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Pigeonpea (*Cajanus cajan* L.) and Cowpea (*Vigna unguiculata* L.) Water as Alternative Growth Medium for *Escherichia coli* and *Staphylococcus aureus* in Laboratory with Minimum Infrastructure

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Abstract

The availability of non-synthetic media from natural ingredients is needed to answer the needs in laboratories where the price of nutrient media is quite expensive and there are limited supplies of material ware houses. Cowpea (*Vigna unguiculata* L.*)* and pigeonpea (*Cajanus cajan* L.) are the local foods of people of NTT (East Nusa Tenggara) which have a high enough nutritional content which has the potential to be developed into cheap, easy and simple non-synthetic media in making. The purpose of this study was to determine whether the agar media contained nutrient from cowpea and pigeonpea water can be used as a alternative for nutrient agar for the growth of *Escherchia coli* and *Staphylococcus aureus* bacteria. This research is a true experiment with posttest-only control design. The growth rate of *S. aureus* bacteria on pigeonpea medium, cowpea medium, nutrient agar medium, were 164 CFU/mL (SD=3,13), 161 CFU/mL (SD=3,02) and 164 CFU/mL (SD=3,21), respectively. The average growth of *E. coli* on cowpea medium, pigeonpea medium, and nutrient agar control medium were 163 CFU/mL (SD=2,79), 167 CFU/mL (SD-2,63) and 164 CFU/mL (SD=2,75) respectively. Test results ANOVA between pigeonpea medium, cowpea medium and nutrient medium in order to obtain p value = 0.145 (p> 0.05) for the growth of *E. coli* bacteria and p value = 0.393 (p> 0.05) for growth *S. aureus.* It was concluded that there was no difference between the number of bacterial colonies of *E. coli* and *S. aureus* on three medium. The pigeonpea medium and cowpea can be used to grow and alternative nutrient agar in order to grow bacteria *E. coli* and *S. aureus.*

Keywords

Cowpea Water, Pigeonpea Water, Alternative Medium, *E. coli*, *S. aureus*.

INTRODUCTION

Cultivation medium is a nutrient medium that is prepared to grow bacteria on a laboratory scale (1,2). Most of the bacteria can grow well on any media, while others require special media (3). The cultivation medium must be able to provide the energy needed for bacterial growth (4). Bacteria that are inoculated on a cultivation medium are called inoculum and bacteria that have grown and reproduced are called bacterial grow (5).

Microbiology laboratories really need bacterial growth media for growing, isolating, counting the number, and testing the physical properties of bacteria so that a bacterium can be identified (6,7). The nutrients needed by microorganisms for their growth include carbon, nitrogen, non-metal elements such as phosphorus, metal elements such as Ca, Na, Fe, vitamins, water, and energi (8,9). Synthetic media commonly used to grow microorganisms in laboratories such as bacteria is a nutrient agar medium (10,11).

Some conditions that must be requirements by the bacterial growth medium are that it must contain the right nutrients for the specific bacteria to be grown, the humidity must be sufficient, the pH is appropriate, and the oxygen content is good enough, the growth media must be sterile and does not contain other microorganisms, the media is incubated at room temperature certain (9,12,13). Synthetic media contain high nutrition, consisting of meat extract, yeast, plant extracts, proteins, vitamins, minerals, other organic materials. Anaerobic media is a media used to grow anaerobic bacteria, containing sodium thioglycolate (14,15). Selective and differential media were used to detect the presence of specific bacteria, suppress the growth of unwanted bacteria, for example Mac conkey. MacConkey Agar media is a selective medium and a differential medium used to isolate Gram-negative bacteria based on the ability of bacteria to ferment lactose or not. MacConkey Agar media is used primarily for the Enterobacteriaceae family such as *E. coli* (15,16). Contains *crystal violet* that can selectively inhibit Gram-positive bacteria (8,17).

The availability of synthetic media requires high costs, time to order to the industry, limited stock in the material warehouse, thus limiting repeated use, especially for research purposes (17). This condition requires non-synthetic media which is considered practical, cheap, easy to obtain and can be produced by the laboratory itself. Non-synthetic media is an alternative media that uses ingredients found in nature (10). The chemical content of these ingredients is not known in detail but can be used because they are abundant in nature, easy to prepare and cheap (18).

