JOURNAL OF INTERDISCIPLINARY PERSPECTIVES

ISSN Print: 2984-8288, ISSN Online: 2984-8385
Vol. 3 No. 8, 369-379, August 2025

Determining the Effect on the Crude Extract of *Psophocarpus tetragonolobus* (Winged Bean) as Protein Source Replacement for Tryptic Soy Broth

Patricia Angela Bianca T. Tordesillas*, Nikson R. Patawaran, Arwin J. Aparente, Lance Edilbert G. Veloso, Jeri Nicole Bautista, Daniel Marl Berches, Eunica Jade Manalang, Angelene Padual, Krizzelle Mae Rañada
National University, Philippines

*Corresponding Author Email: pttordesillas@nu-moa.edu.ph

Date received: May 22, 2025
Date revised: June 15, 2025
Date accepted: July 9, 2025

Originality: 90%
Grammarly Score: 99%
Similarity: 10%

Recommended citation:
Tordesillas, P. A. B., Patawaran, N., Aparente, A., Veloso, L. E., Bautista, J. N., Berches, D. M., Manalang, E. J., Padual, A., & Rañada, K. M. (2025). Determining the effect on the crude extract of *Psophocarpus tetragonolobus* (winged bean) as protein source replacement for Tryptic Soy Broth. *Journal of Interdisciplinary Perspectives*, *3*(8), 369-379. https://doi.org/10.69569/jip.2025.441

Abstract. This study explores the potential of *Psophocarpus tetragonolobus* (Winged Bean) crude extract as a protein source replacement for TSB, aiming to address the need for sustainable and cost-effective culture media. This research evaluated the growth of *E. coli* and *S. aureus* in modified TSB containing *P. tetragonolobus* (Winged Bean) crude extract at concentrations of 2%, 5%, and 10%. The TSB standard served as the positive control, and NSS served as the negative control. The media were inoculated with a crude extract prepared from the leaves of *P. tetragonolobus* (Winged Bean). Growth of bacteria was measured using the bacterial smear count method microscopically in terms of CFU/mL. The results obtained were statistically evaluated using ANOVA and multiple post hoc tests to identify a significant difference across different concentration treatments. The findings of this study indicated that a 5% concentration of *P. tetragonolobus* (Winged Bean) crude extract was the most viable concentration, with a mean count of 136.67 CFU/mL for *E. coli* and 145.33 CFU/mL for *S. aureus*. These values were comparable to those of TSB at 131 CFU/mL and 260.33 CFU/mL for E. *coli* and *S. aureus*, respectively. The growth was moderate, at 2%, while it significantly decreased at a 10% concentration, likely due to the inhibitory effects caused by the high solute concentration. These results confirmed that *P. tetragonolobus* (Winged Bean) crude extract was indeed a feasible source of practical and sustainable protein replacement for TSB.

Keywords: *Escherichia coli; Psophocarpus tetragonolobus*; *Staphylococcus aureus*; Tryptic Soy Broth.

1.0 Introduction

Microorganisms typically grow in a wide range of natural and practical environments, requiring essential sources of protein, food energy, carbon, nitrogen, phosphorus, and other minerals to maintain bacterial growth and reproduction. As such, the need to examine microbiological studies in a laboratory setting must have the dependency on maintaining and cultivating such microorganisms on a preferably provided, appropriate culture medium, to which it is a material used in microbiology laboratory experiments and is designated to supply nutrient ingredients needed for bacterial growth (Raju, 2020). However, related studies on microbial cultivation for identifying and isolating bacterial growth face immense constraints and limitations due to the high expense of culture medium nutrient ingredients used for laboratory purposes. To overcome the constraints of cultivating

microorganisms in the laboratory, researchers formulated an alternative medium that replaces the protein content typically found in culture media with a more sustainable plant-based protein source.

Psophocarpus tetragonolobus, sometimes referred to as "winged bean," is an adaptable tropical leguminous plant found in the tropics of Southeast Asia, in Philippines, and is primarily underutilized for its other characteristics and beneficial sources, excluding it as an edible, natural protein legume food source (Bepary et al., 2023). Despite related evidence that this plant has a relatively high protein content, it makes a viable choice to replace or enhance conventional protein sources in laboratory settings (Bassal et al., 2020). The leaves of *P. tetragonolobus* are found to have high nutritional value in their nutritional vitamin content, while its seeds are rich in calcium, zinc, iron, and phosphorus. The winged bean can bridge several significant gaps in the protein landscape, providing a resource-efficient alternative to meet the growing need for protein while lowering dependency on livestock farming methods (Jain et al., 2022).

Tryptic Soy Broth (TSB) is a commonly used selective enrichment liquid medium for growing and identifying bacteria in qualitative microbiology laboratory processes, which involve a range of biochemical testing and bacterial isolation analyses, including those for fastidious and non-fastidious bacterial strains. Its dependability and adaptability have made it a staple used in laboratory settings (Tankeshwar, 2023). Moreover, Tankeshwar (2023) further stated that, with its distinctive compositional nutrients, specifically amino acids and longer-chained peptides, TSB can be incubated at different temperatures to support the growth of microorganisms, such as *Escherichia coli* and *Staphylococcus aureus*.

