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Comparative Efficacy of Saga Seed-Derived Media in Supporting *Staphylococcus aureus* and *Escherichia coli* Growth

Lestari Rahmah[1], Vuan Sindra Noor[2], Tania Regita Sari[1], Geminsah Putra[1], Febri Sembiring[1*]

[1]Politeknik Kesehatan Kementerian Kesehatan Medan, Indonesia
[2]Medical Technology Laboratory, Poltekkes Kemenkes Medan, Indonesia

*Correspondence email: febrisembiring.kemenkes@gmail.com

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ABSTRAK

Biji saga memiliki kandungan protein yang tinggi dan berpotensi sebagai media alternatif yang hemat biaya untuk aplikasi mikrobiologi. Penelitian ini mengevaluasi kelayakan penggunaan biji saga (Adenanthera pavonina) sebagai media kultur alternatif untuk pertumbuhan Escherichia coli dan Staphylococcus aureus. Dua metode persiapan diuji, yaitu media berbasis tepung dan media berbasis infus. E. coli dan S. aureus dikultur pada media biji saga dan dibandingkan dengan Tryptic Soy Agar (TSA) sebagai media kontrol. Jumlah koloni dihitung dan dianalisis menggunakan uji T sampel independen. Secara fisik, media infus saga menunjukkan larutan yang jernih tanpa endapan, berbeda dengan media berbasis tepung yang masih menyisakan endapan. Morfologi koloni pada kedua jenis media saga (tepung dan infus) berbentuk bulat, berwarna putih, dan berdiameter sekitar 3 mm, konsisten dengan koloni yang tumbuh pada media kontrol. Berdasarkan kriteria tingkat produktivitas, kedua jenis media saga memenuhi ambang batas minimum sebagai media non-selektif. Setelah dikonversi berdasarkan pengenceran, pertumbuhan E. coli pada media tepung saga mencapai 1,06 x 10^5 CFU/ml dan tidak menunjukkan perbedaan signifikan dibandingkan TSA. Namun, pada media infus saga, pertumbuhan E. coli lebih rendah, yaitu sebesar 0,94 x 10^4 CFU/ml. Untuk pertumbuhan S. aureus, tidak terdapat perbedaan signifikan antara media tepung saga (1,08 x 10^5 CFU/ml) dan media infus saga (1,04 x 10^5 CFU/ml) dibandingkan dengan kontrol. Kesimpulannya, media tepung biji saga merupakan alternatif yang layak dan efektif untuk pertumbuhan E. coli dan S. aureus. Namun, penyempurnaan lebih lanjut diperlukan untuk meningkatkan kelarutan dan kejernihan fisik media tepung saga.

Kata Kunci: Media alternatif, Pertumbuhan mikroba, Non-selektif, Rasio produktivitas, Benih saga (*Adenanthera pavonina*)

ABSTRACT

Saga seeds possess a high protein content, presenting a potentially economical substrate for microbiological medium development. This study evaluated the suitability of saga seeds (Adenanthera pavonina) as an alternative culture medium for propagating Escherichia coli and Staphylococcus aureus. The objective was to determine whether medium derived from saga seeds could sustain bacterial proliferation at levels comparable to conventional medium. It was postulated that both flour-based and infusion-based saga seed medium would yield bacterial growth statistically indistinguishable from that on Tryptic Soy Agar (TSA). Two formulation methods were examined: a flour-based medium and an infusion-based medium. E. coli and S.

aureus were cultured on these alternative media and benchmarked against TSA. Colony-forming units (CFUs) were quantified and statistically analyzed using an independent samples t-test. The infusion-based medium displayed complete solubility with no residue, whereas the flour-based variant showed sedimentation. Colonies on both saga-based media appeared white, circular, and approximately 3 mm in diameter, consistent with those on the control medium. Based on productivity rate standards, both the saga seed medium fulfilled the minimum criteria for a non-selective medium. After adjusting for dilution, E. coli reached 1.06 × 10 ^{5} CFU/ml on the flour-based medium, exhibiting no statistically significant deviation from TSA. In contrast, the infusion-based medium supported lower E. coli growth at 0.94 × 10^{4} CFU/ml. S. aureus growth on the flour-based (1.08 × 10 ^{5} CFU/ml) and infusion-based medium (1.04 × 10 ^{5} CFU/ml) did not significantly differ from the control. The flour-based saga medium offers a promising and effective alternative for cultivating E. coli and S. aureus. Nonetheless, further optimization is advised to improve the flour-based formulation's solubility and visual clarity.