Deivanayaki *et al*. (19) conducted research on bacterial growth media from vegetables such as carrots, tomatoes,

cabbage, and pumpkin. These vegetables showed good results for bacterial growth in both liquid and solid medium (19). Some fruits are also used as a medium for bacterial growth, such as avocados, beets, and cucumbers and orange peels (20). A part from grains, vegetables, and fruit, bacterial growth media can also be made from various types of tubers that are rich in carbohydrates (21).

Some researchers have succeeded in showing the growth of good bacteria in growth media from various carbohydrate sources such as cassava (*Manihot utilissima*) (18,22), potatoes (*Solanum tuberosum*), palmirah (Borassus) and sagu (*Metroxylon sagu*) (17,23). Utilization of legumes, local names is red beans (*Phaseolus vulgaris L)*, green beans (*Phaseolus radiatus* L.) and black soy beans *(Glycine max* L. *Merr)* has also been reported (3,10). Alternative media from natural ingredients such as cassava starch, tunggak beans (*Cajanus cajan*), green beans (24), black soy beans and soy bean, ganyong (*Canna discolor*), gembili (*Dioscorea esculenta*), garut (*Maranta arundinacea*), have been reported to be used for the growth of *Aspergillus niger* and *Fusarium oxysporum* (25,26).

Timor Island is one of the areas in East Nusa Tenggara (NTT), Indonesia, which is rich in local nuts (27). It is recorded that there are about 29 types of local beans that are there. Several types of local nuts that are commonly used in managing agriculture in Timor, especially as food, are pigeonpea (*Cajanus cajan* or pigeon pea) and cowpea (*Vigna unguiculata* L.) Both types of beans are used as local food or daily food ingredients by the local community. Both of been is available in large quantities, cheap and easy to find both in traditional markets and in the home page of residents (27).

The absence of research on the use of local NTT legumes in the manufacture of bacterial growth media has attracted researchers to make bacterial growth media using local legumes from Timor Island, NTT, namely cowpeas which in everyday life the people of Timor Island call them cowpea and pigeonpea. The Medical Laboratory Technology Study Program is one of the study programs under the Poltekkes of the Ministry of Health Kupang, which has a Microbiology, Mycology and Parasitology laboratory. These three laboratories require a large number of bacterial growth media for student practicum and research by lecturers and students. The availability of synthetic media such as nutrients is limited because the delivery process from outside the province often experiences obstacles causing vacancies in the laboratory. The study were aimed to find out whether or not cowpea medium and pigeonpea medium can be used as substitution media of nutrient agar for *E.coli* and *S. aureus* bacteria's growth expecially in the laboratory with minimum infrastructure.

MATERIALS AND METHODS

The research method used was *true experiment* with a posttest-only control design in which the control group and sample group were randomly selected and the effect of the treatment was analyzed using a different test, namely Independent test - sample t-test and one-way ANOVA test. This research was carried out in February - March 2020 in the bacterial laboratory, majoring in medical laboratory technology, Poltekkes of the Ministry of Health Kupang, Indonesia. The variables in this study were the bacterial growth media and the number of *E. coli* (ATCC 25922 and *S. aureus* (ATCC 25953) colonies growing on the growth media. *S. aureus* bacteria used in this study were taken from pus samples patients at Prof. Dr. W. Z. Johannes Kupang general hospital. *S. aureus* was maintenance in Mannitol Salt Agar media. Process of making cowpea medium and pigeonpea medium according to Khaerunisa (22) and it show in Figure 1;

