



Antibacterial Activity of Apple Vinegar Against the Growth of *Streptococcus pyogenes*

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Abstract

Apple cider vinegar is a liquid containing acid from the alcoholic fermentation process and acetate fermentation which contains flavonoids and tannins. This study aims to determine the antibacterial activity of apple cider vinegar in inhibiting gram-positive bacteria *Streptococcus pyogenes*. The method used is a quantitative method using disc paper, this stage starts from sample preparation, and sterilization of media manufacture, bacterial experiments, rejuvenation and manufacture of bacterial suspensions, preparation of sample concentrations, and antibacterial tests. Apple cider vinegar used were brands X, Y, and Z with concentrations of 10%, 20%, 40%, 80% respectively, positive control using 25 g Amoxicillin and negative control using sterile distilled water. The results of this study are the zones of inhibition caused by each brand are; X 6.53 mm, 7.5 mm, 12.01 mm, 16, 66 mm, Y brand 7.41 mm, 8.62 mm, 11.69 mm, 16.82 mm, Z brand 6.35 mm, 11.86mm, 14.25mm, 20.17mm. Data analysis using Two Way Anova, from the results of data analysis it can be said that bacteria have antibacterial activity against *Streptococcus pyogenes* with the best zone of inhibition in brand Z apple cider vinegar with a concentration of 80%.

Keywords: Antibacterial, *Streptococcus pyogenes*, Apple cider vinegar, Difusi Disk, Amoxicillin.

1. Introduction

The skin is the outermost layer of the human body (Astuti et al., 2018) which has the function of protecting the surface of the body, regulating body temperature and protecting the body from pathogens (Setiawan et al., 2013). Skin infection in humans is a disease that occurs very often and is included in infectious diseases because it can be transmitted from one individual to another (Azizah et al., 2020). One example of a skin infection caused by bacteria is impetigo, where impetigo is caused by the bacterium *Streptococcus pyogenes* (Indahsari, 2021).

Impetigo is common in toddlers, tetepi also occurs in adults (Nasyuha et al., 2020). Impetigo consists of two types, namely bullous impetigo and nonbulose impetigo. Bullous impetigo is characterized by the formation of bula and often occurs in children and adults with the most frequent areas on the face, neck and extremities, while nonbulose impetigo is characterized by vesicles or pustules that quickly break and become crusts such as honey (Hidayati et al., 2019).

One of the ingredients that can treat impetigo disease is quercetin contained in apple vinegar (Syafina et al., 2020). Apple vinegar is easy to obtain, and its manufacture is quite simple by converting sugar from apple extract into alcohol, then with the addition of acetobacter to produce apple vinegar. Apple vinegar contains quercetin which is a powerful antioxidant that is effective as an antimicrobial (Syafriana et al., 2020).



2. Methods

The tools used are: Laminar Air Flow (Kojair Clean Wizard v130), autoclave (Hirayama HVA-85), incubator (Memmert type INB 500), Thermo Scientific Vortex (Maximix II), analytical scales (Shimadzu), Hotplate, erlenmeyer (Pyrex), measuring cup (Pyrex), petri dish, test tube (Pyrex), test tube rack, caliper term, bunsen, micro pipette (Eppendorf), tweezers, ose wire, L rod, spatel, stirring rod, caliper term, aluminum foil, plastil wrap, filter paper, wipes, matches, cotton wool, yarn.

The ingredients used are: apple vinegar obtained at one minimarket in Jambi City with brands X, Y and Z, *Streptococcus pyogenes* bacteria from the University of Indonesia Laboratory, aquadest (amidis) as a negative control, disc paper containing amoxicillin 25 μ g / disk (oxid) as a positive control, blank disc paper (oxid), nutrient agar (Merck), mueller hinton agar (Merck).

2.1 Sampling

The sample used was apple vinegar with brands X, Y and Z which was obtained at one minimarket in Jambi City, then apple vinegar was diluted with aquadest so that a concentration of 10%, 20%, 40%, 80% was obtained.

2.2 Media Creation

Creation of Nutrient Agar: Done by dissolving 1 gram of NA powder into 50 mL of aquadest in the erlenmeyer, then dissolved on a hotplate and sterilized using an autoclave (Yanti & Mitika, 2017).

Creation of Mueller Hinton Agar: Done by dissolving 9.5 grams of MHA powder and dissolved with 250 mL of aquadest on a hotpalte, then sterilized using an autoclave with a temperature of 121 $^{\circ}$ C for 15 minutes (Yusmaini & Bahar, 2018).

