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Alternative Media from Mangrove Fruit for the Growth of *Shigella dysentery*, *Escherichia coli*, and *Salmonella typhi* bacteria

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ABSTRACT

Objectives: Mangroves are rich in bioactive substances that support bacterial growth. The fruit of the mangrove tree can serve as a valuable source of carbohydrates, making it a potential substitute for Nutrient Agar (NA) as a growth medium.

Methodology and Results: The samples in this study were suspensions of *Shigella dysentery*, *Escherichia coli*, and *Salmonella typhi* with 16 replications. Thus, the total number of samples in this study was 94 samples. The samples were isolated and identified as colony growth on alternative Mangrove and NA media. The results showed that in alternative media prepared from Mangrove fruit flour, there was a significant growth difference (p-value <0,01) between *S. dysentery* bacteria with an average of 61 colonies, *E. coli* bacteria with an average of 2 colonies, and *S. typhi* bacteria with an average of 2 colonies. The comparison between media of Mangrove and NA also showed a significant growth difference with p-value < 0,01.

Conclusions: Mangrove media has been found to be less conducive to the growth of *S. dysentery*, *E. coli*, and *S. typhi* bacteria, despite being a more cost-effective option compared to NA. Notably, *S. dysenteriae* bacteria exhibit greater potential for propagation in this medium.

Keywords: Alternative, culture, mangrove, media, nutrient agar

1. INTRODUCTION

Mangroves are plants that can adapt to salinity and sea tides. In general, Mangroves grow along tropical coasts around the world. Mangrove trees can adapt physiologically to adjust the salt conditions in their tissues. Most Mangroves have floating seeds produced in large quantities each year. Moreover, the plants float until they move to a new place to live in groups. At a distance from functioning as a green pathway, Mangroves also act as sources of carbohydrates, proteins, fats, and secondary metabolites. It is well known that Mangrove ecosystems are a source of various microbes capable of producing enzymes and molecules beneficial to human life, agriculture, fisheries, industry, and bioremediation. (Dourado *et al*., 2012).

Bacteria are thought to play a role in the weathering process of organic matter from plants in the forest in the form of wood or leaves. Generally, bacteria are taken from sediment, leaf litter, and weathered wood in the Mangrove environment (Prartono *et al*., 2022; Sauer *et al*., 2022). Bacteria need adequate space and nutrients to grow and develop (Gill, 2017). Several previous studies discussing the isolation of bacteria from Mangrove plants explained that the main component of Mangrove litter is

leaves (Aida *et al*., 2014; Andrianto *et al*., 2015). Meanwhile, some studies demonstrate that Mangrove leaves have an antibacterial effect against *E. coli* (Mouafi *et al*., 2013).

In a study by Prihanto *et al.*, the results of the enzyme screening showed that isolates from Mangrove leaves were isolates of bacteria that could grow well (Prihanto *et al*., 2018). In addition, these isolates produced the best gelatinase enzymes. Leaf endophytic bacteria in the micro bacteria system test were identified as *Paenibacillus alvei* with negative oxidase results. Thus, these bacteria are grouped into types of Gram-positive bacteria (Prihanto *et al.*, 2018). A research by Triyanto *et al.,* (2008) explained that 41 isolates from the group of bacteria were successfully isolated from the two locations, Cilacap Regency 29 isolates (70.7%) and Indramayu Regency 12 isolates (29.3%). Based on these data, it can be concluded that many bacteria in the Mangrove area are predicted as denitrifying bacteria. The denitrifying bacteria were successfully isolated from sediment samples at a depth of about 5 cm (Rösch *et al*., 2002).

Microbiology laboratory tests need land to develop in the form of growth media. Microbial growth media is a material that contains components or nutrients needed for microbial growth. Microbes utilize media nutrients in the form of small molecules that are assembled to compose cell components. Using growth media makes it possible to isolate microbes into pure cultures. The media must contain the elements necessary for cell metabolism (Basu *et al.,* 2015).

Nutrient Agar (NA) is a semi-natural medium; semi-natural media consists of natural ingredients added to chemical compounds. Based on its use, NA is a shared media type most often used to grow some bacteria. Using NA for bacterial growth is expensive. IDR 500,000 to 1,500,000. The high price of NA is because the media has been clinically tested for bacterial growth and is a universal medium. Expensive NA is a severe problem, especially for researchers in developing countries (Uthayasooriyan *et al*., 2016).

