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Antibacterial Activity of Aloe Vera Gel-based Edible Coating with the addition of Gum Arabic and Ascorbic Acid

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A B S T R A C T

Aloe vera gel has antibacterial properties. The content of antibacterial compounds in aloe vera gel is saponins, anthraquinones, tannins, aloin, and acemannan. Aloe vera gel has the potential as an edible coating for food products. The Processing carried out goes through a heating process that does think to damage the antibacterial compounds in the aloe vera gel. This study aims to determine the antibacterial activity of aloe vera gel formulated as an edible coating on *S. aureus, S. mutans, E. coli* and *K. pneumoniae* bacteria. The research method used is the disc diffusion method. Based on the test results, pure aloe vera gel has a larger inhibition zone diameter than aloe vera gel processed into an edible coating. Diameter of pure aloe vera gel inhibition zone in *S. aureus, S. mutans, E. coli*, and *K. pneumoniae* bacteria. respectively 10.966 ± 0.573 mm; 11.806 ± 0.215 mm; 10,860 ± 0.675 mm, and 10.686 ± 0.081 mm. Aloe vera gel formulated into an edible coating with antibacterial ability, namely at a concentration of 100%, which has anti activity with the inhibition zone's diameter in *S. aureus, S. mutans E. coli*, and *K. pneumoniae* bacteria, respectively. is 10,470 ± 0.213 mm; 10,673 ± 0.127 mm; 10,113 ± 0.040 mm, and 9.676 ± 0.604 mm.

1. INTRODUCTION

Aloe vera (*Aloe vera*) is a functional plant that has health benefits. Aloe vera gel has antibacterial properties. Aloe vera is known to contain anthraquinones which have previously show to have an antibacterial activity where anthraquinones can inhibiting protein synthesis so that the bacteria cannot grow in media containing aloe vera extract [1]. Apart from that, aloe vera also contains saponins. Saponins can act as an antiseptic that kills or prevents microbial growth [2]. Saponins work as antibacterials by disrupting the stability of the bacterial cell membrane causing bacterial cell lysis, so the mechanism of action of saponins does include in the antibacterial group that disrupts the permeability of bacterial cell membranes,

The antibacterial activity of aloe vera gel juice has does investigated by the diffusion method against bacteria, fungi and yeast. Aloe juice exhibits antibacterial activity against Gram-negative bacteria (*Pseudomonas aeruginosa, Klebsiella pneumoniae, E. coli, Salmonella typhimurium*) and *Candida albicans* (in vitro). Antibacterial activity of aloe vera gel juice against Gram-positive bacteria (*Mycobacterium smegmatis, Staphylococcus aureus, Enterococcus faecalis, Micrococcus luteus*, and *Bacillus sphericus*) [4].

Aloe vera is a material that has been widely used as an edible coating. Aloe vera gel is tasteless, colourless and odourless [5]. The many types of polysaccharides in aloe vera gel are glucomannan and lignin, which can withstand the loss of fluid from the skin surface, reducing the senescence rate (wilting / wrinkling), respiration rate, and maintaining fruit freshness [6]. Aloe vera gel contains antibacterial compounds [1] to inhibit microbial growth in fresh fruit. Aloe vera gel-based edible coatings have does show to prevent moisture loss, control respiration rate and progression of ripening, delay oxidative browning, and reduce the proliferation of microorganisms in fruits such as cucumber [7], tomato [8], Bidara [9], and mango [10]. On the one hand, Aloe vera gel can be used as a coating but has its drawbacks. Aloe vera gel is easily oxidized, quickly changing colour, odour and viscosity [11].

In making edible coatings, aloe vera goes through several processing processes such as heating and adding additives, namely ascorbic acid and Arabic gum stabilizer. The heating process can destroy the bioactive content in a material [12]. One of the active compounds that act as antimicrobials is saponins which are easily oxidized due to the heating process [13]. Aloe vera contains aloin compounds that function as antioxidants and antibacterials. Aloin is heat intolerant and hydrolyzes when heated [14. Heating to a temperature of 30-80^{o}C can reduce the aloin compounds in aloe vera skin [15]. In making an Aloe vera gel edible coating, the stabilizer is also added to maintain the aloe vera gel's stabilisation during storage. Arabic gum has the characteristics that it does not affect the colour of the ingredients

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added even though it is in high concentrations, dissolves in hot water and cold water, increases the viscosity of the solution, is dissolved and is stable in acidic and alkaline conditions, and is not easily degraded by enzymes [16]. The combination of aloe vera and gum arabic is expected to be an excellent edible coating. With the addition of other ingredients to aloe vera gel, the ability of aloe vera gel, which does formulation as an edible coating, can be investigated to see whether the aloe vera gel edible coating still has antibacterial properties. Edible coating of aloe vera gel with the addition of ascorbic acid and gum arabic still has antibacterial activity at a concentration of 50% -100% in S. *aureus, S. mutans, E. coli,* and *K. pneumoniae* bacteria.