The researchers aim to find alternative protein sources for the TSB composition, focusing on plant-based sources with high protein content. Hence, the utilization of *P. tetragonolobus* as a sustainably and economically feasible protein source replacement practically instigated significant implications to focus on determining the yielding concentration effect of the crude extract protein of *P. tetragonolobus* using the Protein Crude Extraction Method, and thereby replacing such initial protein component of TSB, primarily the Tryptone (Pancreatic digest of casein) and Soytone (Peptic digest of soybean), with the extracted crude protein of *P. tetragonolobus* (Franca-Oliveira et al., 2021). The researchers would prepare 0.5 McFarland standard broth as a reference for turbidity during the process of preparing microorganism suspensions in modified TSB. The bacterial spp., *E. coli* and *S. aureus*, would be added on the modified TSB (positive control) with three different test tubes in their corresponding different concentrations (2%, 5%, and 10%) of *P. tetragonolobus* (Winged Bean) crude extract, and consequently incubated with a specific time-period. The process of cultivating of both bacterial strains of *E. coli* and *S. aureus* in its liquid broth medium culture, would therefore, be determined its growth inhibition using bacterial smear preparation counting method from broth culture (TSB), evenly and lightly smeared on the glass slide with heat fixation and simple staining, for manually counting the number of bacterial colonies through microscopic identification (Truckee Meadows Community College, 2024).

The manufacturing cost and sustainability of using culture media, that is, TSB, prompted the exploration of alternative or plant-based sources, with the transparent reason that TSB often relies on animal-derived proteins, creating an easily accessible, and affordable, alternative culture media with a satisfactory to produce the desired bacterial colony pure culture in comparison to standard media (Gamit et al., 2023). In this context, there is a growing interest in exploring plant-based alternatives to address these challenges. Contrastingly, the ability to comprehend and make sense of the performance of *P. tetragonolobus* extract in such use as a protein source replacement for TSB can contribute to sustainable practices in biological research and industrial applications, with its advantageous location outgrowing distributed in Southeast Asian countries, particularly in the Philippines (Ho et al., 2024). The outcomes of this study will give stakeholders valuable insights into the ongoing discourse surrounding environmentally conscious choices in laboratory practices, aligning with the contemporary drive towards ecologically friendly and sustainable methodologies in scientific endeavors.

2.0 Methodology

2.1 Research Design

The study is designed as experimental research to investigate the effect of using a crude extract of *P. tetragonolobus* as an alternative protein source for TSB in promoting the growth of *E. coli* and *S. aureus*. The research consists of multiple steps, involving the preparation of the specimen, crude extraction of *P. tetragonolobus*, preparation of the

bacterial inoculum, production of TSB with varying concentrations of *P. tetragonolobus*, and determination of bacterial growth.

The study will involve several entities, involving National University – Mall of Asia (NU-MOA), Adamson University Technology Research and Development Center (AUTRDC), Arenas Farm, Jose Vera Santos Memorial Herbarium, University of the Philippines - Diliman (PUH-UPD), and the University of the Philippines – Manila, College of Public Health (UPM-CPH). Moreover, the experimental methodology involves modifying the different concentrations (2%, 5%, and 10%) of *P. tetragonolobus* in TSB as the independent variable, with bacterial growth serving as the dependent variable. The control groups are TSB without *P. tetragonolobus* and would utilize a Normal Saline Solution (NSS) as the negative control, and TSB with usual sources of protein replaced by the three different concentrations of *P. tetragonolobus* crude extract as the positive control. Bacterial growth will be assessed using the bacterial smear preparation method, which involves a standard microscopic bacterial count. The result of the study would determine the viability of *P. tetragonolobus* as a protein source in TSB compared to conventional protein sources.

2.2 Research Process and Analysis

Sampling Collection Scheme

In this study, the plant *P. tetragonolobus*, used as a protein source replacement for TSB, is grown and harvested from Arenas Farm, located in Saguday, Quirino, Isabela. The researchers opted to harvest a freshly, and authentic plant to acquire the precise extracted protein content of the plant needed for the experiment, and to abstain from the following chemical properties and fertilizers that are extracted by the botanic expertise to make the plant longer and healthier as well as to reduce the possible reaction that may affect the future results for this research study.

Regarding the pathogens required (E. coli and S. aureus), the researchers have concluded that it is best to purchase from the University of the Philippines–Manila, College of Public Health (UPM-CPH), an educational institution renowned for its quality bacterial culture repository. The specimen obtained will later be cultured and preserved in a suitable growth medium.

Protein Crude Extraction of P. tetragonolobus (Winged Bean)

Proteins are utilized as reagents in a variety of scientific applications, suggesting that the reliability and repeatability of experimental findings will be heavily reliant on the quality of the proteins, which should undergo rigorous structural and functional controls. Accordingly, Thomas (2023) provides crucial insight into the procedures and methods of extracting crude proteins through isolation and purification, which enables understanding of the protein's physical and biochemical composition, as well as the acquisition of accurate and desired protein content molecules.

The procedure for Protein Crude Extraction of *P. tetragonolobus* (Winged Bean), as stated by Thomas (2023), is meticulously detailed yet straightforward, comprising several essential steps. Before extracting protein matter from the bean material, it must first be cleared of any debris, dirt, or other foreign objects, rinsed with water, and dried. To make extraction easier later on, the bean material is ground into a fine powder to break down the material. Cell lysis, achieved through either mechanical or chemical means, is then employed to break down cells and release their contents. Afterward, more debris is further removed through the process of centrifugation.

Subsequent centrifugation steps help to remove unwanted debris, ensuring a cleaner extract without the non-protein components. The material is then subjected to heating, which facilitates the separation of proteins from non-protein components and other unwanted substances. The crude protein extract still has purity issues, as it contains other non-protein materials within the extract. To isolate pure protein from the crude protein extract, a precipitation process is employed, where the extract is mixed with a highly concentrated salt solution. Precipitation of proteins is induced using ammonium sulfate, followed by filtration of the precipitated solution through dialysis or chromatography to purify the extract. Final chromatography purification is conducted to achieve the highest level of purity. Additionally, the extract may be further purified using immunoblotting with antibodies to ensure absolute purity (Thomas, 2023).

