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Almond as Alternative Media for Growth of *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT

The high cost of media for microbial culture encourages researchers to create alternative media using local raw materials at low prices. One of the most important media compositions for bacterial growth is protein. This content can be obtained from nuts, one of which is almonds (Prunus dulcis). The purpose of this study is to find natural media that has the potential for the growth of microorganisms at a low price. The research method used is an experimental method to determine the growth of Escherichia coli and Staphylococcus aureus bacteria on natural media of almonds. The results showed the growth of Escherichia coli and Staphylococcus aureus bacteria in this alternative media of almonds. Based on the results of the study, it can be concluded that almond nut media can be applied as an alternative medium in laboratory research, especially in the field of_microbiology. It is suggested that further research should be carried out on natural media which are sources of carbohydrates and proteins as alternative growth media for bacteria.

INTRODUCTION

Microorganisms require nutrients, energy sources and specific environmental conditions to grow and multiply. Microbes will easily adapt to the most suitable habitat for their temporary needs in the laboratory, these requirements must be considered by the culture medium (Abd-Alameer Alkhfaji, 2018),

Bacteria can grow well in a medium if the medium contains all the nutrients that are easily used by bacteria, has an appropriate pH, does not contain inhibitory substances, and must be sterile (Peraturan Menteri Kesehatan RI, 2013). Nutrients needed by microorganisms for growth include carbon, nitrogen, non-metallic elements such as sulfur and phosphorus, metal elements such as Ca, Zn, Na, K, Cu, Mn,

Mg, and Fe, vitamins, water, and energy (Cappuccino & Sherman, 2014)

Nutrient Agar is the media most often used in microbiological examinations as a universal media that has a composition of 0.8% protein, 1.2% agar and the rest is water (brand). Along with the increasing demand for microbiological examinations in the laboratory, the number of uses of Nutrient Agar media has also increased, and while the price of the media is quite expensive.

Some researchers managed to find alternative media for the growth of microorganisms from natural ingredients that are easily found in nature. As some researchers have also conducted research on bacterial growth media from various carbohydrate sources such as sweet potato vines, cassava, potatoes and palmirah tubers, even sago. Alternative media from vegetables are tomatoes, cabbage, carrots, and pumpkin. From fruit, beetroot and avocado. Some types of nuts can also be a source of vegetable protein that can replace animal protein sources on Nutrient Agar (Anisah & Rahayu, 2015).

Almonds are one of the natural ingredients that are easy to find. Compared to other types of nuts, almonds have a higher nutritional value. Almonds have many properties that can be utilised into various types of preparations.

So that vegetable protein from almonds is expected to be an alternative media to replace peptone and meat extract which is a source of animal protein on Nutrient Agar media. Therefore, researchers conducted research on almonds as a growth medium for *Staphylococcus aureus* and *Escherichia coli.*

METHODS

This type of research is experimental research or experiment (experimerntal research), namely by replacing animal protein sources on Nutrient Agar by using vegetable protein from almonds for the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria. The samples used in this study were raw almonds sold in the market.

Research procedure

NA Media Preparation

NA media was made by weighing 8.4 grams of NA, then dissolved in 300 mL of distilled water. Then sterilisation was carried out with an autoclave for 15 minute.

Next, it was waited until the temperature was ± 40ºC, so that it could be poured aseptically into a sterile Petri dish as much as ± 20 mL. Allowed to cool and freeze (Ahmed et al., 2019)

Alternative Media Preparation

The materials used in this study are solidification material (agar), powdered sugar, almonds, distilled water, BaCl2, H2SO4, NaCl 0.9% and pH indicator.

Almond alternative media is made from 100 grams of almonds that have been separated from the skin, washed and boiled using 500 mL of distilled water until boiling. Almonds boiled water was mixed with 10 grams of agar flour and 10 grams of sugar. Heated on a hot plate while stirring until homogeneous and dissolved in neutral media pH conditions. Sterilisation was carried out with an autoclave for 15 minutes at 121ºC. After sterilising the media was lifted and waited until the temperature on the media dropped ± 40º. Then poured aseptically on a sterile

Petri dish as much as ± 20 mL. Left to cool and freeze (Rizki & Syahnitya, 2019).

Preparation of Mc Farland Standard Solution

Mc Farland 0.5 standard solution is made by pipetting 0.05 mL of 1% Barium Chloride ($BaCl_2$) solution which is added to 9.95 mL of 1% Sulfuric Acid (H_2SO_4) solution, then homogenised until completely mixed (Dalynn, 2014). Measurement of the Number of Bacteria by Turbidity Method (Spectrophotometry) Prepared McFarland 0.5 standard solution and a suspension of test bacteria that will be measured at a wavelength of 625 nm. The first to be measured, McFarland 0.5 solution and then the test bacterial suspension. If the absorbance value of the test bacterial suspension is greater, then dilution is carried out so that the absorbance value of the test suspension is the same as the McFarland standard solution. However, if the absorbance value of the test bacteria is smaller, the concentration of test bacteria is added (Rahmadani, 2015).