2. MATERIALS AND METHODS

2.1. Materials and tools

The edible coating material was Aloe barbadensis Miller (1-year-old) from Br. Let Tegalalang Village, gum arabic (food grade), ascorbic acid The materials used in the antibacterial activity test were *S. aureus, S. mutans, E. coli*, and *K. pneumoniae* isolate bacterial isolates. Nutrient Agar (NA) medium (Merck, Germany), 10 µg / disk ampicillin antibiotic disc (Oxoid, UK), 70% alcohol, physiological NaCl solution (Oxoid, UK), distilled water, FeCl3, magnesium, hydrochloric acid and reagent Mayer. The tools used in the analysis are dropper pipettes, volumetric pipettes, goblets, Petri dishes, measuring cups, Erlenmeyers, hockey sticks, test tubes, callipers, analytical balances (Ohaus, USA), incubators (Memmert, Germany), Autoclave. (Hirayama, Japan), Laminar Air Flow (JSCB-900SL, Korea).

2.2. The Process of Making Aloe Vera Edible Coating Gel

The aloe vera used is the one-year-old Aloe barbadensis Miller type. Aloe vera is cut at the base, namely the third to the fourth midrib from the bottom. Aloe vera soaked in chlorine 200 ppm solution. After that, the aloe vera is washed. The aloe vera is then filled in to get the aloe vera meat. The aloe vera meat is then blended by dipping the meat in hot water (100−90ºC) for 1 minute. After that, the aloe vera meat is in the blender for 5 minutes. The crushed aloe vera meat is then heated at 60ºC for 10 minutes. After that, the addition of 0.15% ascorbic acid and 2% gum arabic.

2.3. Phytochemical Screening

Qualitative phytochemical screening was carried out on pure aloe vera gel and aloe vera gel edible coating to determine the content of secondary metabolites contained therein. The phytochemical screening carried out was the phenol test, flavonoid test, saponin test, alkaloid test and tannin test. Phenol test: as much as 2 mL of the sample pipette and add a few drops of 1% FeCl3 solution—positive phenolic when the co is blue. Flavonoid test: 1.3 mL of sample mixed with 0.5 g of magnesium, then boiled for 5 minutes. The change in colour from orange to red indicates the presence of flavonoids. Saponin test: the sample is shaken vigorously in the test tube for a few moments. Saponins are positive if foam forms for 3-5 minutes. Alkaloid test: 1 ml of the sample is dissolved with HCl, then added with Mayer's reagent. The presence of white deposits indicates the presence of flavonoid compounds. Tannin test: 1 ml of the extract is added with a few drops of 10% iron (III) chloride solution. If there is a dark blue or greenish-black colour, it indicates the presence of tannins.

2.4. Antibacterial Activity Testing

The bacteria used were *S. aureus, S. mutans, E. coli,* and *K. pneumoniae*. The bacteria does rejuvenated first, and then a microbial suspension is made. The samples used were pure aloe vera gel and aloe vera gel to formulation into an edible coating. The samples were made at concentrations of 100%, 75%, 50%, and 25%. The 200 µL suspension of the tested bacteria was dropped on NA media and then flattened. 10 µL of the test sample was taken and then dropped on disc paper, then placed on the inoculum media and incubated for 24 hours at 37˚C. Microbial growth does observe, and the clear zone formed around the disc was measured using a caliper. A comparison used discs dripped with distilled water for negative control and positive control discs containing 10 µg ampicillin antibiotic.