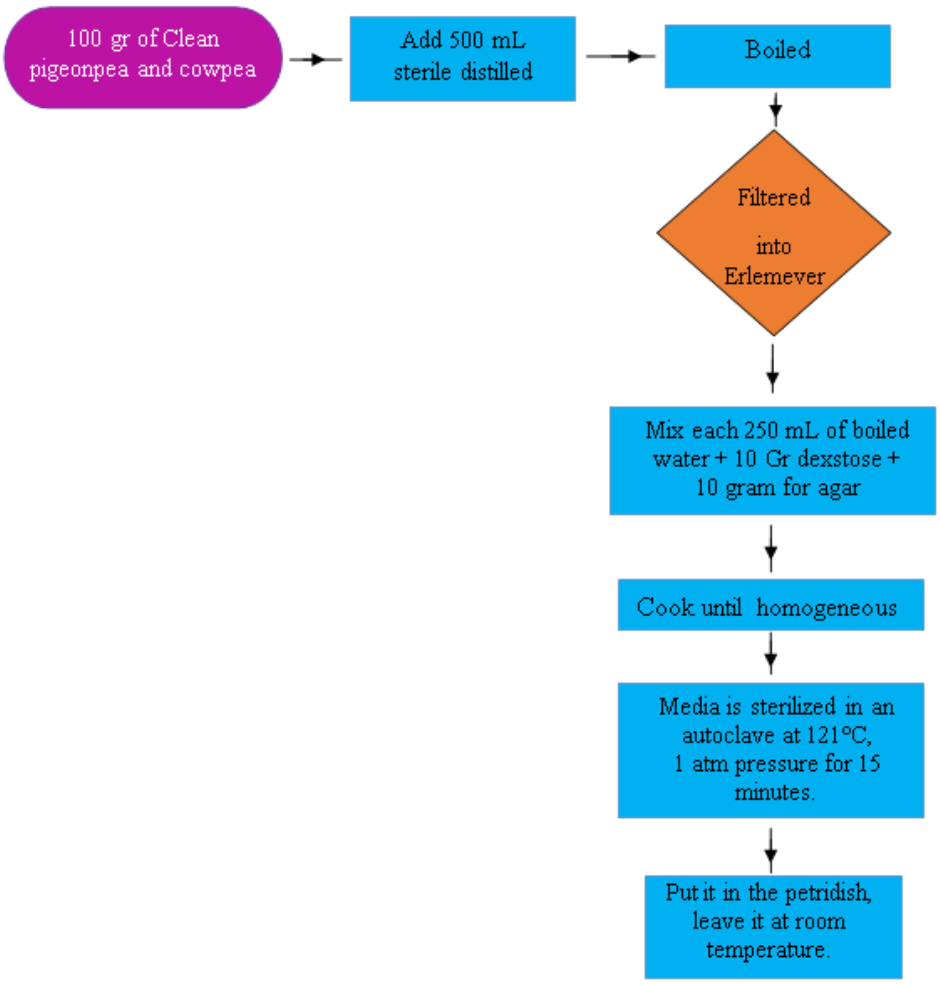

Figure 1. Procedure of making pigeonpea and cowpea medium

Suspensions of *S. aureus* and *E. coli* were diluted with a concentration equivalent to 0.5 MC Farland. Immediately after being suspended inoculated as much as 0.1 mL on each medium for cowpea, for pigeonpea medium and nutrient agar medium. We did six iterations in each test. Incubated in a temperature incubator 37^0C for 24-48 hours then the size of the colony was measured and followed by the calculation of the number of colonies using the colony counter. Nutrient agar to be used as a control medium. Nutrient agar is a general purpose medium supporting growth of a wide range of non-fastidious organisms. It typically contains (mass/volume): 0.5% Peptone (this provides organic nitrogen), 0.3% beef extract/yeast extract (the water-soluble content of these contribute vitamins carbohydrates, nitrogen, and salts), 1.5% agar (this gives the mixture solidity), 0.5% Sodium Chloride (this gives the mixture proportions similar to those found in the cytoplasm of most organisms), distilled water (water serves as a transport medium for the agar's various substances), pH adjusted to neutral (6.8) at 25°C (77°F) (28).

Primary data obtained from examination of total plate numbers from alternative media for pigeonpea and cowpea as well as nutrient agar media, then the data were displayed in tabular form. In addition, to obtain differences in the number of bacteria, the data obtained were processed using statistical tests, namely the independent test - sample T test and ANOVA to see the differences in bacterial growth in each medium.

RESULTS

General description of *E. coli* and *S. aureus* growth based on the number of bacterial colonies on cowpea medium, pigeonpea medium and nutrient agar medium can be seen in Table 1. Table 1 shows that the average growth rate of *S. aureus* bacteria on pigeonpea medium was 164 CFU/mL, on cowpea medium was 161 CFU/mL and on nutrient agar as much as 164 CFU/mL on media. The same table shows the average growth of *E. coli* bacteria on cowpea medium as much as 163 CFU/mL, pigeonpea medium as much as 167 CFU/mL and nutrient agar medium as much as 164 CFU/mL.