The solution to solving the cost problem above requires media derived from natural materials that are cheap and easy to obtain. Several studies have used natural materials derived from carbohydrates and protein as alternative media, including those from potatoes, purple yam, green beans, and soybeans (Sawiphak *et al*., 2021; Uthayasooriyan *et al*., 2016). In previous studies, the use of natural ingredients as alternative media could be used to grow the bacteria *E. coli*, *Pseudomonas sp*., *Bacillus sp*., *Staphylococcus sp*., and *Klebsiella sp*. but only the bacteria *Klebsiella sp*., which can grow better than other bacteria (Uthayasooriyan *et al.,* 2016). Another study recommends the nutritional composition of alternative media that can be used for standard media, containing at least 3g of protein needed by bacteria (Arulanantham *et al*., 2012). In addition, compositions such as cellulose are also needed for the energy needs of bacteria, especially for processes related to the metabolic system (Gamit *et al*., 2023). The advantages of using alternative media from previous research are easier preparation and less expensive costs for laboratory experiment-based research. However, some things can be detrimental, including unpredictable composition or even killing bacteria because it is toxic (Basu *et al.,* 2015).

Except for that, fruit media has also been used. Although, in general, fruit contains some antibacterial antioxidant compounds (Suriyaprom *et al.,* 2022). One of the studies is the use of kepok bananas. The results obtained were *Streptococcus* and *Pseudomonas aeruginosa* bacteria at a concentration of 5% (Marbun & Supartiningsih, 2021). Another fruit that can be recommended as material for the growth of bacterial media is Mangrove fruit. Mangrove plants can complement additional choices as an alternative bacterial breeding medium. The reason is that mangrove plants provide good nutrition for the surrounding

environment (Reef *et al*., 2010). Meanwhile, in the fruit part, Mangroves contain various bioactivities such as carbohydrates, proteins, and fats. The amount of carbohydrates, protein, and fat in Mangrove fruit ranges from 10 to 500 mg/g (Budiyanto *et al*., 2022). The use of Mangrove fruit from the study by Harahap *et al*. (2022) found that Mangroves inhibit bacterial growth. This is contrary to the composition of Mangrove, which contains components that support bacterial growth. In this regard, further exploratory studies are needed to identify the potential of Mangrove fruit for bacterial growth and whether its composition stimulates or inhibits bacterial growth.

Pathogenic bacteria can be used to test the effectiveness of using Mangrove fruit as an alternative medium, which generally triggers widespread digestive diseases in West Kalimantan, including *S. dysenteriae*, *E. coli*, and *S. typhi.* Health analysis often find this bacteria when examining samples in several laboratories in West Kalimantan, especially samples from children and teenagers. Based on data obtained from the West Kalimantan Provincial Government, 1,801 cases of diarrhea were found in 2022 (Kalbarprov, 2022). Diarrhea is generally caused by the pathogenic bacteria *S. dysenteriae* and *E. coli*. Meanwhile, 539 cases of disease caused by *S. typhi* bacteria were found in 2012 based on reports from the Regional General Hospital, dr. Soedarso Pontianak (Nurlaila *et al*., 2014).

Although several studies have examined the use of natural materials as alternative materials for the reproduction of bacteria, exploratory studies using other plants, including the use of Mangrove fruit, are still needed. Therefore, this study explained the differences in the growth of bacterial colonies on Mangrove fruit powder and NA media.

2. MATERIALS AND METHODS

Research designs

The research method used was quasi-experimental with a purposive sampling technique. The samples in this study were suspensions of *S. dysentery*, *E. coli*, and *S. typhi* bacteria with 16 replications. Thus, the total number of samples in this study was 94 samples. Then, the samples were isolated and identified as colony growth on alternative Mangrove flour and NA media. This study uses bacteria as research subjects, so ethical clearance is unnecessary.

Mangrove fruit preparation

The Mangrove fruit utilized in our production is sourced from Mempawah Regency, located in West Kalimantan, Indonesia. We hold the highest standards in quality, and therefore, only select Mangrove fruit that is fresh, green in color, free from any signs of wilting or rot, and free from any animal or caterpillar damage.

Mangrove media preparation

Mangrove flour powder was weighed as much as 10 g, put into a 500 mL Erlenmeyer plus 10 g of sugar and 10 g of pure agar, dissolved with 500 mL of distilled water, heated, and homogenized. Then, the media composition was sterilized in an autoclave at 121°C for 15 min. After it cooled down, 15 mL was poured into a petri dish and allowed to freeze.

Production of NA

As much as 10 g of NA is weighed using an analytical balance and then dissolved in 500 mL of distilled water. Heat the solution while stirring until completely dissolved. Please put it in Erlenmeyer, then cover it with cotton. Continue with sterilization using an autoclave for 15 min at 121°C.

Preparation of bacterial suspension

To prepare for the analysis of three sample groups, a total of 16 test tubes were sterilized and filled with 5 mL of sterile NaCl each. From pure cultures, colonies of *E. coli*, *S. typhi*, and *S. dysentery* were carefully taken using sterile round loops and added to the respective test tubes containing sterile NaCl. The mixture was homogenized and compared against the Mc-Farland 0.5 turbidity standard.

