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Evaluation of Mung Bean (Vigna radiata) Flour and Infusion as Alternative Growth Media for Staphylococcus aureus

Evaluasi Tepung dan Infusum Kacang Hijau *(Vigna radiata)* sebagai Media Alternatif Pertumbuhan *Staphylococcus aureus*

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Abstract. *Limited access to and the high cost of commercial growth media have driven the development of alternative media based on locally available materials. Although several legume-based materials have been reported to support bacterial growth, the use of mung beans (Vigna radiata) as an alternative medium—particularly in the form of flour and infusion—and comparisons of their effectiveness against standard media remain limited. This study aims to evaluate the potential of these two forms as growth media for Staphylococcus aureus. This was an experimental study conducted from March to May 2024 at the Microbiology Laboratory of the Medan Public Health Polytechnic (Poltekkes Kemenkes). The method used was growth analysis via the Total Plate Count (TPC) method. The results showed that the average TPC of S. aureus on mung bean flour medium was 1.07 × 10 ^{7} CFU/mL, and on mung bean infusion medium it was 4.7 × 10 ^{6}CFU/mL, whereas TSA medium, as the standard medium, yielded 5.8Z× 10 ^{6} CFU/mL. This indicates that both forms of the mung bean alternative media were capable of supporting optimal bacterial growth, with the flour medium yielding a higher cell count compared to the standard medium (TSA). Morphologically, the colonies on the alternative media were smaller (±2 mm, yellowish-white) compared to those on TSA (±3 mm, yellow). Thus, it can be concluded that mung bean flour has the potential to be an effective alternative medium for growing S. aureus compared to the infusion medium, which is less optimal due to the possible degradation of nutrients during the heating process.*

Keywords: *alternative_media; mung_bean_flour; Staphylococcus_aureus; total_plate_count; Vigna_radiata*

Abstrak. Keterbatasan akses dan tingginya biaya media pertumbuhan komersial mendorong pengembangan media alternatif berbasis bahan lokal. Meskipun beberapa bahan legum telah dilaporkan dapat mendukung pertumbuhan bakteri, pemanfaatan kacang hijau *(Vigna radiata)* sebagai media alternatif, khususnya dalam bentuk tepung dan infusum, serta perbandingan efektivitasnya terhadap media standar masih terbatas. Penelitian ini bertujuan mengevaluasi potensi kedua bentuk tersebut sebagai media pertumbuhan *Staphylococcus aureus*. Jenis penelitian adalah eksperimental yang dilakukan pada Maret–Mei 2024 di Laboratorium Mikrobiologi Poltekkes Kemenkes Medan. Metode yang digunakan adalah analisis pertumbuhan menggunakan metode *Total Plate Count* (TPC). Hasil menunjukkan bahwa rerata TPC *S.aureus* pada media tepung kacang hijau adalah 1,07 × 10^{7} CFU/mL, dan pada media infusum yaitu 4,7 × 10^{6} CFU/mL, sedangkan media TSA sebagai media standar

diperoleh 5,8 × 10^6 CFU/mL. Hal ini berarti bahwa kedua bentuk media alternatif kacang hijau mampu menyokong pertumbuhan bakteri secara optimal, di mana media tepung menghasilkan jumlah sel yang lebih tinggi dibandingkan media standar (TSA). Secara morfologi, koloni pada media alternatif berukuran lebih kecil (±2 mm, warna putih kekuningan) dibandingkan koloni TSA (±3 mm, kuning). Sehingga dapat disimpulkan bahwa tepung kacang hijau berpotensi efektif sebagai media alternatif, untuk menumbuhkan *S. aureus* dibandingkan media infusum yang kurang optimal akibat kemungkinan degradasi nutrisi selama proses pemanasan.