Preparation of Broth Media (TSB)

TSB is primarily utilized as an initial growth medium and has initial materials and compositions filtered down to per a hundred milliliters, which are mainly composed of the following: A pancreatic digest of casein with amount of (1.7 gm), a peptic digest of soybean meal with amount of (0.3 gm), sodium chloride of (0.5 gm), dibasic potassium phosphate with (0.25 gm), glucose monohydrate with (0.25 gm), and a (100 mL) of distilled water. The inclusion of casein and soy peptones makes the medium more nourishing by delivering organic nitrogen, specifically amino acids containing longer-chained peptides, plus sodium chloride to maintain osmotic equilibrium, and a solidifying agent, such as broth.

For the alternative protein source for TSB, the researchers selected *P. tetragonolobus*, which can be found on farms and in local markets. In addition, the researchers must purchase laboratory items which include test tubes from a medical supply store to use as broth medium containers, as well as sodium chloride (0.5 gm), dibasic potassium phosphate (0.25 gm), glucose monohydrate (0.25 gm), and distilled water (100 mL) filtered down to per a hundred milliliters for the production of broth, added with the alternative protein source of crude extract of *P. tetragonolobus* (2 gm) with different concentrations of 2%, 5%, and 10% as the positive control for the conducting research study. Meanwhile, the negative control for this experiment would be added with NSS as the dilution blank for the culture suspension, as there is no swelling or shrinking of the cells of the chosen bacteria, thereby minimizing the chances of bacterial death. Furthermore, the researchers will adapt the media containing alternative protein sources for TSB, which will predominantly consist of *P. tetragonolobus* with varying concentrations of components and compositions.

To prepare the TSB medium with varying concentrations of *P. tetragonolobus*, the following simple procedure is followed: Firstly, in a large flask or beaker, the *P. tetragonolobus* crude extract is combined with distilled water. Then, 0.5 g of sodium chloride, 0.25 g of dipotassium phosphate, and 0.25 g of glucose monohydrate are added to each solution, with thorough stirring. The final pH of the solution should be adjusted to 7.3 at a temperature of 25°C, and monitored using either a pH meter or pH indicator strips. Lastly, the broth solutions are sterilized by autoclaving them at 121°C for 15 minutes.

Bacterial Inoculum Preparation

The researchers will employ a 0.5 McFarland standard, consisting of 0.5 mL of barium chloride dihydrate solution and 99.5 mL of sulfuric acid. The utilization of the 0.5 McFarland standard guarantees a consistent size for the bacterial inoculum. McFarland standards serve as turbidity references in the process of preparing microorganism suspensions. The 0.5 McFarland standard is one of the most used methods for estimating microbial populations. McFarland standards serve as turbidity benchmarks for preparing microbial suspensions. The McFarland method uses a turbidity scale to estimate bacterial concentrations. This scale consists of a series of calibrated tubes with optical densities created by the precipitation of barium sulfate. These absorbance values are compared to bacterial populations, with the widely used value being 0.5 on the scale, representing a population of 1.5×10^8 CFU/mL (colony-forming units per milliliter).

The process of preparing the McFarland standard involves several steps: To prepare the sulfuric acid solution, begin by adding around 90 mL of distilled water to a 100-mL volumetric flask. Then, using a 1.0-mL volumetric pipette, carefully add 1.0 mL of concentrated sulfuric acid to the flask. Top off the solution with distilled water to achieve a final volume of 100 mL, and then thoroughly mix the solution. This prepared sulfuric acid solution can be stored in a measured glass bottle or beaker at room temperature for up to one year. For the barium chloride solution, weigh out 1.175 g of barium chloride and place it in a 100-mL volumetric flask. Add about 50 mL of distilled water and mix well until the barium chloride is completely dissolved. Then, fill the flask with distilled water to reach a final volume of 100 ml and mix thoroughly. This prepared barium chloride solution can also be stored in a glass bottle or beaker at room temperature for up to one year. In a clean container, mix 0.5 mL of the barium chloride solution with 9.95 mL of the sulfuric acid solution, stirring constantly to create a barium sulfate precipitate that matches the desired turbidity standard (Lonsway, 2023).

Incubation and Cultivation

The prepared culture media would supplement the extracted *P. tetragonolobus* using modified TSB to facilitate the growth of the necessary microorganisms, *E. coli* and *S. aureus*, and apply and test the extracted crude protein in a total of six test tubes simultaneously. Moreover, the chosen bacteria would be incubated in modified TSB at 37

degrees Celsius for 48 hours with three different concentrations of the extracted crude protein of *P. tetragonolobus* (2%, 5%, and 10%), respectively, as the methodological processing of incubating the modified TSB would be strictly done through batching at different periods. The first batch, comprising three test tubes, should be inoculated with the modified TSB supplemented with the bacterial strain of E. coli, with an incubation period of approximately 48 hours at 37 degrees Celsius. On the other hand, the remaining three test tubes containing another modified TSB were inoculated with the bacterial strain of *S. aureus* and incubated for the same period at the duplicate temperature in the indicated latter part (Tankeshwar, 2023).

Waste Disposal

The importance of proper waste management in laboratory activities is critical, as it requires researchers to follow procedures strictly after experimentation is complete. It is imperative to educate each researcher on the proper management and disposal of various laboratory findings they will encounter during practical experimentation.