***Keywords**: Alternative media, Microbial growth, Non-Selective, Productivity Ratio, Saga seeds (Adenanthera pavonina)*

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INTRODUCTION

Microorganisms assimilate nutrients from the culture medium through small molecules, which are subsequently incorporated into cellular components. The nutritional requirements vary among microbial species; hence, culture media differ in both composition and form based on the specific organisms being cultivated. An ideal culture medium should meet several criteria: cost-effective, straightforward to prepare, and practical. These media are typically available in liquid or solid formulations (Girase et al., 2022).

Bacterial proliferation necessitates key nutrients, including carbon, nitrogen, mineral salts, and water. Among these, carbon—the most prevalent element in bacterial cells—plays a vital role in the biosynthesis of essential organic compounds such as lipids, proteins, nucleic acid, and carbohydrates. Bacteria can utilize inorganic carbon sources, such as carbon dioxide, and organic sources, including alcohols and sugars. Moreover, carbon functions as the primary substrate in their metabolic processes, thereby serving as a fundamental nutrient source (Anastassiadis, 2016; Amaliah et al., 2018)

The addition of agar solidifies this alternative medium, with glucose serving as a carbon source for bacterial growth (Anisah & Rahayu, 2015). Bacteria, as single-celled organisms, require specific nutrients to thrive. Essential macronutrients, including N, O, P, S, K, H, C, Fe, Ca, and Mg, support synthesizing cellular components like carbohydrates, lipids, proteins, and nucleic acids, with several

acting as cations for cell functions. Microorganisms also depend on micronutrients (Cu, Mo, Mn, Zn, Co, Ni) for enzymatic processes and may need organic growth factors. Such nutrient requirements are fundamental to microbial cultivation and contribute to advancing microbiological research (Gamit et al., 2023; Hassan et al., 2020). Cultivating bacteria requires expensive growth media due to intricate nutritional requirements. The substantial cost associated with conventional culture medium has prompted researchers to explore alternative substrates that are more readily accessible and cost-effective. These alternative media must possess a sufficient concentration of essential nutrients, particularly carbohydrates and proteins, to support optimal bacterial proliferation ; Gamit et al., 2023)

Various protein-rich sources have been effectively utilized as alternative culture media to support microbial proliferation. Leguminous grains such as cowpea (*Vigna unguiculata*), mung bean (*Vigna radiata*), black soybean (*Glycine max*), soybean (*Glycine max*), and jackfruit (*Artocarpus heterophyllus*) seeds have demonstrated potential in promoting the growth of diverse bacterial genera, including *Pseudomonas*, *Staphylococcus*, *Bacillus*, and *Escherichia coli* (Foekh et al., 2024; Juariah et al., 2018). (Uthayasooriyan et al., n.d.) Further reported alternative substrates such as *Setaria italica*, *Zea mays*, *Cicer arietinum, Lens culinaris*, *Oryza sativa,* and natural soy flour as viable culture medium. Their findings revealed that *Klebsiella* exhibited enhanced growth in chickpea-based medium, while *Pseudomonas* proliferated more robustly in natural soy flour than in conventional Nutrient Agar (NA).

Additionally, date palm flour was found to be a promising substrate for cultivating *E. coli* and *Bacillus cereus*, yielding colony counts of 5.4×10^6 CFU/g and 4.5×10^6 CFU/g, respectively (Rini & Saidi, 2023). Seeds of the saga tree, which resemble petai in morphology with their small, vibrant red appearance, have also emerged as a prospective medium (Usmani et al., 2016). This leguminous tree is well-adapted to marginal lands, can thrive without fertilization, and exhibits natural resistance to pests and invasive weeds, eliminating the need for chemical pesticides (Fajar et al., 2024). Furthermore, saga plants contribute positively to sustainable agriculture and can be cultivated alongside other crops (Dwitanti et al., 2020). Nutritionally, saga seeds are characterized (g 100 g^{-1}) by a high protein concentration of 2.44, a fat content of 17.99, and essential minerals, while

maintaining relatively low sugar levels of 8.2 and starch of 41.95. Their protein content is notably higher than many other legumes, positioning saga seeds as a promising protein source.