Bacterial inoculation on NA

Immerse a sterile swab into a suspension of *E. coli*, *S. typhi*, and *S. dysentery* bacteria. Next, place the cotton against the side of the tube, allowing the liquid to drain. Gently press the sterile swab onto the top of the liquid, rotating it as necessary to ensure proper drainage. Then, rub the swab onto the surface of the NA media three times, turning the petri dish at approximately 60 °C for each swabbing process. This will ensure that the inoculum suspension is evenly distributed across the media's surface. Leave the medium for 10 min incubation at 37 °C for 24 h.

Interpretation of results

The bacterial colonies that developed in each sample were subjected to Gram staining. Subsequently, a colony counter was used to determine the count of *E. coli*, *S. typhi*, and *S. dysentery* colonies grown on Mangrove fruit flour media.

3. RESULTS AND DISCUSSIONS

Table 1 shows that the lowest number of *S. dysentery* bacteria colonies on NA was 78, and the highest number was 230, with an average of 136 colonies. The statistical analysis results using the Mann-Whitney test obtained a p-value < 0.05. There is a difference in the number of *S. dysentery* bacteria colonies that grow on NA media and the number of *S. dysentery* bacteria colonies that grow on alternative media of Mangrove flour.

In microbiological examinations, more use of agar media for culture isolation or bacterial growth. The most commonly used agar medium is NA. In general, NA is a media in the form of a yellowish-white powder in solid form because it contains agar as a solidifier. The most important composition of NA is carbohydrates and proteins contained in meat extracts and peptones according to the needs of most bacteria (Uthayasooriyan *et al.*, 2016).

Table 1: Results of Mann-Whitney Statistical Test of Differences in the Number of *Shigella dysenteries* Bacterial Colonies on NA and Mangrove Flour Alternative media

Colonies	n	Mean	p-Values
S. dysenteriae on NA	16	136	0,000
S. dysenteriae on Mangrove	16	61	

NA: Nutrient Agar *S. dysenteriae*: *Shigella dysenteriae*

In this study, alternative media using Mangrove fruit flour resulted in higher growth of *S. dysentery* bacteria compared to other bacteria, namely an average of 61 colonies (Figure 1). Like living things in general, bacteria need carbohydrates and proteins to support growth (Stokes *et al*., 1991). *S. dysentery* bacteria have the same characteristics as *Shigella* bacteria in general. This type of bacteria has enterotoxins ShET-1 and ShET-2, which can change electrolytes and water, triggering diarrhea and dehydration problems (Zhu *et al.*, 2021). Various multilevel metabolic processes control the mechanism of carbohydrate digestion in bacteria. The process is highly coordinated and regulated as needed by involving small regulatory RNAs (Svetlana *et al*., 2018). The simple part of monosaccharides from carbohydrates, namely glucose, supports bacteria in producing lactic acid. In addition, another form of glucose, namely D-allulose, also gives similar results (Al-Baarri *et al*., 2018).

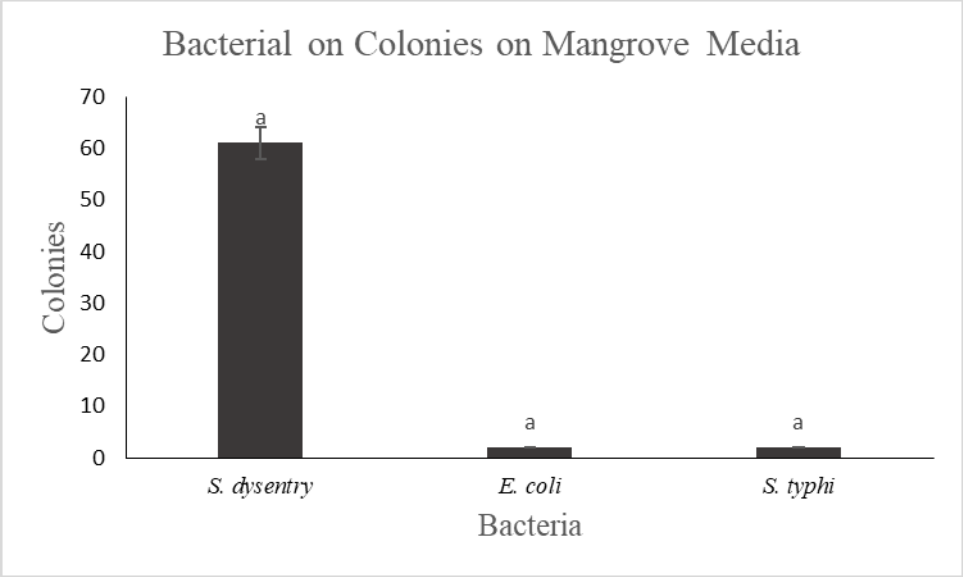

Figure 1: The comparison of growth of bacterial colony numbers in Mangrove flour alternative media
a indicates p-value < 0,05 from Friedman Test
S. dysentery*: *Shigella dysentery* *E. coli*: *Escherichia coli* *S. typhi*: *Salmonella typhi