Inoculation of *Escherichia coli* and *Staphylococcus aureus* Bacteria on NA Media and Almond Nut Alternative Media

Inoculation of bacteria on NA media and alternative almonds was carried out by pipetting 0.1 mL of *Escherichia coli* and *Staphylococcus aureus* bacterial suspensions that were equivalent to Mc Farland 0.5, which were then poured into NA media and alternative almonds. Bacterial suspensions were spread using a bent rod (spider) evenly. Furthermore, it was incubated for 24-48 hours at 37ºC in an incubator (Arulanantham et al., 2012)

Calculation of Bacteria Using the Total Plate Count Method

Calculation of bacteria used is TPC by observing the number of colonies that grow. To meet the standards of Plate Count, the number of colonies that grow on Petri dish media ranges from 30-300 CFU/mL (Waluyo, 2016).

According to (Tyas et al., 2018) basically this TPC method is a method used to grow live microbial cells on media so that these cells can live well and can form colonies that can be seen directly by the eye without using a microscope. Bacterial colonies can be counted using a hand counter. Calculation of bacterial colonies on a petri dish can be done by dividing the petri dish into four parts on a petri dish with a non-permanent marker to facilitate calculation.

Data analysis

The data in this study are primary data, namely all data obtained directly from research conducted by researchers. Primary data collection was carried out by treating the samples and then counting the number of colonies of *Escherichia coli* and *Staphylococcus aureus* bacteria in the samples. The data of the research results are then presented in tabular form and equipped with narrative explanations.

RESULTS AND DISCUSSION

The manufacture of alternative media with the basic ingredients of almonds obtained a good solid, with a pH of 7, this fulfils the requirements of bacterial growth media, which must have a neutral pH in accordance with the pH of NA (Nutrient Agar) control media, which is 7-7.4. In this study, researchers made 11 media, this aims to be repeated when inoculating bacteria on the media. In each bacterium, 5

times repetitions were carried out so that 10 media were needed and 1 was designated as negative control media. The bacteria used are the results of culture and already known species. The first step is to perform Gram staining. The results of Gram staining showed *Staphylococcus aureus* bacteria with purple cocci-shaped cell morphology and *Escherichia coli* bacteria with red rod-shaped cell morphology (Figure 1).

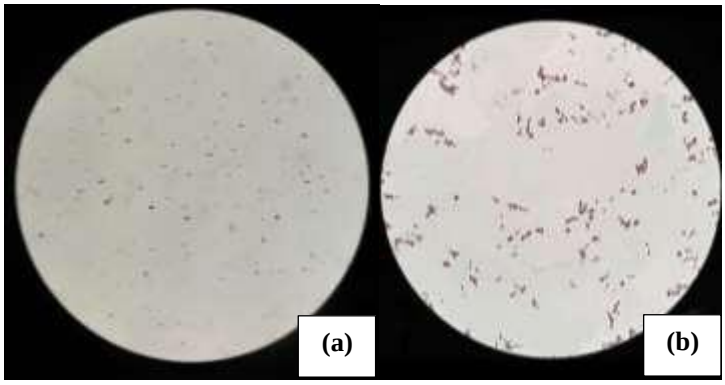

Figure 1. Gram staining. (a) *Staphylococcus aureus* (b) *Escherichia coli*

Figure 2 (a) shows that the characteristics of *Staphylococcus aureus* bacterial colonies that grow on almond media have a circular shape, which is round with edges, with a size of 1-4 mm, white colonies, have a smooth texture, growing colonies have a distance from other colonies, not overlapping so as to facilitate the counting process. In Figure (b) shows the characteristics of *Staphylococcus aureus* bacterial colonies that grow on Nutrient Agar media as a control medium in a round shape, large in size, white in colour, colonies that grow in one type, flat edges and negative mucus.