The following measures can be taken to ensure proper waste disposal management, as recommended by the National Research Council (US) Committee (2011) guidelines. Firstly, for the disposal and disinfection of bacterial pure culture, use a 20% bleach solution or 70% isopropyl alcohol to kill bacteria in the plate tube or bottle, and then dispose of it in the biohazard trash. Autoclave at about 121 degrees Celsius for 15 minutes in the autoclave equipment. For disinfecting materials and laboratory surroundings, apply Lysol, bleach, or alcohol to the working tables and benches to kill any contaminated foreign microorganisms. Soak or rinse the used culture tubes and inoculation sticks in a dilute solution of Lysol or bleach before disposing of them in the biohazard trash. Lastly, as the researchers observed the proper attire of PPE (Gloves, Lab gown, Mask, and Head cap) during the laboratory experiment, with the hair being tied to avoid contamination, the used gloves, mask, and head cap would be disposed of in the infectious waste.

2.3 Data Gathering Procedure

Bacterial Growth Determination

The exact measurement of living microorganisms is a crucial component of microbiological research. Traditional approaches, while undemanding and profitable, such as the CFU method on agar plates, require a significant amount of time and workforce, often demanding a couple of days for the agar to solidify until colonies form. Hence, the researchers employed a bacterial smear preparation counting method for microscopic viewing on glass slides using an oil immersion objective, as well as a bacterial growth count of *E. coli* and *S. aureus* in TSB, providing a more comprehensive and accurate method for counting microorganisms.

Counting CFUs is based on the principle that each colony originates from a single viable microbial cell. By tallying the colonies on the incubated liquid broth medium cultures and factoring in the dilution used, the researchers determined the initial number of microorganisms in the sample, measured in CFU/mL. The normal range for a typical bacterial count on the CFU/mL scale using Tryptic Soy Broth (TSB) is 30-300 CFU/mL. To prepare a smear for staining, bacteria must be firmly adhered to a glass slide. In this process, there are two important factors to consider. The bacteria must first be spread evenly and delicately on the slide. An excessive number of bacteria will cluster together, making it challenging to see the individual bacterial cell shape. Large clumps of bacterial cells also stain incorrectly, producing unreliable results (TMCC, 2024).

First, the bacteria in the broth must be suspended in the culture tube by properly mixing them before making a smear from it. Label the slide using a marker on the side part of the frosted slide, and then aseptically transfer a loopful of the organism. Spread out the broth drop by smearing it in a swirling, circular motion around the slide with the flat portion of the loop. The smear is kept evenly dispersed and prevents beading up on the surface of the slide due to the protein component of the broth. Then, set the slide away to air dry. Lastly, to perform a Gram stain, label the slide and arrange the smears as follows: Gram-negative bacteria on the left, the unknown sample in the middle, and Gram-positive bacteria on the right. Methanol fixes the slide, then cover the smears with crystal violet for one minute, ensuring complete coverage without dripping. Rinse with water, avoiding direct spraying on the smears. Apply iodine mordant for one minute, then decolorize with Gram's decolorizer until the solution is barely transparent. Blot off the excess, and apply safranin for one minute. Rinse gently with water, air dry, and observe under oil immersion. Gram-positive bacteria will appear purple, and Gram-negative bacteria will appear pink (TMCC, 2024).

2.4 Data Analysis

To evaluate and comprehend the results, the researcher used one-way ANOVA. According to Bevans (2023), use one-way ANOVA to investigate mean differences of an independent variable having at least three levels of concentration (2%, 5%, and 10%), that is the crude extract of *P. tetragonolobus*, and one quantitative dependent variable, that is, TSB, to examine the collected data. This method of statistical analysis utilizes data collected from samples to conduct an inference test of hypotheses and draw conclusions on whether there is a significant difference in determining the minimum concentration of using *P. tetragonolobus* as a protein source replacement for TSB.

2.5 Ethical and Safety Considerations

The ethical considerations for this research project will delve further into the safety and precautions that each researcher needs to take for themselves. This research project has not involved any human subjects or animals that have been harmed, exploited for financial gain, tested, or exposed to any unethical practices. The necessary permissions, informed consent forms, and other ethical prerequisites are not needed as these factors are not pertinent to the objectives at hand.

In addition, *E. coli* and *S.* aureus were the chosen targets of the microorganisms in the experiments. These species are recognized as detrimental to human health, particularly to the health of researchers. Furthermore, it is essential to use personal protective equipment (PPE) within the laboratory, as researchers also ensured that every piece of laboratory equipment used had been handled carefully and underwent an autoclaving process for adequate cleaning and sterilization. Completed and assigned to a designated location, specimen storage is carried out in a suitable manner as well. The researchers would closely monitor all safety protocols to ensure complete safety, adhering to ethical guidelines and completing the requested compliance forms, in order to prevent potential harm and improper use of the substance that could lead to serious situations.

3.0 Results and Discussion

The bacterial smear counting method involves preparing uniform smears on glass slides from each culture condition, staining the samples, and examining them microscopically to determine bacterial counts (CFU/mL). To ensure accuracy, smears were standardized in terms of volume and spread, and bacterial counts were averaged over one field of view per slide to minimize variability. Each experimental condition, involving the microscopical counting of bacteria on glass slides with different concentrations of P. tetragonolobus (Winged Bean) crude extract, was replicated twice to assess consistency. The TSB (positive control) and NSS (negative control) were also included to benchmark the performance of the crude extract. After thorough experimentation, the data were submitted to a statistician for analysis using ANOVA to identify significant differences among treatments. Post hoc Tukey's HSD tests were conducted for pairwise comparisons to identify which concentrations differed significantly.