***Kata Kunci**: media_alternatif; Staphylococcus_aureus; tepung_kacang_hijau; total_plate_count; Vigna_radiata*

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1. Introduction

In microbiology, growth media are essential materials containing a balanced mix of nutrients used to cultivate and study microorganisms, including fungi and bacteria. The efficacy of a growth medium in supporting microbial growth hinges on several critical factors: it must be incubated at a specific temperature, maintain adequate humidity, possess optimal pH levels, ensure sufficient oxygen levels, be sterile prior to seeding, free from inhibitory substances, and provide all essential nutrients required by microorganisms [1], [2]. Microorganisms require a variety of nutrients for their growth and metabolic activities. These include carbon, nitrogen, non-metallic elements like sulphur and phosphorus, and metallic elements such as calcium, zinc, sodium, potassium, copper, manganese, magnesium, and iron. Additionally, vitamins, atmospheric gases, and sources of energy are vital for their physiological processes [3].

Among these nutrients, carbohydrates and proteins play pivotal roles as they serve as primary sources for microbial synthesis, particularly in bacteria [4]. These nutrients support essential cellular functions, including growth, reproduction, and the production of enzymes and structural components necessary for microbial survival and proliferation [5]. Understanding the precise nutritional requirements of microorganisms and optimizing growth media formulations are crucial in microbiological research [6]. By ensuring that growth media provide a balanced and supportive environment, researchers can effectively study microbial behaviours, interactions, and responses to various experimental conditions. This knowledge not only advances basic understanding but also informs practical applications in fields such as medicine, agriculture, and environmental science [7]. In conclusion, the composition and quality of growth media significantly influence the outcomes of microbiological studies. Rigorous attention to these factors enhances the reliability and reproducibility of experimental results, facilitating deeper insights into microbial physiology and potential applications in diverse scientific disciplines.

In microbiology, growth media can be categorized into two main types based on their constituent materials: synthetic media and natural media. Synthetic media are composed of ingredients with precisely known compositions, such as Tryptic Soy Agar. These media are formulated using purified chemicals and are standardized to provide consistent nutrient levels for microbial growth. On the other hand, natural media consist of ingredients derived from natural sources such as potato extract, carrot juice, nuts, and tubers [1]. These media utilize complex organic compounds present in natural materials, offering a more diverse array of nutrients compared to synthetic counterparts. Natural resources and waste utilization offer promising alternatives as growth media for microorganisms derived from natural materials, which are cost-effective and easy to maintain [8]. Previous studies by Zamilah, Ruhimat, & Setiawan [9] on peanuts and Nurhidayanti [10] on soybeans

have demonstrated that peanut flour and soybean flour can effectively serve as growth media for *Staphylococcus aureus* bacteria. Similarly, mung beans, renowned for their high protein content, exhibit similar potential. These natural materials provide a rich source of nutrients essential for microbial growth, including carbohydrates, proteins, and various micronutrients. They can support microbial proliferation while offering environmental and economic benefits through the repurposing of agricultural by-products and waste materials. The utilization of mung bean flour, in particular, underscores its suitability as a nutrient-rich substrate for cultivating *Staphylococcus aureus* under laboratory conditions. Such research contributes to expanding the repertoire of sustainable and accessible growth media options in microbiological studies.

Mung beans, a staple crop in tropical regions and a member of the legume family (*Fabaceae*), offer numerous benefits in everyday life due to their high vegetable protein content. Renowned for their nutritional profile, mung beans are an excellent source of protein, fiber, minerals, and vitamins. They also contain a variety of bioactive compounds, including polyphenols, polysaccharides, and peptides, which contribute to their health benefits [11]

The average green bean production in North Sumatra in 2017 was 2,874.3 tons, an increase of 703 tons from the previous year. Langkat, Serdang Bedagai, and Deli Serdang districts are the main production areas for green beans in North Sumatra. Green bean production in Deli Serdang District reached 662 tons with a harvested area of 571 hectares [12].