Research has shown that using vegetables as a media source can promote strong bacterial growth, with protein being a notable source of nourishment (SANTOS *et al*., 2022). Typically, bacteria require only around 14-15% of their total nutritional needs to be met by protein (Stokes *et al*., 1991). *S. dysenteriae* has been identified as a potential cause of diarrhea, as it can invade colonic epithelial cells and multiply intracellularly. Interestingly, this bacterium appears to grow particularly well in Mangrove media, potentially due to the intracellular multiplication process, despite the minimal protein content of this medium (Keusch *et al*., 1981).

The lowest number of *E. coli* colonies on NA was 68, and the highest was 249, with an average of 140 (Table 2). The statistical analysis results using the Mann-Whitney test obtained a P-value < 0.05. There is a difference in the number of *E. coli* bacteria colonies that grow on NA and the number of *E. coli* bacteria colonies that grow on Mangrove flour alternative media.

Table 2: Results of Mann-Whitney statistical test differences in the number of *E. coli* bacterial colonies on NA and Mangrove Flour Alternative media

Colonies	n	Mean	p-Values
E. coli on NA	16	140	0,000
E. coli on Mangrove	16	2	

NA: Nutrient Agar *E. coli*: *Escherichia coli*

The three bacteria from this study showed a significantly lower difference in the number of colonies on Mangrove media than on NA. The existence of a higher protein composition sourced from meat extract is thought to be the cause of the high colonies on NA. Similar comparative studies complement the reasons for the findings of this study. Research conducted by Shareef *et al.* (2019) showed that the growth of the number of colonies of *E. coli* bacteria was also lower in alternative media compared to colonies on NA. However, the difference is not significant (Shareef, 2019). The study by Raju *et al.* (2018) also gave similar results when alternative media with a higher protein composition than other media. The result was that the number of *E. coli* bacteria colonies would also grow more.

The lowest number of *S. typhi* bacterial colonies in Mangrove flour media was 1 colony, and the highest number was 2 colonies with an average of 2 colonies. The statistical analysis results using the Mann-

Whitney test obtained a p-value < 0.05. There is a difference in the number of *S. typhi* bacteria colonies that grow on Nutrient Agar and the number of *S. typhi* bacteria colonies that grow on Mangrove flour alternative media (Table 3).

Table 3: Results of Mann-Whitney Statistical Test Differences in the Number of *S. typhi* Bacterial Colonies on NA and Mangrove Flour Alternative Media

Colonies	n	Mean	p-Values
S. typhi on NA	16	78	0,000
S. typhi on Mangrove	16	2	

NA: Nutrient Agar *S. typhi*: *Salmonella typhi*

In this study, *S. typhi* grew more on NA than Mangrove media. Better breeding of *S. typhi* on NA follows the results of previous studies. When grown on NA and then incubated at 37 °C , the *Salmonella* chain changes size to medium, and an increase in diameter occurs (Wang, 2022). *S. typhi* propagation in Mangrove flour media differs from previous studies' results, which explained that glucose is the primary energy source for *S. typhi* multiplication. Nonetheless, there are several different findings about factors supporting the proliferation of *S. typhi* bacteria. A study by Bowden *et al*., (2009) explained that S. *typhi* needs glucose as an energy booster for cell replication in macrophages. In addition, gluconeogenic substrates such as amino and tricarboxylic acids (TCAs) are also required by *S. typhi* during the infection mechanism. Meanwhile, in the human epithelial cell line (Hela), glucose is not needed by *S. typhi* (Bowden *et al*., 2014). The presence of iron and manganese also affects the ability of a bacterium to survive in host cells. When the concentration of iron in the host cell is reduced, it will induce a variety of bacterial toxins (Röder *et al*., 2021).

This study still has limitations; the researchers did not analyze the composition of glucose and protein in the media. Therefore, based on previous research, the causes of non-optimal bacterial growth in *E. coli* and *S. typhi* colonies can only be correlated. Examining protein levels and other nutritional compositions is recommended to ensure the factors that trigger differences in the results of the proliferation of each bacterial colony.

CONCLUSION

The alternative Mangrove media showed significantly lower growth of *S. dysentery*, *E. coli*, and *S. typhi* bacteria compared to NA. Additionally, the number of *S. dysenteriae* colonies was significantly higher than that of *E. coli* and *S. typhi* on Mangrove alternative media. Therefore, it can be concluded that while Mangrove media is cheaper than NA, it is less efficient for the growth of these three bacteria. However, *S. dysenteriae* shows potential for propagation in this media and further research is needed.

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