Table 1. Growth results of *E. coli* and *S. aureus* bacteria on cowpea medium and pigeonpea medium

Bacteria	Media Type	Repetition	Min CFU/mL	Max CFU/mL	Mean	SD
E. coli	Nutrient agar	6	160	168	164	2,75
	Cowpea	6	160	168	163	2,63
	Pigeonpea	6	163	170	167	2,79
S. aureus	Nutrient agar	6	160	170	164	3,21
	Cowpea	6	158	168	161	3,02
	Pigeonpea	6	157	166	164	3,13

Based on the number of *E. coli* bacterial colonies growing on these three media, it can be seen that the average number of *E. coli* bacteria colonies grows more on pigeonpea medium, namely as much as 170 CFU/mL while *S. aureus* bacteria grow more on nutrient control medium so that is as much as 170 CFU/mL. Based on the homogeneous test, it shows a significant value> 0.05 so that it means that the data is homogeneous and can be continued with the ANOVA test.

In addition to growing pure cultures of *E. coli* and *S. aureus*, we also carry out tests on clinical specimen from pus samples from patients. It was found that the growth of *E. coli* could be identified properly. Our findings indicate that pigeonpea medium and cowpea medium can be used as alternative media to grow *E. coli*. The form of bacterial growth on both media is shown in the image below:

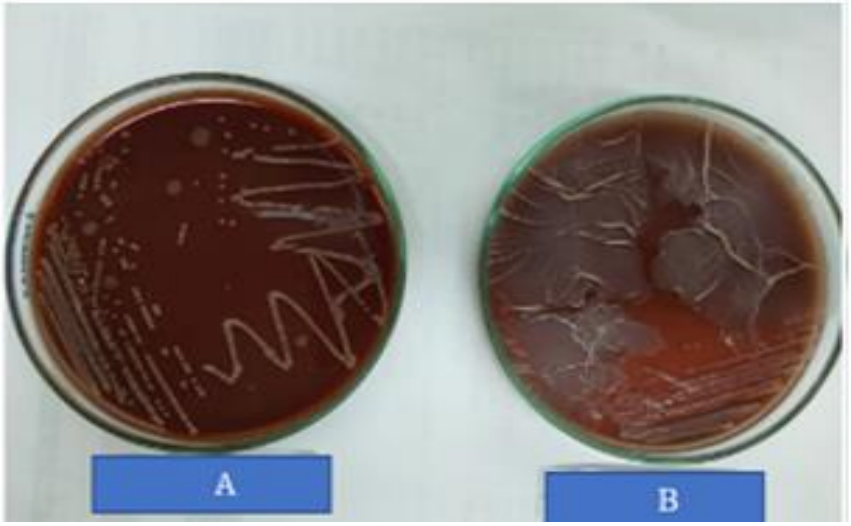

Figure 2. Growth of *E. coli* bacteria from pus samples. (A) on cowpea and (B) on pigeonpea medium

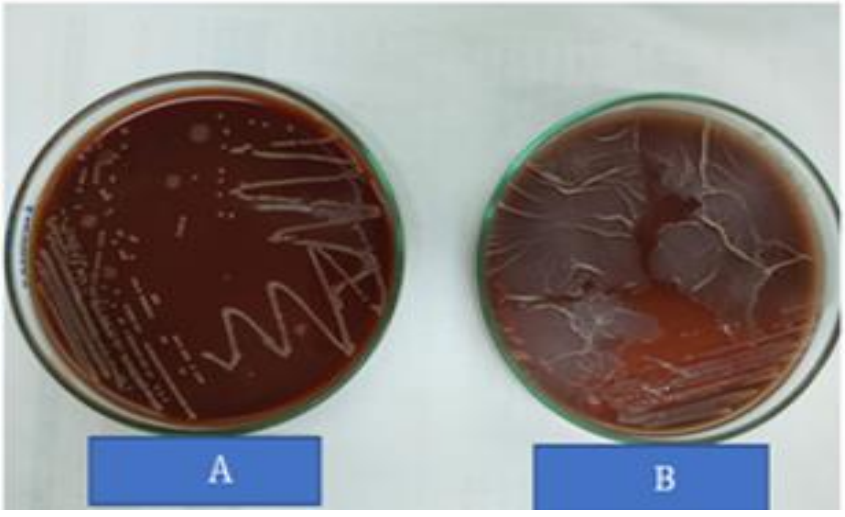

Figure 3. Growth of pure *E. coli* bacteria (A) on cowpea media and (B) on pigeonpea media

Figure 2 above shows that the *E. coli* bacteria from pus clinic samples can grow well, the form of colonies that grow is different and the specific growth is found in cowpea medium, namely large, slimy and wetter colonies while in pigeonpea medium, the form of colonies is dry, small and spread. Figure 3 show pure cultures of *E. coli* bacteria can grow well and show specific growth, in the medium of cowpea the form of large colonies, spreading and wetter, while in the medium of pigeonpeas the form of small colonies and drier.