Additionally, they contain substantial amounts of total fatty acids, predominantly linoleic and oleic acids, comprising approximately 70.7% of the total lipid profile (Dwitanti et al., 2020). Despite their rich nutritional profile, the potential of saga seeds as a culture medium for bacterial growth has not yet been systematically explored in scientific studies. Thus, the potential of saga seeds as a substrate for bacterial growth is investigated in this work. E. coli and S. aureus were employed as indicator microorganisms in the early stages to assess the success in developing bacterial culture medium. This study aims to evaluate the effectiveness of saga seed-derived medium, specifically infusion-based medium and flour-based medium, in supporting the growth of two indicator bacteria, with the hypothesis that both medium possess comparable capabilities to TSA.

METHOD

Bacterial Strain and Culture Conditions

To evaluate the efficacy of saga seed medium as an alternative culture medium, the bacterial strains *S. aureus* ATCC 6538 and *E. coli* ATCC 25952 were employed as reference organisms. Both strains were initially cultured on Tryptic Soy Agar (TSA) (Merck, Germany), serving as the reference medium. They were incubated for 24 hours at 37°C under aerobic conditions to ensure optimal growth.

Preparation of Saga Seed Flour Media

Saga seeds were initially processed using a mechanical grinder, after which the fragments were manually separated from their outer coats. The decorticated seeds were then dried in an oven at 60°C for approximately 6 to 8 hours. Upon reaching the desired dryness, the seeds were finely ground using a blender and filtered through a 48-mesh sieve to produce a consistent flour. An amount of 22.5 g L^{-1} of this saga seed flour was mixed (g L^{-1}) with agar (15), NaCl (5), and specified amounts of yeast extract, meat extract, and peptone (8). The pH of the solution was then adjusted to 7.0 using either NaOH or HCl, as required, to create an optimal environment for bacterial growth. The finalized medium was sterilized via autoclaving at 121°C for 15 minutes and poured into Petri dishes, allowing it to solidify at room temperature (Hayek, 2013).

Preparation of Saga Seed Infusum Media

One hundred grams of cleaned saga seeds were boiled in water (500 mL). The resulting filtrate was then collected, and the extract (250 mL) was combined with agar (10 g). To dissolve this combination solution, it was heated while being stirred. Before sterilization, the pH of the final medium was carefully adjusted to 7.0 to maintain neutrality and promote consistent bacterial proliferation (Hayek, 2013).

Growth Assessment for *E.coli* and *S.aureus* Bacteria

Preserved cultures of *E. coli* and *S. aureus* stored at −80°C were reactivated by streaking onto Nutrient Agar (NA) plates, followed by incubation at 37°C for 18 hours. Distinct colonies from the NA medium were then inoculated into TSB and incubated accordingly. The resulting bacterial suspension, with an optical density (OD) of 0.755—equivalent to approximately 10^9 CFU/mL as determined by spectrophotometric analysis—was subsequently subjected to a series of tenfold dilutions ranging from 10^{-1} to 10^{-4}. A 10^{-4} dilution was plated onto TSA and saga seed agar, which had been prepared using both flour and infusion techniques. Plates were incubated at 37°C for 24 hours, and bacterial proliferation was quantified by enumerating the colony-forming units (CFU) per milliliter (Nurmalasari et al., 2022; Zituni et al., 2014). Sterile distilled water was used as a negative control to validate that any observed bacterial growth was due to the nutritional contribution of the saga seed-based medium.