3. RESULT AND DISCUSSION

3.1. Phytochemical Screening

In this study, aloe vera gel's edible coating adds ascorbic acid and gum arabic as a stabilizer. The addition of ascorbic acid aims to prevent the oxidation of aloe vera gel. An ascorbic acid concentration of 0.15% can prolong the aloe vera gel's stability for 5 days at cold temperature and 2 days at room temperature. However, there is still a phase separation in the gel [11]. The separation of the phase in the gel was overcome by gum arabic, which functions as a stabilizer. The concentration of gum arabic added was 2%. Aloe vera gel is a hydrophilic polysaccharide, so it is not good at holding water and air vapour. At the same time, Arabic gum has an good ability to bind water. The binding capacity of water in a material can be affected by the number of hydroxyl groups (-OH) and the molecular mass. Water bound to gum arabic will form a gel, so trapped moisture is challenging to evaporate [17]. Gum arabic has arabinogalactan protein (AGP) and glycoprotein (GP) groups which act as emulsifying, binding, stabilizing and protective agents. Some of the advantages of Arabic gum are that it has high solubility and low viscosity and has two sides, namely a hydrophilic side and a hydrophobic side which is helpful as an emulsifying and stabilizing agent [18], so it is good to be used as an additive in making aloe vera gel edible coating. Aloe vera gel has the potential to be used as an edible coating on food products. The edible coating creates a thin layer that protects the desired food commodity, such as fruit. The edible coating can cover the fruit's pores by forming a barrier on the skin of the fruit that can prevent water loss and reduce direct contact with O_2, thereby inhibiting the rate of fruit respiration. The edible coating also functions to carry active compounds that protect food product commodities such as antioxidant compounds and antibacterial compounds. Antibacterial compounds are one of the active compounds that are important in maintaining the safety of food products because they can inhibit microbial growth in food products. Aloe vera gel naturally contains antibacterial compounds, namely saponins, anthraquinones, tannins, aloin and acemannan. The phytochemical screening test results, phenol test, flavonoid test, saponin test, alkaloid test, and tannin test on aloe vera gel can be seen in Table 1.

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Table 1. Results of Phytochemical Screening Test

Phytochemical Screening	Pure Aloe Vera Gel	*Edible Coating* Aloe Vera Gel	Information
Phenol	+	+	Give a blue to blackish colour
Flavonoids	+	+	It gives a pink to reddish colour
Saponins	+	+	Formed foam
Alkaloids	+	+	A white precipitate is formed
Tannin	+	+	It gives a greenish-brown colour

Based on Table 1, aloe vera gel and aloe vera gel edible coating contain phenolic compounds, flavonoids, saponins, alkaloids and tannins. This shows that Processing aloe vera gel into edible coating Aloe vera does not remove the bioactive compounds in the aloe vera gel. During Processing, there is a heating process with a temperature of 60ºC so that it does not cause much damage to the bioactive compounds. Heating that carries out does not use high temperatures because too high a temperature can cause damage to the material does process [19].

3.2. Antibacterial Activity Testing

The edible coating of aloe vera gel made in concentrations of 100%, 75%, 50% and 25%, and then tested for antibacterial activity. Edible coating with a concentration of 100%, namely aloe vera gel without dilution, edible coating with a concentration of 75%, namely 75 ml of aloe vera gel with 25% distilled water, edible coating with a concentration of 50%, namely dilution of 50 ml of aloe vera gel with 50% distilled water and edible coating with a concentration of 25%, namely dilution of 25 ml of aloe vera gel with 75% distilled water. Aloe vera gel formulated into an edible coating does test for antibacterial activity on *S. aureus, S. mutans, E. coli* and *K. pneumoniae* (Table 2 and Figure 1).

Table 2. Antibacterial activity of aloe vera gel edible coating with the addition of gum arabic and ascorbic acid on *S. aureus, S. mutans, E. coli,* and *K. pneumonniae* bacteria

Concentration	Inhibition Zone Diameter (mm)			
	S. aureus	*S. mutans*	*E. coli*	*K. pneumonniae*
K +	17.266 ± 0.430	17.253 ± 0.497	17.843 + 0.708	16.213 ± 0.350
K-	-	-	-	-
Pure gel	10.966 ± 0.573	11.806 ± 0.215	10.860 ± 0.675	10.686 ± 0.081
2% Gum Arabic Solution	-	-	-	-
Edible coating Aloe vera gel				
100% concentration	10.470 ± 0.213	10.673 ± 0.127	10.113 ± 0.040	9.676 ± 0.604
Concentration 75%	9.133 ± 0.098	9.630 ± 0.566	8.763 ± 0.423	8.593 ± 0.431
50% concentration	8.893 ± 0.136	8.656 ± 0.276	8.22 ± 0.091	8.146 ± 0.064
25% concentration	-	-	-	-