In pigeonpea medium, the size of the *S. aureus* bacteria colony was smaller than the colonies that grew on the control media. The colony size of *E. coli* on cowpea medium was the same as the colony size of *E. coli* in the control medium, while the colony size of *E. coli* on pigeonpea medium was smaller than the colony size of *E. coli* that grew on the control medium.

The differences in the size of bacterial colonies are caused by several factors including nutritional factors, temperature, and osmotic pressure (23). Our literature study shows that the nutrients that can be used by bacteria for growth are sources of energy, carbon, nitrogen, minerals, sulfur, and vitamins (22). The difference in nutrient content in the three media was one of the causes of the difference in colony size(20). In addition to nutritional factors and incubation temperature, another influential factor is the adaptation phase. The adaptation phase is a situation when bacteria are transferred to a new environment, the bacteria will undergo an adaptation process including the synthesis of new enzymes from the previous growing medium (21). In addition, Figure 5 shows the growth of *E. coli* and *S. aureus* on nutrient agar as a control medium.

Figure 4. Growth of pure culture of *S. aureus* bacteria (A) on cowpea and (B) on pigeonpea media.

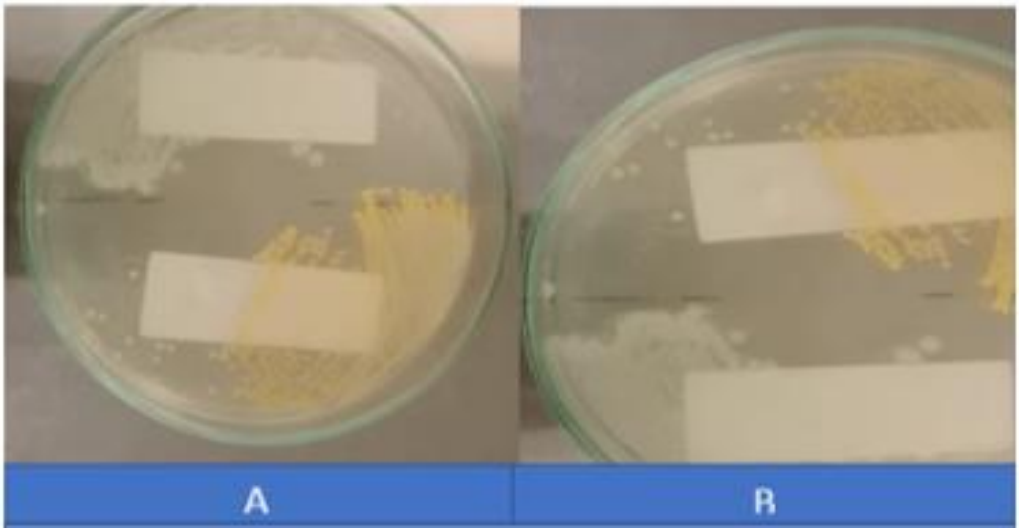

Figure 5. Growth of pure culture of *S. aureus* bacteria (A) on cowpea and (B) on nutrient agar media.

The analysis was continued with the ANOVA test and obtained p value = 0.145 (>0.05) for the number of *E. coli* bacteria and p value = 0.393 (>0.05) for the number of *S. aureus* bacteria so that there was no significant difference number of bacteria in the cowpea, pigieonpea and nutrient agar media. It was found that the significant value of cowpea medium and pigeonpea medium could substitute the use of nutrient agar medium.

DISCUSSION

Factors that can affect bacterial growth include nutritional factors, temperature, pH and osmotic pressure (6,10). The nutrients contained in cowpea are incomplete compared to the nutrients contained in pigeonpea and in nutrient medium so that some of the nutrients needed for bakery growth cannot be fulfilled, as a result, it is difficult for bacteria to grow on cowpea medium. Based on our literature study, we found that pigeonpea contain nitrogen, Vitamin B12, iron/Fe, phosphorus and fiber which are not found in cowpea. The nutrients needed by microorganisms for their growth include carbon, nitrogen, non-metal elements such as sulfur and phosphorus, metal elements such as Ca, Zn, Na, K, Cu, Mn, Mg, and Fe, vitamins, water, and energi (19,21). Our literature study also shows that pigeonpea beans and cowpea beans contain nutrients including, water, calories, protein, fat, carbohydrates and calcium which are needed for bacterial growth (19).