Data Analysis

This study applied a quantitative experimental design with two replications for each treatment group. Descriptive statistics were used to calculate mean CFU/ml values, which were subsequently analyzed to compare bacterial growth across medium types. An independent samples t-test was utilized to assess the statistical significance of differences in bacterial colony counts between the saga seed-based medium and the TSA control. The analysis was performed using SPSS Statistics, applying a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

This study assessed the performance of two formulations of saga seed-based culture medium—flour-derived and infusion-derived—in supporting the proliferation of *S. aureus* and *E. coli*, benchmarked against conventional TSA. As illustrated in **Figure 1**, the two medium types are visually represented, with the

flour-based variant depicted on the left and the infusion-based on the right, emphasizing their distinct physical characteristics. The flour-based medium displayed considerable sedimentation, reducing visual clarity due to the accumulation of particulate matter at the bottom. Conversely, the infusion-based medium exhibited enhanced transparency; nevertheless, neither medium attained the optical clarity necessary for precise bacterial growth assessment through spectrophotometric methods.

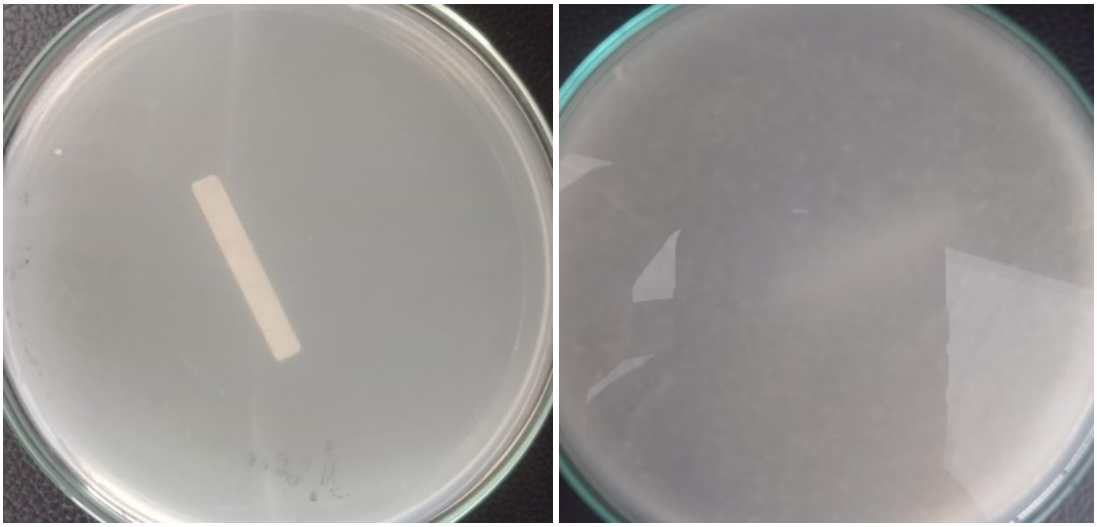
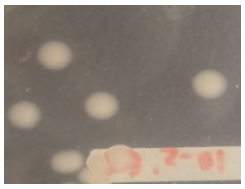
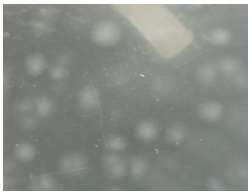
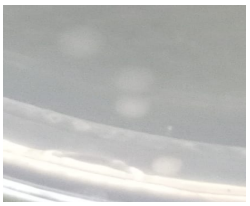
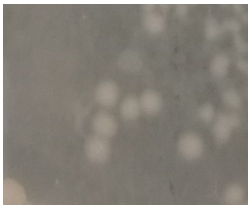


Figure 1. Saga seed medium: (left) flour-based, (right) infusion-based

The nutrient composition of a culture medium significantly influences bacterial colony size (Bonnet et al., 2020). **Table 1** provides a comparative evaluation of media formulated from saga seeds, including flour and infusum preparations, alongside TSA, in their ability to support the growth of S. aureus and E. coli. The data indicated that the infusum medium and TSA supported *E. coli* colony development with an average diameter of 3 mm. In contrast, the flour-based medium produced marginally smaller colonies, averaging 2.4 mm. The pigmentation of colonies on the flour medium ranged from cream to off-white. At the same time, those on the infusum exhibited hues from cream to light beige, closely resembling the colony appearance observed on TSA. For *S. aureus*, colony diameters on the infusum and TSA averaged 3 mm, with slightly reduced sizes on the flour medium at 2.8 mm. Colonies cultured on the saga seed-derived medium displayed pale white to translucent coloration, presenting morphological traits comparable to those observed on TSA. In contrast to Deslate et al., (2019) study on alternate medium with corn skin extract, it was discovered that *S. aureus* produced

slightly larger, ream-colored colonies, whereas *E. coli* flourished with white colonies.