2.3 Bacterial Identification

It is done by taking 10 μ L of bacteria by using a micro pipette from the bacterial suspension, then dripped on the center of the object glass and fixation is carried out until it dries. Give 1 drop of crystal violet solution and leave for 1 minute, then wash with aquadest and dry with a tissue. Then drip the lugol solution and leave for 1 minute, after 1 minute drain the color blanching solution (alcohol 70%) and washed using aquadest, then dried using a tissue. Then dripped safranin 1 drop, then let stand for 45 seconds, wash with aquadest and dry using a tissue. Then it was observed using a microscope with a magnification of 100X. If you see purple cell color, it indicates gram-positive bacteria, if red cell color then it includes gram-negative bacteria (Daeng & Azis, 2019).

2.4 Rejuvenation and Manufacture of Bacterial Suspension

Bacteria are rejuvenated using NA oblique media that has been sterilized by poking 1 ose bacteria from the bacterial stock then inoculation into NA media and incubated for 24 hours (Yanti & Mitika, 2017).

Making a bacterial suspension is carried out by preparing 0.9% NaCL as much as 5 mL in a test tube then several bacterial ose is taken from bacterial rejuvenation and inoculated into 0.9% NaCl to a turbid suspension. Suspension turbidity was measured using a UV-Vis spectrophotometer with a wavelength of 580 nm with a transmitting value of 25% (Azizah et al., 2020).

2.5 Antibacterial Activity Testing

Testing of antibacterial activity using the Disc Diffusion method or disc diffusion using MHA media. Pour the MHA media into the petri dish wait until it solidifies, then put 100 μ L of bacterial suspension into the test media and flatten it using an L rod. Next, the blank disc paper is soaked into apple vinegar with a concentration of 10%, 20%, 40%, and 80% and aquadest as a negative control, then the disc paper is taken and allowed to stand until the solution does not drip, then place it on the MHA media while gently pressed. Then incubated for 1x24 hours (Suhartati & Roziqin, 2017).

3. Research Results

Based on the results of studies that have been carried out on microscopic identification, it can be seen that purple bacteria are coccus-shaped or round with an arrangement attached like a chain.



Figure 1: Microskopik Identification

Antibacterial activity testing used concentrations of 10%, 20%, 40% and 80% with negative aquadest control used as a comparison against research samples that had no effect on the formation of inhibitory zones, and positive controls using discs containing amoxicillin 25 µg / disk as a comparison against research samples that had an effect on the formation of inhibitory zones. Amoxicillin is used as a positive control because it is a penicillin group antibiotic with a broad spectrum, so that it can be used to inhibit the growth of gram-positive and negative bacteria, amoxicillin has a mechanism of action by damaging the peptidoglycan layer of the bacterial cell wall (Nisaummahmudah *et al.*, 2016).

Table 1 Bacterial inhibition Zones

Concentration	Apple Vinegar X	Apple Vinegar Y	Apple Vinegar Z
10%	6,53 mm	7,41 mm	6,35 mm
20%	7,5 mm	8,62 mm	11,86 mm
40%	12,01 mm	11,69 mm	14,25 mm
80%	16,66 mm	16,82 mm	20,17 mm

The inhibition zone formed is caused by the content contained in apple vinegar, namely flavonoids, and tannins which are antibacterial (Dimariwu *et al.*, 2020). Flavonoids contained in apple vinegar are quercetin which has antibacterial activity and has the ability to increase the permeability of bacterial cell membranes, so that it can change the structure and function of cell membranes that cause denaturation of membrane proteins so that cell membranes will be damaged and lysis, and quercetin is hydrophilic and lipophilic, so that it can lower the surface tension of cells that cause bacterial destruction (Herslambang *et al.*, 2015). Tannins work by forming polysaccharide complex compounds that can damage the cell wall of bacteria, thereby disrupting the permeability of bacterial cells and causing cells to be unable to carry out life activities, as a result of which bacterial growth will be inhibited and cause bacteria to die (Bamasri, 2021). Apple vinegar has very good benefits for skin health, apple vinegar can be used to rejuvenate skin cells (Chan, 2016).

The results of the analysis of inhibition zone diameter data showed that the application of apple vinegar brands X, Y, and Z to *Streptococcus pyogenes* there was a noticeable difference (P-Value <0.05) of apple vinegar brand X and Y was different from brand Z. Results of the concentration analysis used in *Streptococcus pyogenes* bacteria had a noticeable difference in each concentration used marked by a P-Value value of <0.05.



4. Conclusion

Based on the results of the study, it can be concluded that Apple Vinegar brands X, Y, and Z in *Streptococcus pyogenes* in vitro with a concentration of 10%, 20%, 40%, and 80% has antibacterial activity.

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