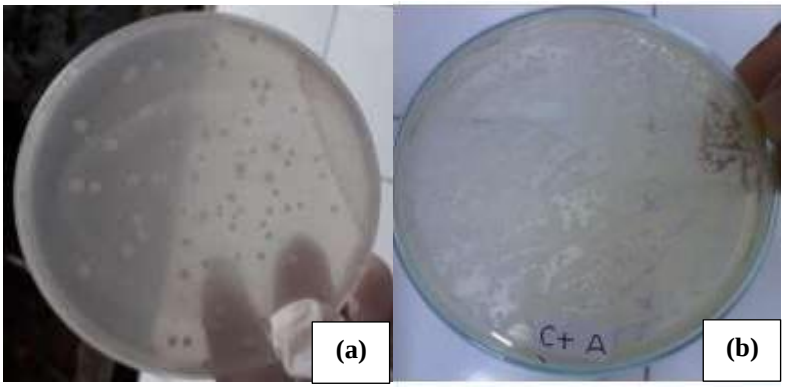

Figure 2. Colonies of *Staphylococcus aureus* bacteria grown on (a) Almond Nut, (b) Nutrient Agar media

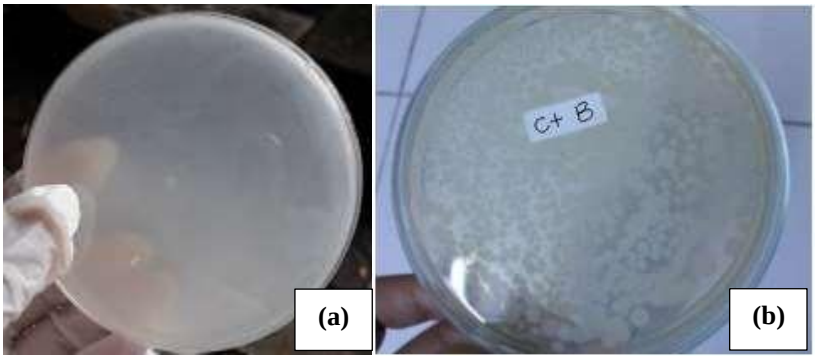

Figure 3. Colonies of *Escherichia coli* bacteria grown on (a) Almond Nut, (b) Nutrient Agar media

Based on Figure 3 (a) it can be seen that the characteristics of *Escherichia coli* bacterial colonies growing on almond media have a circular shape, which is round with edges, white in colour, has a smooth texture, with a size of no more than 1mm. Colonies that grow mostly accumulate, so that even though they grow a lot they are still considered one colony, only a small part of the colonies are small and grow separately. Figure (b) shows the characteristics of *Escherichia coli* bacterial colonies that grow on Nutrient Agar media as control media are round, medium and small in size, white in colour, colonies that grow in one type, flat edges and negative mucus.

Table 1. Growth Results of *Escherichia coli* and *Staphylococcus aureus* Bacteria on Almond Alternative Media

Nama Bakteri	Kode Label	Perhitungan Pada Media Kacang Almond (CFU)					Total Koloni	Rata-rata	NA CFU Control Positif	NA CFU Control Negatif
		1	2	3	4	5				
EC	A	108	52	120	96	122	498	100	132	-
SA	B	48	1	1	16	4	70	14	204	-

Based on table 1, it can be seen that the number of colonies of *Staphylococcus aureus* bacteria shows a number of 100 CFU while the colonies of *Escherichia coli* bacteria that grow on almond media show a number of 14 CFU. The media sterility test was also carried out by incubating at the same temperature of 37º C for 2 x 24 hours, and after being observed there was no bacterial growth, this indicates that the almond media is sterile.

Table 1 shows that there is growth of *Escherichia coli* and *Staphylococcus aureus* bacteria on natural almond media. Observation of the number of colonies on almond media is less when compared to NA media as a positive control. Repetition of the sample was done five times for each bacteria. *Escherichia coli* and *Staphylococcus aureus* bacteria received the same treatment at the time of this study, this aims to make the characteristics of each colony close to the same. Bacterial isolates grown on almond natural media are young bacteria because they have been rejuvenated beforehand. According to (Hamdiyati, 2011) and (Soedarto, 2015) bacterial rejuvenation is a way to treat bacteria to keep it good. The age of bacterial culture can be determined from the length or time of incubation. If the culture media conditions are optimal for

the growth and life of bacteria, there will be maximum growth. At the time of observation and counting, the colony of *Staphylococcus aureus* bacteria showed a number of 100 CFU while the colony of *Escherichia coli* bacteria growing on almond media showed a number of 14 CFU. This number meets the standard plate count calculation, which is the number of colonies that grow on a Petri dish media ranging from 30-300 colonies (Waluyo, 2016). The number of colonies of *Escherichia coli* bacteria is less than 30 because the colonies that grow mostly accumulate, so that even though they grow a lot they are still considered one colony, only a small part of the colonies are small and grow separately. Escherichia coli bacteria grow more than Staphylococcus aureus bacteria, this is because Escherichia coli bacteria are able to double their bodies within 15-20 minutes, while *Staphylococcus aureus* bacteria require a division time of 25-28 minutes on a simple medium. So the growth of *Escherichia coli* bacteria is faster than *Staphylococcus aureus* bacteria because *Escherichia coli* has twice the reproductive ability (Wulandari & Sulistyani, 2016).