Table 1. Characteristics of Colonies on each medium

Species	Medium	Colony Appearance	Colony Size (mm)	Colony Color
E.coli	Flour-based		2.4	Cream to off-white
	Infusum-based		3	Cream to light beige
	TSA		3	Cream to light beige
S.aureus	Flour-based		2.8	Pale white, clear
	Infusum-based		3	White, clear
	TSA		3	Pale white, clear

This study confirmed that the saga seed-based medium fulfilled the fundamental requirements for non-selective microbiological culture medium, as evidenced by the successful overnight growth of *S. aureus* and *E. coli* at 37 °C, following guidelines from the Australian Society for Microbiology. Slight variations

in colony size were observed, likely attributable to differences in nutrient availability. Overall, saga seed medium—especially in its infusum form—represents a viable alternative for microbiological research, promoting sustainability through using locally available and readily accessible materials for bacterial cultivation.

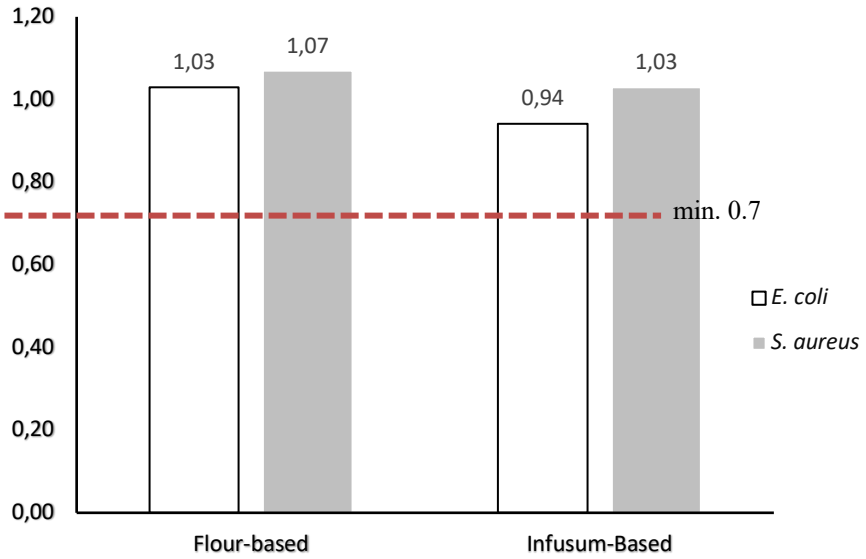


Figure 2. Productivity rate of flour-based and infusion-based saga seed medium

Figure 2 illustrates that *S. aureus* exhibited higher productivity rates than *E. coli* on both types of medium evaluated, indicating a possible preferential support of *S. aureus* growth by these medium formulations. Nonetheless, both preparations of saga seed-based medium met the required productivity rate threshold, with PR values exceeding 0.7, as established by (Madajczak et al., 2012). These findings hold substantial relevance for advancing alternative culture media in microbiological applications. In line with Kambuno et al. (2021), Pigeonpea and cowpea water medium showed no significant difference in growth rates for *E. coli* and *S. aureus* compared to NA.. According to Condrillon et al. (2024), the majority of the inexpensive protein sources—soybean protein, cowpea, lentil, chickpea, mung bean, and pea—were successful in encouraging the growth of the tested microorganisms, which included *Pseudomonas aeruginosa, Bacillus cereus, E. coli,* and *S. aureus*.