In addition to nutritional factors, these bacteria are in an adaptation phase, which is when the bacteria is transferred to a new environment, it will undergo an adaptation process including the synthesis of new enzymes that are different from the previous

growth media and recovery of toxic metabolics such as acids, alcohols and alkalis (8,10). The adaptation response can be due to nutrient deficiencies in cowpea medium and pigeonpea medium, indicated by the small size of the bacteria (18,19).

The protein hydrolysis process is carried out because the protein molecules are too large to be able to enter through the bacterial cell membrane, so the bacteria excrete the protease enzyme which hydrolyzes the protein into simpler peptides (22). Then the peptides formed with the help of peptidases are converted into amino acids, so that the amino acids formed can enter bacterial cells (20). In bacterial cells the amino acids that are formed are catalyzed by the enzyme lactic acid dehydrogenase and reduced by NADH to produce energy, so that it can be used for growth in colony size (1,29).

Research by Rizki & Syahnitya (24) which utilizes jicama and bean sprouts as growth media for *S. aureus* and *E. coli* bacteria shows that the growth of *S. aureus* and *E. coli* bacteria is more in jicama media, this is because jicama has a higher starch content than the starch sprouts contained in jicama is composed of 2 kinds of carbohydrates, namely amylose and amylopectin (22,24).

The same thing was conveyed by Khaerunisa (5) which used boiled water for yellow tubers and purple tubers as an alternative medium for the growth of *S. aureus* and *E. coli* bacteria, the results showed that the number of *S. aureus* and *E. coli* bacteria grew more on media made using yellow tuber boiled water, because the carbohydrate content of yellow tuber boiled water is higher than purple tuber boiled water, where the carbohydrate contained in yellow tubers is raffinose. Raffinose is a trisaccharide consisting of the monomers fructose, glucose and galactose which are used as an energy source to increase the number of bacterial growth (22).

Widya's research (23) states that bacterial growth in alternative media is influenced by several factors, namely nutrient content, extract-making process, fiber content, storage and the effect of changes in pH after autoclaving sterilization (23). Purwati (21) reports that the use of different natural sources of carbohydrates can be used to substitute nutrient agar (NA) media for bacterial growth. Suweg tuber (*Amorphophallus paeoniifolius*), taro tuber (*Cyrtosperma merkusii*), and kimpul tuber (*Xanthosoma sagittifolium*) can be used as nutrient substitution media for the growth of Gram-positive and Gram-negative bacteria however, the best medium is the medium from the Suweg tuber (*Amorphophallus paeoniifolius*) (21).

Test results of ANOVA between pigeonpea media and nutrient media in order to obtain p value = 0.145 ($p > 0.05$) for the growth of *E. coli* bacteria and p value = 0.393 ($p > 0.05$) for the growth of *S. aureus* bacteria. These results indicate that there is

no significant effect between the number of bacterial colonies *E. coli* and *S. aureus* grown on pigeonpea media, cowpea and nutrient agar as control media. The statistical test shows that non-synthetic media from cowpea and pigeonpea can replace the use of nutrient media for daily use. The results of this study, we recommend the use of natural media for the needs of student practicum and lecturer / student research. In the initial isolation stage that requires large amounts of media, we recommend the use of these two media. We hope that the use of these two media will help with laboratory activities and make it easier to prepare and use them.

Several previous studies have reported the use of natural materials as a substitute for nutrient agar to grow *S. aureus* and *E. coli* (12,15,16,22). Anisa (12) has also developed several different carbohydrate sources including canna tubers (*Canna discolor*), gembili tubers (*Dioscorea esculenta*), and garut tubers (*Maranta arundinacea*) as an alternative medium for bacterial growth. Jannah (16) has also conducted research and showed that sweet potato (*Ipomoea batatas* (L). Lam) cilembu as a substitute for carbohydrates in potato dextrose agar (PDA) media for the growth of the fungus *Trichophyton rubrum*. Indrayana (15) also proved that sweet corn cobs waste flour (*Zea mays*) can be used for the growth of *E. coli* and *S. aureus* bacteria.

Safitri *et al*. (13) have also developed a tofu whey substrate as a growth medium for *Pediococcus pentosaceus* lactic acid bacteria. The development of specific media that grows *E. coli* bacteria using pure head water was developed by Wulandari, it was found that there were differences in the number of colonies at each concentration, *E. coli* growth was found in young coconut water media with concentrations of 20%, 40%, 60%, 80%, while there was no growth at concentrations of 0% and 100%. The potential of red beans and green beans to grow *Lactobacillus acidophilus* bacteria was developed by Kurniasih *et al*. (5), the results showed that mung bean juice was more effective as a growth medium for *Lactobacillus acidophilus* than red bean juice (5).