Table 1. *P. tetragonolobus (Winged Bean) Crude Extract against E. coli*

Samples	Trial 1	Trial 2	Mean	SD
2%	96.00	124.00	109.00	14.11
5%	122.00	165.00	136.67	24.54
10%	83.00	102.00	92.67	9.50
TSB (Tryptic Soy Broth Positive Control)	126.00	140.00	131.00	7.81
NSS (Negative Control)	0.00	0.00	0.00	0.00

Table 1 presents the bacterial growth of E. coli, which varied significantly across different concentrations of *P. tetragonolobus* (Winged Bean) crude extract in modified TSB. This variation was observed in two trials, conducted and examined microscopically on glass slides using the bacterial smear counting method.

At a 2% concentration of *P. tetragonolobus* crude extract in modified TSB, the bacterial growth in trial one and trial two had a mean count of 96 and 124 CFU/mL, respectively, with a standard deviation (SD) of 14.11. Meanwhile, the 5% concentration of *P. tetragonolobus* crude extract in modified TSB showed comparatively significant bacterial growth in both trials, with a mean count of 136.67 CFU/mL and a standard deviation of 24.54, yielding the highest values in all aspects. Lastly, for the 10% concentration of *P. tetragonolobus* crude extract in modified TSB, its

bacterial growth declined significantly, with a mean of 92.67 CFU/mL, SD at 9.50, and a marking count in both trials at 83 and 102 CFU/mL. As for comparison, the positive control (TSB) for *E. coli* had a marking count in trials 1 and 2 at 126 and 140 CFU/mL, a mean of 131 CFU/mL, and an SD of 7.81. Moreover, the negative control (NSS) showed no bacterial growth, as it was used to confirm the necessity of protein-rich media.

Table 2. *P. tetragonolobus (Winged Bean) Crude Extract against S. aureus*

Samples	Trial 1	Trial 2	Mean	SD
2%	120.00	132.00	127.67	6.66
5%	141.00	148.00	145.33	3.79
10%	82.00	94.00	89.67	6.66
TSB (Tryptic Soy Broth Positive Control)	232.00	277.00	260.33	24.66
NSS (Negative Control)	0.00	0.00	0.00	0.00

Table 2 also revealed similar trends regarding the significant variations in bacterial growth across different concentrations of the P. tetragonolobus (Winged Bean) crude extract for S. aureus, as observed microscopically in glass slides using the bacterial smear counting method.

At 2% concentration of *P. tetragonolobus* crude extract in modified TSB, bacterial growth yielded a mean of 127.67 CFU/mL and SD of 6.66, with both trials in proximity of marking count at 120 and 132 CFU/mL, respectively. Moreover, the 5% concentration of *P. tetragonolobus* crude extract in modified TSB recurrently showed as the most significant bacterial growth among the test concentrations, with a mean of 145.33 CFU/mL, trials 1 and 2 at 141 and 148 CFU/mL, and SD of 3.79, respectively. Lastly, for the 10% concentration of *P. tetragonolobus* crude extract in modified TSB, its bacterial growth dropped significantly, with a mean of 89.67 CFU/mL, SD at 6.66, and a marking count in both trials at 82 and 94 CFU/mL. As for comparison, the positive control (TSB) for *S. aureus* had a marking count in trials 1 and 2 at 126 and 140 CFU/mL, a mean of 131 CFU/mL, and SD of 7.81 Similar to *E. coli*, the negative control (NSS) showed no growth, validating the role of protein content in bacterial proliferation.

Table 3. *ANOVA on the Different Concentrations of E. coli and S. aureus*

		ANOVA					
		Sum of Squares	***df***	**Mean Square**	***F***	**Sig.**	**Interpretation**
Escherichia coli	Between Groups	36756.40	4	9189.10			
	Within Groups	1905.33	10	190.53			
	Total	38661.73	14		48.23	p <.001	Significant
Staphyloccocus aureus	Between Groups	106824.93	4	26706.23	187.72	p <.001	Significant
	Within Groups	1422.67	10	142.27			
	Total	108247.60	14				

Table 3 presents the analysis of variance (ANOVA) results for the growth of *E. coli* and *S. aureus* across Different concentrations of *P. tetragonolobus* (Winged Bean) crude extract. The results demonstrate a significant variation in bacterial growth among the groups, as indicated by a p-value of 0.000 for both organisms. The high F-values (48.228 for *E. coli* and 187.720 for *S. aureus*) indicate that variations between groups were primarily due to the treatment conditions, not random error. This confirms that the varying concentrations of the crude extract and the controls have a statistically significant impact on bacterial growth.

For *E. coli*, the between-group sum of squares (36,756.400) was substantially larger than the within-group sum of squares (1,905.333), emphasizing that the observed differences in growth are primarily attributed to the treatment conditions rather than random variations within groups. The degrees of freedom (df) for the analysis were distributed as follows: 4 for the between-group comparison, accounting for the three concentrations and the two controls; 10 for within-group variability, representing random variation within each group; and a total of 14 degrees of freedom. The mean square between groups (9,189.100) reflects the average variation caused by the treatment conditions, while the mean square within groups (190.533) accounts for random error. The high F-value (48.228) further confirms that the variation between groups is significantly greater than within groups, highlighting the strong influence of the treatment conditions on *E. coli* growth.

Similarly, the ANOVA results for *S. aureus* revealed a significant impact of the different concentrations of crude extract and controls on bacterial growth. The between-group sum of squares (106,824.933) was markedly greater

than the within-group sum of squares (1,422.667), indicating that the treatment conditions played a substantial role in the observed differences. The df followed the same distribution as for *E. coli* (4 between groups, 10 within groups, and 14 total). The mean square between groups (26,706.233) demonstrated the average variation due to treatment, while the mean square within groups (142.267) indicated the effect of random variation. The extremely high F-value (187.720) for *S. aureus* suggests an even more substantial impact of the treatment conditions on growth compared to *E. coli*.

