an oven at 60°C for 48 hours obtaining 500 grams of dry potato peel. This result indicates that the dry weight was produced from 10% of the wet weight of the potato peel. Extraction of dried potato peel with 70% ethanol solvent obtained 15 grams of extract. This result indicates that the viscous extract was produced from a 3% dry weight of potato peel.

The selection of 70% ethanol solvent in the extraction process was aimed to withdraw all the antibacterial compounds contained in potato skins. Ethanol is a universal solvent that can attract non-polar and polar compounds (Padmasari et al., 2013).

This study used MRSB and MRSA as the media because these two are selective media that can grow only lactic acid bacteria, so they are suitable for use to observe the growth of *L. acidophilus*. MRSB is a liquid medium that can facilitate lactic acid bacteria in releasing bacteriocins in the media. Meanwhile, MRSA is a solid medium for growing and isolating lactic acid bacteria that grow in colonies (Ningsih et al., 2018).

Phytochemical screening

The potato peel ethanol extract positively contained flavonoids, anthocyanins, alkaloids, tannins, and terpenoids. However, the extract showed negative results for steroid compounds. This may be due to the different types of solvents used. According to previous research by Noor, et al.

(2017), potato peel extract contains steroids when dissolved in distilled water, absolute ethanol, and 80% ethanol (Noor et al., 2017). The difference in polarity of the solvents used may cause steroids to not be withdrawn from the extracts in this study. In addition, the absence of steroid compounds in the potato peel extract was probably because the potatoes used in this study did not contain steroids. The levels of active compounds contained in a plant may be affected by genetic factors, planting place, light, temperature, radiation, drought, and soil salinity, as well as pathogenic organisms (Cirak & Radusiene, 2019).

Antibacterial activity

The solution of the liquid dilution test for the antibacterial effectiveness of the ethanol extract of potato peel against *L. acidophilus* (Figure 1) in the tube with a concentration of 100% and 50% appeared dark black. The solution in the 25% and 12.5% concentration tubes appeared dark brown. The solution in the tube with a concentration of 6.25% and 3.125% was yellowish brown. Meanwhile, the solution in the positive control tube (CHX 2%) was clear yellow and in the negative control tube (DMSO) the solution was cloudy yellow. MIC is the lowest concentration of the experimental substance that can inhibit bacterial growth after 24 hours of incubation, which is calculated visually by observing the turbidity of the ethanol extract of potato peels that have been treated with the test bacteria in a test tube. The color of the extract before and after being given bacteria is similar. This was likely due to the dark color of the potato peel ethanol extract, which resulted in a blackish-brown appearance when suspended with bacteria. Consequently, determining the concentration at which it turned clear became challenging, leading to the inability to determine the MIC value. These results are different from research conducted by Rahmadhany (2021) and Nurhayati (2017) which obtained the MIC value of potato peel extract against *S. aureus* (Nurhayati, 2017; Rahmadhany, 2021). The difference is probably caused by differences in the antibacterial test method used. The two previous studies used the diffusion method. The agar diffusion method is less precise for testing the antibacterial effectiveness of an extract because the volatile components of the extract tend to evaporate during the incubation process (Golus et al., 2016). In addition, the amount of substance that diffuses into the agar media cannot be known (Balouiri et al., 2016).

After the MIC test was carried out, the test material was planted from each tube into solid media for the MBC test (Figure 2). MBC was



Figure 1. MIC test tube of potato peel ethanol extract against *L. acidophilus*

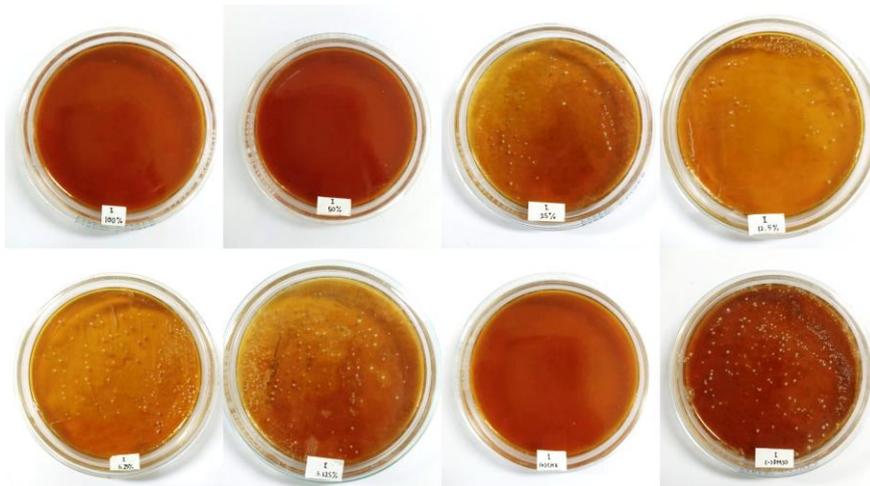


Figure 2. Results of the MIC test of potato peel ethanol extract against *L. acidophilus*.

determined by counting the number of bacterial colonies that grew on MRSA after being given the test material and incubated for 24 hours. *L. acidophilus* growth was not found in the groups with concentrations of 50%, 100%, and positive control. This shows that there is an antibacterial activity in the three groups. The lowest concentration that could kill *L. acidophilus* was 50%, so this concentration was designated as the MBC in this study. When compared with CHX 2%, the MBC value with a concentration of 50% is just as effective in killing *L. acidophilus*. The full results of the MBC test for ethanol extract of potato skins against *L. acidophilus* can be seen in Table 1.