Other natural ingredients that have also been reported to have potential as sources of nutrition, carbohydrates, protein, vitamins and minerals for the growth of several types of bacteria include tofu industrial wastewater (4) for *Bacillus* sp. bacteria, soybeans for *Pseudomonas aeruginosa* (3), Industrial Liquid Waste Tapioca for lactic acid bacteria *Pediococcus pentosaceus* (2), cassava, white sweet potato, yellow sweet potato for *E. coli* and *Bacillus* sp. (23), bacteria, tofu pulp flour as a growth medium for *Serratia marcescens* (17), bacteria, Jicama for the growth of *E. coli* and *S. aureus* (24), suwes tubers, taro tubers, and kimpul tubers as a substitute for nutrient agar media (21), Arum Manis Mango Seeds (*Mangifera indica* L.) for the growth of *Candida albicans* and *Aspergillus* sp. (20).

The results of our study provide recommendations to the microbiology laboratory which is difficult to obtain nutrient agar media from the industry due to budget constraints, the use of natural ingredients such as pigeonpeas and cowpeais one solution. Especially for student practicum needs that require bacterial cultivation, as well as for research purposes for students and lecturers who need it.

CONCLUSIONS

There was no difference between the number of bacterial colonies of *E. coli* and *S. aureus* on pigeonpea, cowpea and nutrient agar medium. Agar medium contained nutrient from Cowpea water and pigeonpea water can be used as a alternative medium to substitute nutrient agar in order to grow bacteria *E. coli* and *S. aureus*.

AUTHOR CONTRIBUTIONS

Norma Tiku Kambuno: conceptualized the study, developed the proposal, and performed sample collection, data management, analysis, and manuscript writing. Ninda P. Y. Amtaran: participated in laboratory work and performed sample collection. Sherly Dewu: participated in laboratory work and performed sample collection. Kuntum E. Nurdin: participated in result interpretation, data analysis, data management and manuscript writing. Ni Made Susilawati: participated in result interpretation, study supervision, and manuscript writing and editing. Yoan Novicadlitha: participated in result interpretation, data analysis, data management and manuscript writing.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

REFERENCES

1. Fachraniah, Fardiaz D, Idiyanti T. Peptone production from soybean press cake and yeast by papain enzyme for the bacterial growth media. J Teknol dan Ind Pangan. 2002;XIII(3):260–6.
2. Wulan R, Meryandini A, Sunarti TC. The potential of tapioca industrial liquid waste as a growth media for starter lactic acid bacteria *Pediococcus pentosaceus* E.1222. J Sumberd Hayati. 2017;3(1):27–33.