Based on the given results on Table 1 and Table 2, both bacterial species exhibited the highest growth at 5% concentration of *P. tetragonolobus* crude extract in *E. coli* (mean: 136.67 CFU/mL) and *S. aureus* (mean: 145.33 CFU/mL), closely approximating the growth observed in the positive control (TSB) of *E. coli* (mean: 131 CFU/mL) and *S. aureus* (mean: 260.33 CFU/mL). This indicates that the 5% concentration is the most effective and optimal concentration necessary for supporting bacterial growth, showing its potential as a viable protein replacement for TSB, whereas higher (10%) or lower (2%) concentrations of *P. tetragonolobus* (Winged Bean) crude extract are less effective. Further analyses of the results show multiple comparisons of the different concentrations of *P. tetragonolobus* (Winged Bean) crude extract, along with the positive and negative controls, against E. coli (Table 1) and S. aureus (Table 2).

Table 4. *Multiple Comparison Analysis against E. coli*

Multiple Comparisons against *Escherichia Coli*				
I	***J***	**Mean Difference (I-J)**	**Sig.**	**Interpretation**
2%	5%	-27.67	0.178	Not Significant
	10%	16.33	0.613	Not Significant
	TSB (Tryptic Soy Broth Positive Control)	-22.00	0.352	Not Significant
	NSS (Negative Control)	109.00*	$p < .001$	Significant
5%	2%	27.67	0.178	Not Significant
	10%	44.00*	0.019	Significant
	TSB (Tryptic Soy Broth Positive Control)	5.67	0.985	Not Significant
	NSS (Negative Control)	136.67	$p < .001$	Significant
10%	2%	-16.33	0.613	Not Significant
	5%	-44.00*	0.019	Significant
	TSB (Tryptic Soy Broth Positive Control)	-38.33*	0.042	Significant
	NSS (Negative Control)	92.67*	$p < .001$	Significant
TSB (Tryptic Soy Broth Positive Control)	2%	22.00	0.352	Not Significant
	5%	-5.67	0.985	Not Significant
	10%	38.33*	0.042	Significant
	NSS (Negative Control)	131.00*	$p < .001$	Significant
NSS (Negative Control)	2%	-109.00*	$p < .001$	Significant
	5%	-136.67*	$p < .001$	Significant
	10%	-92.67*	$p < .001$	Significant
	TSB (Tryptic Soy Broth Positive Control)	-131.00*	$p < .001$	Significant

The growth of *E. coli* varied across the different concentrations and both controls of the experiment in Table 1, with significant differences in effectiveness. Regarding the 2% concentration of *P. tetragonolobus* (Winged Bean) crude extract, E. coli growth was moderate, with a mean of 10^9 CFU/mL. The statistical analysis revealed that this concentration failed to produce bacterial growth levels comparable to the positive control, TSB (mean: 131 CFU/mL), nor was it significantly different from the 10% concentration (mean: 92.67 CFU/mL). Table 4 further compares the multiple concentrations of 2% against other concentrations. With a mean difference of -27.67 (p-value = 0.178) compared with the 5% concentration, and a mean difference of 16.33 (p-value = 0.613) with the 10% concentration, neither of these concentrations was statistically significant, indicating relatively comparable growth with no substantial variation. Meanwhile, the mean difference of -22.00 (p-value = 0.352) between the 2% concentration of *P. tetragonolobus* (Winged Bean) crude extract and the positive control (TSB) was also not significant, indicating that the 2% concentration was moderately effective, but still less so than the positive control of TSB.

The 5% concentration of *P. tetragonolobus* (Winged Bean) crude extract for *E. coli* yielded the highest bacterial growth (mean: 136.67 CFU/mL) among all the other concentrations. It was comparable to the positive control (TSB). Multiple comparison analysis of Table 4 indicated that the bacterial growth difference between the 5% concentration and the TSB positive control, with a mean difference of 5.67 (p-value = 0.985), was not statistically

significant, confirming its effectiveness as an alternative protein source for TSB. Additionally, the 5% concentration was significantly more effective than the 10% concentration, with a mean difference of 44.00 (p-value = 0.019), demonstrating its superior ability to support *E. coli* growth. In contrast, the 10% concentration may have inhibitory effects.

In contrast with the 10% concentration of *P. tetragonolobus* (Winged Bean) crude extract for *E. coli*, bacterial growth had significantly declined (mean: 92.67 CFU/mL). Multiple comparisons on Table 4 revealed that the 0.5% concentration was significantly less effective than both the 2% and 5% concentrations, with mean differences of -16.33 (p-value = 0.613) and -44.00 (p-value = 0.019), respectively. The bacterial growth at a 10% concentration is also significantly lower than that of the TSB positive control, with a mean difference of -38.33 (p-value = 0.042). This result indicates a potential inhibitory effect of the crude extract at higher concentrations.

Table 5. *Multiple Comparison Analysis against S. aureus*

Multiple Comparisons against *Escherichia Coli*				
I	***J***	**Mean Difference (I-J)**	**Sig.**	**Interpretation**
2%	5%	-17.67	0.417	Not Significant
	10%	38.00*	0.019	Significant
	TSB (Tryptic Soy Broth Positive Control)	-132.67*	$p < .001$	Significant
	NSS (Negative Control)	127.67*	$p < .001$	Significant
5%	2%	17.67	0.417	Not Significant
	10%	55.67*	0.001	Significant
	TSB (Tryptic Soy Broth Positive Control)	-115.00*	$p < .001$	Significant
	NSS (Negative Control)	145.33*	$p < .001$	Significant
10%	2%	-38.00*	0.019	Significant
	5%	-55.67*	0.001	Significant
	TSB (Tryptic Soy Broth Positive Control)	-170.67*	$p < .001$	Significant
	NSS (Negative Control)	89.67*	$p < .001$	Significant
TSB (Tryptic Soy Broth Positive Control)	2%	132.67*	$p < .001$	Significant
	5%	115.00*	$p < .001$	Significant
	10%	170.67*	$p < .001$	Significant
	NSS (Negative Control)	260.33*	$p < .001$	Significant
NSS (Negative Control)	2%	-127.67*	$p < .001$	Significant
	5%	-145.33*	$p < .001$	Significant
	10%	-89.67*	$p < .001$	Significant
	TSB (Tryptic Soy Broth Positive Control)	-260.33*	$p < .001$	Significant