Information K + = 10 µg ampicillin antibiotic, K- = distilled water

It can be seen that pure gel, namely aloe vera gel without Processing, has greater antimicrobial activity than aloe vera, which has formulation into an edible coating on 4 types of bacteria (Table 2). The diameter of the inhibition zone of pure aloe vera gel in S. *aureus, S. mutans, E. coli*, and *K. pneumoniae* were 10.966 ± 0.573 mm, respectively; 11.806 ± 0.215 mm; 10,860 ± 0.675 mm and 10.686 ± 0.081 mm. Meanwhile, aloe vera gel that has formulation into an edible coating at a concentration of 100% has antibacterial activity with the inhibition zone diameter of *S. aureus, S. mutans, E. coli* and *K. pneumoniae*, respectively 10.470 ± 0.213 mm; 10.673 ± 0.127 mm; 10,113 ± 0.040 mm, and 9.676 ± 0.604 mm.

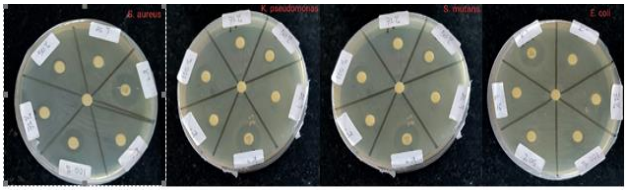

Figure 1. Antibacterial activity of aloe vera gel edible coating with the addition of gum arabic on *S. aureus, S. mutans, E. coli,* and *K. pneumoniae bacteria*

Based on the study results, aloe vera gel has greater antibacterial activity against *S. aureus* and *S. mutans*, which are Gram-positive bacteria, than *E. coli* and *K. pneumonniae*, which are gram-negative bacteria. Gram-negative bacteria have more complex cell walls than Gram-positive bacteria. Gram-negative bacteria can produce B-Lactamase (ESBL) spectrum, which causes resistance to antimicrobial compounds [20].

Aloe vera gel that has process into edible coating has lower antibacterial activity because the processing process goes through a heating process to damage some of the active compounds in the aloe vera gel. The heating process does give to aloe vera gel is the temperature of 60ºC for 10 minutes. The purpose of heating is to deactivate the enzymes so that the aloe vera gel becomes more resistant when stored. E*dible coating* Aloe vera gel still has antibacterial activity seen from the clear zone formed, which means that not all active compounds function as antibacterials damage during the processing process. Aloe vera gel contains anthraquinone as an active compound, which resembles tetracycline in its action mechanism, namely inhibiting bacterial protein synthesis by blocking ribosome sites. Because of this, bacteria cannot grow on media containing aloe vera extract [21]. The antibacterial effect of aloe vera gel is due to active substances such as saponins, tannins, acemannan, and anthraquinone [22] [23]. Saponins as anti-bacteria work to stimulate membranolytics and change the pressure of the extracellular medium. This inhibitory activity is related to the adsorbs process to the microbes themselves. Tannin is thought to have the same mechanism as other phenolic compounds in killing and inhibiting bacterial growth. The mechanism is to react with cell membranes, inactivation of essential enzymes, and destruction or inactivation of genetic material's function [24].

Edible coating Aloe vera gel does test for its antibacterial activity at different concentrations against *S. aureus, S. mutans, E. coli*, and *K. pneumonniae*. The results showed that the higher the concentration of aloe vera gel given, the greater the inhibition zone's diameter (Table 2). The difference in the inhibition zone diameter at each concentration is due to the difference in the active substance contained in it so that the formed inhibition zone

will be different for each concentration. The low concentration of antibacterial compound components will reduce its antibacterial activity. The concentration of aloe vera gel as an antibacterial is a determining factor for the size of the ability to inhibit bacteria [25].

4. CONCLUSION

Based on the research above, it can be concluded that pure aloe vera gel has a larger inhibition zone diameter than aloe vera gel that has been processed into an edible coating. The diameter of the inhibition zone of pure aloe vera gel in S. *aureus, S. mutans, E. coli* and *K. pneumoniae* was 10.966 ± 0.573 mm, respectively; 11.806 ± 0.215 mm; 10,860 ± 0.675 mm and 10.686 ± 0.081 mm. Meanwhile, aloe vera gel that has formulation into an edible coating at a concentration of 100% has antibacterial activity with the inhibition zone diameter of S. *aureus, S. mutans, E. coli* and *K. pneumoniae*, respectively 10.470 ± 0.213 mm; 10.673 ± 0.127 mm; 10,113 ± 0.040 mm and 9.676 ± 0.604 mm. So, aloe vera gel that has process into edible coatings still has antibacterial activity at a concentration of 50% -100%.

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