3. Danela S, Gede LS, Ariami P. Soybeans as an alternative media for *Pseudomonas aeruginosa* bacterial growth bakteri. J Anal Med Biosains. 2019;6(1):73.
4. Juriah S, Sari WP. Utilization of tofu industrial liquid waste as an alternative media for the growth of *Bacillus* sp. J Anal Kesehat Klin Sains [Internet]. 2018;6(1):24–9. Available from: http://jurnal.univrab.ac.id/index.php/klinikal/article/view/525/361
5. Kurniasih N, Rosahdi TD. Comparison of the effectiveness of red bean and green bean extracts as growth media for *Lactobacillus acidophilus*. Pros Semin Nas Sains dan Teknol Nukl. 2013;212–6.
6. Tharmila S, Jeyaseelan EC, Thavaranjit AC. Preliminary screening of alternative culture media for the growth of some selected fungi. Arch Appl Sci Res. 2011;3(3):389–93.
7. Susilawati NM, Kambuno NT, Saputri YI, I Gede Putu Arnawa. Prevalence of negative strain Gram bacteria extended spectrum beta lactamase (ESBL) in Rsud Prof . Dr . W . Z . Johannes Kupang. Pakistan J Med Heal Sci. 2020;14(2):1434–6.
8. Haddish K. Production of single cell protein from fruit of beles (*Opuntia ficus-indica* L.) Peels Using Saccharomyces cerevisiae. J Microbiol Exp. 2015;2(7).
9. Nurdin KE, Olla LRY, Feoh SF, Galla ADP, Istnaini KD, Jonison EPF, et al. Effectivity test of 96% from soe (*Citrus sinensis* L.) sweet orange rind ethanol extract as biolarvaside. J Info Kesehat. 2019;17(2):176–83.
10. Noriko N. Potency of tea leaf (*Camellia sinensis*) and *Acalypha indica* L. earring leaf in inhibiting the growth of *Salmonella typhi*. J Al-AZHAR Indones SERI SAINS DAN Teknol. 2013;2(2):104.
11. Dewu S, Bala R, Kambuno NT, Kupang PK. Differences of preparation examination methods for the number of acid-resistant bacteria. 2020;18:59–67.
12. Anisah A. Alternative Media for bacterial growth using different carbohydrate sources. Vol. 53, Universitas Muhammadiyah Surakarta. 2015.
13. Safitri N, Sunarti TC, Maeryandini A. *Pediococcus pentosaceus* lactic acid bacteria growth media formula using tofu whey substrate. J Sumberd Hayati. 2017;2(2):31–8.
14. Saputra D, Nurhayati T. Production and application of fish peptone for bacterial growth media. J Pengolah Has Perikan Indones. 2014;16(3):215–23.
15. Indrayana FF. Alternative media for nutrient agar (AN) sweet corn cob waste flour (*Zea mays*) for the growth of *Escherichia coli* and *Staphylococcus aureus*. Poltekkes Kemenkes Bandung. 2020;53(9):1689–99.
16. Jannah ANAM. Utilization of sweet potato (*Ipomoea batatas*) Cilembu as a substitute for carbohydrates in potato dextrose agar (PDA) for the growth of *Trichophyton rubrum*. 2020.
17. Rosidah U. Tofu dregs flour as a growth medium for Serratia marcescens bacteria. Skripsi Unimus. 2016;1–63.
18. Al-azzauy AAM, Hassan AM. The beetroot juice as a bacterial growth and maintenance medium for many pathogenic bacteria. Univ Al-Mustansiriyah, Coll Pharm Dept Med Microbiol Biotechnol. 2011;5(3):147–61.
19. Deivanayaki, M.; Antony IP. Alternative vegetable nutrient source for microbial growth. Int J Biosci. 2012;2(5):47–51.
20. Sundari, Wisrakarmila, Marlina D, Faizah. utilization of arum manis (*Mangifera indica* L.) seeds as alternative growth media for *Candida albicans* and *Aspergillus* sp. J Pengelolaan Lab Pendidik. 2021;3(1):14–7.
21. Purwati S. Utilization of different carbohydrate sources (suweg bulbs, taro bulbs, and kimpul bulbs) as substitution of Na (Nutrient Agar) media for bacterial growth. J Chem Inf Model. 2013;53(9):1689–99.
22. Khaerunnisa, Rismaya, Iis K, Dewi N, Asep D. Utilization of boiled water of yellow and purple bulbs as alternative media for the growth of *Escherichia coli* and *Staphylococcus aureus*. J Ris Kesehat Poltekkes Depkes Bandung. 2019;11(1):269.
23. Widya A. Growth of *Escherichia coli* and *Bacillus subtilis* bacteria on cassava, white sweet potato and yellow sweet potato media as substitute for NA media. Skripsi. 2016;1–13.
24. Rizki Z, Syahnitya H. Utilization media bengkuang (*Pachyrrhizus erosus*) and bean sprouts (V*igna radiate*) as alternative media for *Escherichia coli* and *Staphylococcus aureus* bacterial growth. Sel J Penelit Kesehat. 2019;6(1):1–9.
25. Rahmawati R. Fungal growth in kluwih seed media and jackfruit seeds as substitute for PDA media. 2016;2016.
26. Rahma Faradiana. Utilization of Different carbohydrate sources (Suweg Bulbs and Kimpul Bulbs) as substitutes for PDA media for fungal growth. Skripsi [Internet]. 2019;53(9):1689–99. Available from: www.journal.uta45jakarta.ac.id
27. Puspita D, Palimbong S, Pratamaningtyas NL, Nugroho KPA. Proximate Analysis of different

types of legumes growing on the island of Timor-NTT. Semin Nas Tek Kim Kejuangan. 2017;0(0):05.

28. American Public Health Association, American Chemical Society A of OAC (U. S. Standard methods for the examination of water and sewage. In: Association of Official Agricultural Chemists (US). 1920.

29. Martyniuk S, Oroń J. Use of potato extract broth for culturing root-nodule bacteria. Polish J Microbiol. 2011;60(4):323–7.