The growth of *S. aureus* also varied significantly in its effectiveness among the different concentrations tested, alongside both controls of the experiment in Table 2. The mean bacterial growth for 2% concentration of *P. tetragonolobus* (Winged Bean) crude extract for *S. aureus* was 127.67 CFU/mL, comparatively lower than both 5% concentration (mean: 145.33 CFU/mL) and the TSB positive control (mean: 260.33 CFU/mL). While it supported moderate growth, multiple comparison analysis on Table 5 showed that this concentration was highly significant, with a mean difference of -132.67 (p-value = 0.000), indicating that the TSB positive control outperformed the 2% concentration. Meanwhile, compared to a 5% concentration of P. tetragonolobus (Winged Bean) crude extract, the mean difference of -17.67 (p-value = 0.417) was not significant, indicating comparable growth at these concentrations. However, while the 5% concentration supports slightly higher growth, the variation is not enough to conclude a meaningful difference. Regarding the comparison between 2% and 10% concentrations, the difference in bacterial growth is statistically significant, with a mean difference of 38.00 (p-value = 0.019). The 2% concentration supports significantly more bacterial growth than the 10% concentration, indicating reduced effectiveness at the higher concentration.

Similar to *E. coli*, the 5% concentration of *P. tetragonolobus* (Winged Bean) crude extract for *S. aureus* produced the highest growth (mean: 145.33 CFU/mL) among the different tested concentrations, demonstrating its effectiveness as a protein source replacement for TSB. Based on Table 5, multiple comparisons confirmed that it was significantly more effective than both the 2% and 10% concentrations. With a mean difference of 55.67 (p-value = 0.001), the difference in bacterial growth between 5% and 10% concentrations is statistically significant. This implies that the

5% concentration supports significantly more bacterial growth than the 10% concentration, highlighting its superior effectiveness. Moreover, bacterial growth at a 5% concentration is significantly lower compared to the TSB positive control, with a mean difference of -115.00 (p-value = 0.000), making it a statistically significant difference. While a 5% concentration supports notable growth, it does not match the effectiveness of the TSB standard.

In contrast to the 10% concentration of P. tetragonolobus (Winged Bean) crude extract for S. aureus, bacterial growth also dropped significantly, with a mean of 89.67 CFU/mL. Multiple comparisons, as shown in Table 5, revealed that the 10% concentration was significantly less effective than both the 5% and the TSB positive control, with mean differences of -55.67 (p-value = 0.001) and -170.67 (p-value = 0.000), respectively. This result suggests a potential inhibitory effect of the extract at higher concentrations, consistent with the trend observed with *E. coli*.

4.0 Conclusion

TSB is a commonly used selective enrichment liquid medium for growing and identifying bacteria in qualitative microbiology laboratory processes, supplying and performing a range of biochemical testing and bacterial isolation analyses, including those for fastidious and non-fastidious bacterial strains. Its dependability and adaptability have made it a staple used in laboratory settings (Tankeshwar, 2023). The research was conducted to address the growing demand for sustainable and cost-effective alternatives to conventional, animal-derived culture media. Winged bean, known for its high protein content, was hypothesized to provide the essential nutrients required for microbial growth (Bassal et al., 2020).

The methodology involved the preparation of crude extracts from winged bean seeds at three concentrations (2%, 5%, and 10%), which were then tested against the standard TSB (positive control) and normal saline solution (NSS, negative control). Bacterial growth was measured using the bacterial smear counting method, followed by statistical analyses, including ANOVA and multiple comparison tests, to determine significant differences across treatments.

The results revealed that a 5% concentration of winged bean crude extract supported the highest bacterial growth, yielding values comparable to those of TSB for both *E. coli* and *S. aureus*. The 2% concentration demonstrated moderate growth, while the 10% concentration showed significantly reduced effectiveness, possibly due to inhibitory effects caused by high solute concentration or certain phytochemicals in the extract. Statistical analyses confirmed that the observed differences in bacterial growth were significant, underscoring the influence of the extract's concentration on bacterial growth.

These findings prove that winged bean crude extract, particularly at a 5% concentration, is a viable and sustainable alternative to TSB. Its ability to support comparable bacterial growth positions it as a promising substitute for conventional protein sources in microbiological media, addressing economic and ethical concerns associated with animal-derived components. These results demonstrated the potential of using affordable, plant-based options, such as corn and chickpea, for microbiological media. Similarly, Bepary et al. (2023) emphasized the high protein content and nutritional benefits of winged beans, highlighting how plant-based alternatives can lower costs without compromising effectiveness.

In conclusion, this research not only supports the use of being feasible and cost-effective alternatives in laboratory settings but also underscores the practical value of underutilized plants like winged beans. With further exploration, such as testing additional bacterial strains or refining extraction methods to enhance their biochemical composition, this approach could have a significant impact on sustainable microbiological practices for large-scale production and industrial use.

5.0 Contributions of Authors

The authors indicate equal contribution to each section. The authors reviewed and approved the final work.