There is a difference in the number of bacterial colonies that grow on almond media and on NA media because NA media is a media that has been clinically tested good for bacterial growth, so that the process of bacterial growth takes place optimally. Meanwhile, almond media still has more complex nutritional compounds so that growth is not optimal, as in NA media. Complex media is media whose content is not known in detail and uses materials from nature. The complex content in the media can cause bacterial growth to take longer to decompose simple components that can be absorbed by cells and used for cell synthesis and energy (Anisah & Rahayu, 2015). Alternative media making materials used in this study using almond boiling water. During the boiling process of almonds, protein heating occurs in almonds which can cause denaturation reactions, loss of enzyme activity, solubility changes, colour changes, amino acid residues, and peptide bond breaks. This reaction is influenced by the temperature and duration of heating. This reaction also causes protein levels to decrease (Rikardo et al., 2016).

As a result, if the nutrient content in almonds decreases, it can slow down the growth of bacteria. Because if the nutrients needed by bacteria are sufficient, it will accelerate bacterial growth, and vice versa if the nutrients needed are insufficient, the bacteria must adjust to the environment and the formation of enzymes to break down the substrate which takes longer (Anisah & Rahayu, 2015).

NA media has a protein content of 98%, while according to the United States Department of Agriculture (2016), almonds (Prunus dulcis) contain 21.2% protein, 4.4% water content, 49.9% fat, 21.6% carbohydrates. The nutrients in almonds can meet the needs of *Escherichia coli* and *Staphylococcus aureus* bacteria to grow even though the number of colonies is less than the colonies that grow on NA media. In addition, from the type of natural media protein, almonds are vegetable protein while NA is animal protein. Animal protein does contain more complete amino acids than vegetable protein. In addition, the digestibility of animal protein is greater at 95-97% while the digestibility of animal protein is 70-80% (Muchtadi & Ayustaningwarno, 2010).

Researchers also made media without almond water to determine the effect of agar and sugar content on bacterial growth. Then, bacterial culture was planted on the media, then incubated at 37º C for 24 hours. After observation, there was no bacterial growth on the media. Agar agar contains 2% carbohydrate, 0% protein and 0% fat. This shows that the content of agar and sugar alone has no effect on bacterial growth due to insufficient nutrients.

Temperature is also the most important factor that can affect bacterial growth. Bacteria can generally live in the temperature range of -5ºC to 80ºC. For most pathogenic bacteria can grow well at 37ºC (Soedarto, 2015).

Because these two bacteria are pathogenic bacteria, when incubated at the optimum temperature of 37º C the bacteria *Staphylococcus aureus* and *Escherichia coli* can grow well.

In addition, osmosis pressure also affects the growth of bacteria. Osmotic pressure is the pressure required to maintain osmotic equilibrium between a solution and its pure solvent separated by a semipermeable membrane that can be penetrated only by the solvent. The process of moving solvent molecules from the more dilute part to the more concentrated part or from the part with low solvent concentration to high solvent concentration is called osmosis. If the bacteria are in a hypotonic solution (a solution whose solute concentration is smaller than the solvent concentration) then water will enter the bacterial cells. Whereas if the bacteria are in a hypertonic solution (a solution whose concentration is higher than the concentration in bacterial cells), then the possibility that will occur is the release of fluid from bacterial cells through the cytoplasmic membrane called plasmolysis. The most suitable medium for bacterial life is a medium that is isotonic to the contents of bacterial cells (Fatmariza et al., 2019).

Based on this, this almond natural media has a good osmosis pressure for bacterial growth, this is due to the growth of bacterial colonies on almond natural media.

CONCLUSIONS AND RECOMMENDATIONS

Based on the results of the research that has been done, it can be concluded that almond decoction can be used as an alternative medium for the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria. In the alternative media of almond decoction, the number of colonies of *Staphylococcus aureus* bacteria showed a number of 100 CFU while the colonies of *Escherichia coli* bacteria showed a number of 14 CFU. Future researchers are expected to perform multistage dilutions on bacterial suspensions inoculated on alternative media, the aim is to reduce the number of microbes in the liquid to facilitate calculation. Then, conduct a comparative test between alternative media of almond decoction and media without almond decoction content (using only solidification material (agar) and sugar flour) to determine the effect of almond content on the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria on the media.

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