The data were tested for normality with the Shapiro-Wilk test and homogeneity with the Levene test. The results of the Shapiro-Wilk test in Table II show a significance value in the 25% concentration test; 12.5%; 6.25%; and 3.125% more than 0.05 so it can be concluded that the data on the average number of colonies of *L. acidophilus* bacteria is normally distributed. The results of the Levene test in Table II show a significance value of $p=0.001$ ($p<0.05$) so it can be concluded that the data on the average number of colonies of *L. acidophilus* bacteria is not homogeneous.

Because the data were normally distributed but not homogeneous, a non-parametric Kruskal-

Table I. Results of counting the number of colonies of potato peel ethanol extract against *L. acidophilus*

Substances	Concentrations	Repetition (CFU/ml)				Mean (CFU/ml)
		I	II	III	IV	
Potato Peel Ethanol Extract	100%	0	0	0	0	0
	50%	0	0	0	0	0
	25%	73	66	45	70	63,5
	12,5%	133	109	152	147	135,25
	6,25%	178	156	190	170	173,25
	3,125%	235	248	224	203	237,5
Positive Control (CHX 2%)		0	0	0	0	0
Negative Control (DMSO)		358	150	259	306	270,75

Table II. Results of Nirmality and Homogeneity Test

Groups	Normality	Hommogeneity
100%	-	p = 0,001
50%	-	
25%	p = 0,153	
12,5%	p = 0,501	
6,25%	p = 0,995	
3,125%	p = 0,932	
Positive Control	-	
Negative Control	p = 0,888	

Table III. Kruskal-Wallis test result

Groups	Normality	Hommogeneity
100%	-	p = 0,001
50%	-	
25%	p = 0,153	
12,5%	p = 0,501	
6,25%	p = 0,995	
3,125%	p = 0,932	
Positive Control	-	
Negative Control	p = 0,888	

Table IV. Post Hoc test results

	100%	50%	25%	12,5%	6,25%	3,3125%	K+	K-
100%	-	1,000	1,000	1,000	0,237	0,032*	1,000	0,021*
50%	1,000	-	1,000	1,000	0,237	0,032*	1,000	0,021*
25%	1,000	1,000	-	1,000	1,000	1,000	1,000	0,930
12,5%	1,000	1,000	1,000	-	1,000	1,000	1,000	1,000
6,25%	0,237	0,237	1,000	1,000	-	1,000	0,237	1,000
3,3125%	0,032*	0,032*	1,000	1,000	1,000	-	0,032*	1,000
K+	1,000	1,000	1,000	1,000	0,237	0,032*	-	0,021*
K-	0,021	0,021	0,930	1,000	1,000	1,000	0,021*	-

* : significant; K+ : positive control; K- : negative control

Wallis statistical test was carried out. The results (Table III) obtained a significance value of $p=0.000$ ($p<0.05$). This value indicates that changes in the level of concentration of potato peel ethanol

extract gave a significant difference in the average number of *L. acidophilus* colonies.

After that, a *Post Hoc* test was carried out to show the pair of extract concentration groups that

gave and did not give a significant difference. *Post Hoc* test results (Table 4) show that the effect of potato skin ethanol extract between 100% concentration and 3.125% concentration and negative control, and 50% concentration with 3.125% concentration and negative control has a significant difference.

The antibacterial effect of potato peel extract is probably caused by its active compounds. Potato peel contains active substances that can act as antibacterial agents (Noor et al., 2017; Noushad et al., 2020). Flavonoids function as antibacterial by inhibiting the formation of nucleic acids, cell membrane function, and energy metabolism (Pendit et al., 2016). Anthocyanins are derivatives of secondary metabolites of flavonoids. Anthocyanin exposure causes irregularities in the outer membrane and cytoplasmic leakage in bacteria (Nomer et al., 2019). Alkaloids destroy the constituent components of peptidoglycan in bacterial cells, preventing the cell wall layer from forming properly and resulting in bacterial cell death (Ningsih & Zufahair, 2016). The antibacterial effect of tannins works by inhibiting the action of enzymes, deactivating bacterial adhesion, and inhibiting the transport of proteins in the cell envelope (Rahman et al., 2017). Terpenoids function as antibacterial by making strong polymer bonds with transmembrane proteins on the outer membrane of the bacterial cell wall so that it is damaged and reduces the permeability of the bacterial cell wall (Rachmawati et al., 2011). Utilization of the ethanol extract of potato peel as a cavity cleanser requires further study regarding the antibacterial effectiveness of the extract against other bacteria in the cavity and *in vivo* studies to test its toxicity.

This study has proven that ethanol extract from potato peel has antibacterial activity against *L. acidophilus*. However, this study had certain limitations. First, the phytochemical test conducted were only qualitative, necessitating further research with quantitative phytochemical tests to identify which compound has the most dominant role for the antibacterial effect. Second, the study only involved *in vitro* testing, and therefore, *in vivo* research is required to explore the clinical applicability of this material. Third, the MIC value could not be determined, suggesting the need for spectrophotometry in future studies to obtain more accurate results. Fourth, this study had not covered all the bacteria found in dental cavities. Therefore, additional study to test other bacteria is necessary so that the ethanol extract of potato peel can be used as an alternative cavity-cleaning agent.

CONCLUSION

Based on the research that has been done, it can be concluded that the ethanol extract of potato peel (*S. tuberosum* L.) has antibacterial effectiveness against the growth of *L. acidophilus* with MBC at a concentration of 50%.

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