Despite this abundant local availability, the utilization of mung beans as an alternative bacterial growth medium remains extremely limited and poorly documented. Theoretically, the relatively high protein content of green beans makes them comparable to Trypticase Soy Agar (TSA) medium, which consists of agar, tryptone, soytone, and sodium chloride [13]. However, a critical research gap exists regarding how different processing methods affect this nutrient integrity. While various legumes are known to support microbial growth, there is a distinct lack of comparative studies evaluating whether simpler preparation forms, such as raw flour, preserve essential proteins and carbohydrates better for pathogenic bacteria like *Staphylococcus aureus* compared to heated liquid infusions, which risk thermal nutrient degradation. Therefore, this study aimed to evaluate and compare the efficacy of mung bean flour and infusion as alternative growth media for *Staphylococcus aureus*. By addressing these gaps, this research intends to provide a scientifically validated formulation that can significantly reduce the cost of practical learning and research in laboratories with limited infrastructure.

2. Methods

This research was conducted using a laboratory experimental design, which was carried out at the Microbiology Laboratory of the Medan Health Polytechnic Integrated Laboratory from March to May 2024. In this study, Tryptic Soy Agar (TSA) was employed as the standard commercial medium, serving as the positive control group to evaluate and compare the growth efficacy of the alternative mung bean media.

Tools and Materials

The tools used in this research are petri dishes, beaker glass, test tubes, Erlenmeyer, measuring cup, blender, 80 mesh sieve, stirring rod, incubator, Bunsen, balance scale, autoclave, stirrer, oven, Bio Safety Cabinet. The materials used in this study were mung beans, TSA, TSB, BPW, distilled water, NaCl, standard agar and *Staphylococcus aureus* bacteria.

Preparation of Tryptic Soy Agar Media

A total of 3 g TSB was added with 1.5 g standard agar and dissolved into 100

ml of distilled water; the solution was homogenized using a stirrer. The media was sterilized using an autoclave at 121°C for 15 minutes. After the solution is cooled until the temperature reaches 45 ° C, the media is poured into petri dishes [4].

Preparation of Mung Bean Flour

Preparation of mung bean flour refers to research; 100 g of mung beans were washed thoroughly with distilled water. Mung beans that have been washed clean are dried using an oven for 8 hours at 60°C. Mung beans are pulverized using a blender until they become flour, then the mung bean flour is filtered using a sieve.

Preparation of Green Mung Bean Infusum

Preparation of mung bean extract refers to research [9], 100 grams mung beans are washed thoroughly. Then the mung beans are boiled with 500 ml of distilled water at 90 °C for 15 minutes. Next, the boiled water is filtered into an Erlenmeyer.

Making Alternative Media for Mung Bean Flour

A total of 2.25 g mung bean flour, 1.5 g standard agar and 0.5 g NaCl were put into an Erlenmeyer and dissolved in 100 ml of distilled water, the solution was homogenized using a stirrer. The media was sterilized using an autoclave at 121°C for 15 minutes. After the solution was cooled until the temperature reached 45°C, the media was poured into Petri dishes aseptically using a Bunsen and carried out in laminar air flow [8].

Making Alternative Media for Mung Bean Infusum

A total of 100 ml of mung bean infusion, 1.5 g standard agar and 0.5 g NaCl were put into an Erlenmeyer, the solution was homogenized using a stirrer. The media was sterilized using an autoclave at 121°C for 15 minutes. After the solution was cooled until the temperature reached 45°C, the media was poured into Petri dishes aseptically using a Bunsen and carried out in laminar air flow [14].

Calculation of Bacterial Growth Using the TPC Method

S. aureus bacteria were streaked on TSA media and incubated at 37°C for 18 hours. 1 colony that grew was then inoculated back into TSB media. The growing colonies were measured for Optical Density up to 0.725 using a spectrophotometer. Bacteria with Optical Density 0.725 were diluted to and spread on TSA media and alternative mung bean media using the Spread Plate method. Then the media was incubated at 37°C for 24 hours. The growing bacterial colonies were counted and converted using the TPC formula [6], [15].

Data Analysis

Bacterial growth was quantified using the Total Plate Count (TPC) method and expressed as colony-forming units per millilitre (CFU/mL), calculated by multiplying the number of colonies by the reciprocal of the dilution factor. Only plates containing 30–300 colonies were considered valid for enumeration, while plates exceeding this range were recorded as too numerous to count (TNTC) [16]. All experiments were performed in triplicate, and the results were presented as mean ± standard deviation. Differences between groups were analysed using Welch's t-test due to unequal variances. Statistical analysis was conducted using SPSS to ensure the reliability of the comparisons.