Table 2. Colony-forming unit counts of *E. coli* and *S. aureus* at a 10^{-4} dilution level cultured on saga seed medium prepared with flour and infusion, compared to TSA

	***Escherichia coli* (CFU/ml)**			***Staphylococcus aureus* (CFU/ml)**		
	R1	**R2**	$\bar{x}$	**R1**	**R2**	$\bar{x}$
TSA	1×10^5	$1{,}05 \times 10^5$	$1{,}02 \times 10^5$	$1{,}02 \times 10^5$	1×10^5	$1{,}01 \times 10^5$
Flour-based	$1{,}12 \times 10^5$	1×10^5	$1{,}06 \times 10^5$	$1{,}1 \times 10^5$	$1{,}06 \times 10^5$	$1{,}08 \times 10^5$
Infusion-based	9×10^4 [a]	$1{,}03 \times 10^5$	$9{,}6 \times 10^4$	$1{,}06 \times 10^5$	$1{,}02 \times 10^5$	$1{,}04 \times 10^5$

Notes: [a]p value < 0.05; R = Repetition

As outlined in **Table 2**, bacterial growth analysis demonstrated that *E. coli* achieved a mean population of 1.02×10^5 CFU/ml on TSA. At the same time, the saga flour-based medium supported a slightly higher growth level, averaging 1.06×10^5 CFU/ml. This suggests that the flour-based formulation may contain a comparatively more prosperous nutrient matrix. In contrast, the saga infusion-based medium resulted in a moderately lower average count of 9.6×10^4 CFU/ml, indicating a reduced, yet still supportive, growth capacity. A similar trend was observed for *S. aureus*, with TSA supporting an average of 1.01×10^5 CFU/ml, whereas the flour-based medium slightly increased to 1.08×10^5 CFU/ml. Notably, the infusion-based medium yielded 1.04×10^5 CFU/ml for *S. aureus*, marginally exceeding the TSA reference. Statistical evaluation using independent sample t-tests revealed no significant differences in colony-forming unit counts between the flour-based and TSA medium for both *E. coli* and *S. aureus*, as indicated by t-values of 0.80 and 0.12, respectively, both below the critical threshold of 2.45 at a 5% level of significance. These results affirm the potential application of saga seed-derived medium, particularly the flour-based formulation, for routine bacterial culture. The comparatively lower CFU/ml detected in the infusion-based medium may be attributed to its reliance on water-soluble nutrients.

In contrast, the flour-based counterpart likely provides a broader spectrum of nutritional components that better facilitate microbial proliferation. This difference may be attributed to the partial loss of protein and carbohydrate components during the boiling and filtration process used in the infusion preparation, which extracts primarily water-soluble compounds while omitting insoluble but bioavailable macromolecules retained in the flour-based medium. The flour-based medium, by preserving both soluble and insoluble fractions of the saga seeds, rich in protein (2.44 g 100 g^{-1} and starch 41.95 g/100 g^{-1} as mentioned earlier), offers a more complete nutrient matrix that potentially enhances microbial

proliferation (Dwitanti et al., 2020; Usmani et al., 2016). Differences in solubility and nutrient retention likely account for the superior performance of the flour-based medium compared to the infusion-based preparation. Both formulations of saga seed-based medium exhibit significant potential as economical and sustainable substitutes for TSA, especially advantageous in resource-limited environments where access to commercial culture medium is restricted.

CONCLUSIONS

This investigation demonstrates that culture media formulated from saga seeds—infusion-based and flour-based—are viable and environmentally sustainable alternatives to TSA for cultivating *E. coli* and *S. aureus*. The flour-based medium produced significantly high CFU/ml counts, possibly due to its more diverse nutrient profile. In contrast, although yielding marginally lower CFU/ml, the infusion-based medium effectively supported robust bacterial growth. Both formulations generated colonies with morphological traits comparable to those observed on TSA and exceeded the minimum productivity threshold (>0.7), substantiating their suitability for microbial culture applications. These results underscore the potential of saga seed medium as an affordable and practical alternative for microbiological studies, particularly in settings with limited resources, while advancing sustainability through locally available materials. However, this study was limited to two bacterial species and a single incubation condition; future research should explore the performance of saga seed-based medium across a broader range of microorganisms and culture environments.

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