6.0 Funding

This work received no specific grant from any funding agency.

7.0 Conflict of Interests

The authors declare no conflict of interest in the publication.

8.0 Acknowledgment

None.

9.0 References

Basavaraju, M., & Gunashree, B. S. (2023). Escherichia coli: An overview of main characteristics. In M. Starčič Erjavec (Ed.), Escherichia coli—Old and New Insights. IntechOpen. https://doi.org/10.5772/intechopen.105508

Bassal, H., Merah, O., Ali, A. M., Hijazi, A., & Omar, F. E. (2020). Psophocarpus tetragonolobus: An underused species with multiple potential uses. Plants, 9(12), Article 1730. https://doi.org/10.3390/plants9121730

Bepary, R. H., Roy, A., Pathak, K., & Deka, S. C. (2023). Biochemical composition, bioactivity, processing, and food applications of winged bean (Psophocarpus tetragonolobus): A review. Legume Science, 5(3). https://doi.org/10.1002/leg3.187

Bevans, R. (2023). One-way ANOVA | When and how to use it (with examples). Scribbr. Retrieved from https://tinyurl.com/mrpyd2r4

Franca-Oliveira, G., Fornari, T., & Hernández-Ledesma, B. (2021). A review on the extraction and processing of natural-source-derived proteins through eco-innovative approaches. Processes, 9(9), Article 1626. https://doi.org/10.3390/pr9091626

Ho, W. K., Tanzi, A., & Sang, F. (2024). A genomic toolkit for 'the soybean of the tropics' – Winged bean (Psophocarpus tetragonolobus) [Preprint]. Research Square. https://doi.org/10.21203/rs.3.rs-1355353/v1

Kim, I., Kim, C., & Yang, W. (2021). Physiologically active molecules and functional properties of soybeans in human health — a current perspective. International Journal of Molecular Sciences, 22(8), Article 4054. https://doi.org/10.3390/ijms22084054

Laloučková, K., Malá, L., Maršík, P., & Skřivanová, E. (2021). In vitro antibacterial effect of the methanolic extract of the Korean soybean fermented product doenjang against Staphylococcus aureus. Animals, 11(8), Article 2319. https://doi.org/10.3390/ani11082319

Lonsway, D. R. (2023). Clinical microbiology procedures handbook (5th ed.). ASM Press.

Malik, N. H. A., Simarani, K., & Aziz, M. A. (2022). Soybean as an alternative nutrient medium for *Bacillus subtilis* growth. Malaysian Applied Biology, 51(4), 67–74. https://doi.org/10.55230/mabjournal.v51i4.12

Mohanty, C. S., Singh, V., & Chapman, M. A. (2020). Winged bean: An underutilized tropical legume on the path of improvement, to help mitigate food and nutrition security. Scientia Horticulturae, 260, 108789. https://doi.org/10.1016/j.scienta.2019.108789

Mueller, M., & Tainter, C. R. (2025). Escherichia coli infection. In StatPearls. StatPearls Publishing. https://tinyurl.com/ycxbxv3p

Petrova, S. Y., Khlgatian, S. V., Emelyanova, O. Y., Pishulina, L. A., & Berzhets, V. M. (2022). Structure and biological functions of milk caseins. Russian Open Medical Journal, 11(2), e0209. https://romj.org/2022-0209

Puvača, N., & De Llanos Frutos, R. (2021). Antimicrobial resistance in Escherichia coli strains isolated from humans and pet animals. Antibiotics, 10(1), Article 69. https://doi.org/10.3390/antibiotics10010069

Raju, K. (2020). Alternative culture media for cultivation of bacteria. International Journal of Science and Research (IJSR), 9(3), 1174-1176. https://tinyurl.com/6jw6j5wc

Rizkita, A. D., & Dewi, S. A. (2023). Isolation and identification of chemical components of winged bean (Psophocarpus tetragonolobus D.C) leaf diethyl ether extract. Proceedings – International Conference on Religion, Science and Education, 2, 721–725. https://tinyurl.com/2th29d5r

Shrestha, A. (2022). Colony counters: Types, principles and uses. Microbe Online. Retrieved from https://microbeonline.com/colony-counter/

Sriwichai, S., Monkham, T., & Sanitchon, J. (2021). Dual-purpose of the winged bean (Psophocarpus tetragonolobus (L.) DC.), the neglected tropical legume, based on pod and tuber yields. Plants, 10(8), Article 1746. https://doi.org/10.3390/plants10081746

Tankeshwar, A. (2023). Tryptic soy agar (TSA): Composition, preparation, uses. Microbe Online. Retrieved from https://tinyurl.com/yhnx5sbn

Taylor, T. A., & Unakal, C. G. (2023). Staphylococcus aureus infection. StatPearls. https://tinyurl.com/mrxkr6at

Thomas, L. (2023). Protein purification techniques. News-Medical/Life Sciences. Retrieved from https://tinyurl.com/26yhxr9b

Truckee Meadows Community College. (2024). Bacterial smear preparation. TMCC Microbiology Resource Center. Retrieved from https://tinyurl.com/4rcr5tk4

Xie, Z., Peng, Y., Li, C., Luo, X., Wei, Z., Li, X., Yao, Y., Fang, T., & Huang, L. (2020). Growth kinetics of Staphylococcus aureus and background microorganisms in camel milk. Journal of Dairy Science, 103(11), 9958–9968. https://doi.org/10.3168/jds.2020-18616

Yu, D., Banting, G., & Neumann, N. F. (2021). A review of the taxonomy, genetics, and biology of the genus Escherichia and the type species Escherichia coli. Canadian Journal of Microbiology, 67(8), 553–571. https://doi.org/10.1139/cjm-2021-0114