3. Result and Discussion

After conducting research on the growth of *Staphylococcus aureus* bacteria on alternative growth media of mung bean flour and mung bean infusion, the results were obtained.

Table 1. Number of colony growth of *S. aureus* bacteria on mung bean flour alternative media

Sample Repetition	Control (10^{-4}) Colonies/ml	(CFU/ml)	Colony Count (Colonies) 10^{-2}	10^{-3}	10^{-4}	10^{-5}	Colonies 10^{-4} (CFU/ml)
1	35	3.5 x 10^6	TNTC	TNTC	102	22	1.02 x 10^7
2	39	3.9 x 10^6	TNTC	TNTC	103	7	1.03 x 10^7
3	101	1.01 x 10^7	TNTC	TNTC	118	10	1.18 x 10^7
X	58	5.8 x 10^6	-	-	107	13	1.07 x 10^7
SD	-	3.7 x 10^6	-	-	-	-	8.9 x 10^5

TNTC : Too Numerous To Count

The growth of *S. aureus* bacteria on an alternative medium made from mung bean flour showed promising results. The mean bacterial count on mung bean flour medium was 1.07 × 10^6 ± 8.9 x 10^5 CFU/mL, which was higher than TSA (5.83 x 10^5 ± 3.70 x 10^5 CFU/mL) (Table 1). However, the difference was not statistically significant (p = 0.141). The mung bean flour medium demonstrated the ability to support the growth of *S. aureus*, showing numerically higher colony counts than TSA. In 100 grams of mung bean flour, the nutritional content includes 70 grams of carbohydrates, 27 grams of protein, and 2 grams of fat. This rich nutrient profile supports bacterial growth, making mung bean flour a viable alternative to traditional media.

The mean bacterial count on mung bean infusion medium was 4.7 x 10^5 ± 3.5 × 10^5 CFU/mL, which was lower than TSA. However, no statistically significant difference was observed between the two groups (p = 0.720) (Table 2). In contrast, on TSA (Tryptic Soy Agar) media, the average growth of *S. aureus* was 58 colonies, or 5.8 x 10^6 CFU/ml in dilution. The reduced growth of bacterial colonies on the mung bean infusion media compared to mung bean flour media can be attributed to the lower nutritional content of the infusion. This decrease in nutritional value is likely due to the boiling process, which can reduce nutrient levels and cause protein denaturation from high-temperature heating [17].

Comparison between mung bean flour and infusion media showed that the flour medium tended to produce higher bacterial counts; however, the difference was not statistically significant (p = 0.087). These findings suggest that while mung bean infusion media can support the growth of *S. aureus*, it is less effective than mung bean flour media due to the loss of nutrients during preparation. This highlights the importance of maintaining nutrient integrity in the development of alternative

Table 2. Number of colony growth of *S. aureus* bacteria on mung bean infusion alternative media

Sample Repetition	Control (10^{-4}) Colonies/ml	(CFU/ml)	Colony Count (Colonies) 10^{-2}	10^{-3}	10^{-4}	10^{-5}	Colonies 10^{-4} (CFU/ml)
1	35	3.5 x 10^6	TNTC	TNTC	16	9	1.6 x 10^6
2	39	3.9 x 10^6	TNTC	TNTC	40	8	4.0 x 10^6
3	101	1.01 x 10^7	TNTC	TNTC	85	9	8.5 x 10^6
X	58	5.8 x 10^6	-	-	47	9	4.7 x 10^6
SD	-	3.7 x 10^6	-	-	-	-	3.5 x 10^6

TNTC : Too numerous to count

bacterial growth media.

The characteristics of *S. aureus* bacteria that grow on alternative mung bean media show distinct differences compared to those on TSA media. On the alternative mung bean media, the bacterial

colonies measure approximately 2 mm in diameter and exhibit a yellowish-white colour. In contrast, the colonies on TSA media are larger, measuring around 3 mm in diameter, and have a yellow colour.

The smaller size of the colonies on the mung bean media can be attributed to the less complex nutritional composition compared to the control TSA media. Additionally, the bacteria on the alternative media are likely in an adaptation phase, adjusting to the different environmental conditions. This adaptation involves synthesizing new enzymes to utilize the available nutrients, which can temporarily slow down their growth. The combination of these adaptation responses and the limited nutrients in the mung bean media contributes to the smaller colony size [18].

These observations highlight the impact of nutrient composition and environmental adaptation on bacterial growth characteristics, emphasizing the need to consider these factors when developing and using alternative growth media.

Tabel 3. Characteristics of bacteria on mung bean alternative media

Bacteria Name	Media Name	Colony Image	Colony Size	Colony Color
Staphylococcus aureus	TSA		3mm	Yellow
	Mung Bean Flour		2mm	Yellowish White
	Mung Bean Infusum		2mm	Yellowish White

Although mung bean flour medium showed higher bacterial counts compared to TSA, the difference was not statistically significant. This suggests that mung bean flour has comparable potential as a growth medium for *S. aureus*. The average colony count on green bean flour medium reached 1.07×10^7 CFU/ml, while on TSA it was 5.8×10^6 CFU/ml. This indicates that green bean flour contains sufficient nutrients to meet the metabolic needs of *S. aureus*. The relatively high protein, carbohydrate, and fat content in green bean flour (protein 18.4%; carbohydrates 66.2%; fat 1.8%) plays a crucial role in providing the carbon, nitrogen, and energy sources required by bacteria for growth [4], [18].

In contrast, the green bean infusion medium showed lower colony growth, at 4.7×10^6 CFU/ml. This difference is likely due to the reduction in nutrient content caused by high-temperature boiling, which can lead to protein denaturation and the loss of some water-soluble vitamins and

minerals [17]. Overall, statistical analysis indicated no significant differences in bacterial growth among the control, mung bean flour, and mung bean infusion media ($p > 0.05$). Nevertheless, mung bean flour medium demonstrated a consistent trend toward higher bacterial growth compared to the other media.

Differences in the morphology of *S. aureus* colonies were also observed in this study. Colonies on TSA were approximately 3 mm in size with a yellow colour, while those on green bean media (flour and infusion) were approximately 2 mm in size with a pale-yellow colour. These differences in size and colour may be attributed to varying nutrient content. The green bean medium, which has a simpler composition than TSA, may cause bacteria to remain in the adaptation phase longer, resulting in slower colony growth and smaller colony size [19].

These findings reinforce previous studies indicating that legume-based food materials can be used as bacterial growth media [9], [10]. The use of green bean flour as an alternative medium not only has the potential to reduce the cost of microbiology experiments but also utilizes locally available resources. This is particularly relevant for educational or research laboratories with limited funding, thereby reducing dependence on relatively expensive commercial media.

However, this study has several limitations. First, it only tested one type of Gram-positive bacteria *S. aureus*, so the effectiveness of the medium against other bacterial types remains unknown. Second, nutrient content analysis of the medium after production was not conducted, so it is unclear how much nutrients are actually available to bacteria. Third, the concentrations of green bean flour and infusion were not varied to assess formula optimization effects [20].

4. Conclusion

Based on the results of a study on bacterial growth, mung bean (Vigna radiata) flour shows strong potential as an effective alternative growth medium for Staphylococcus aureus, with growth effectiveness comparable to that of the standard commercial medium, Tryptic Soy Agar (TSA). In contrast, mung bean infusion medium is less optimal for bacterial growth, presumably due to the degradation of essential nutrients during the heating or boiling process. From a morphological perspective, both alternative media successfully supported bacterial growth, although they produced colonies that were slightly smaller than those on the standard medium. Therefore, mung bean flour can be recommended as a viable, low-cost alternative growth medium for microbiology laboratory exercises, particularly in laboratories with limited infrastructure.

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