Antimicrobial activity of turmeric leaf extract against *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae*, and *Lactobacillus acidophilus*

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Abstract. Indonesia is a country known for its diversity of plants, especially agricultural products and spices, which have the potential as antimicrobials, such as turmeric leaves (*Curcuma domestica* Val.). This study aimed to determine the antimicrobial activity of turmeric leaf extract. The method used was a completely randomized design with two factors, namely extraction method with maceration using water, methanol and ethyl acetate as the solvent, and concentration of turmeric leaf extract of 20%, 40%, 60%, and 80%. Turmeric leaf extract gave an inhibitory effect to the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Shigella dysenteriae*, and did not give inhibitory effect to the growth of *Lactobacillus acidophilus*.

Keywords: turmeric leaf, antimicrobial, bacteria

1. Introduction

Natural materials extracted from plants had been increasingly used for both pharmaceutical and agricultural purposes, because in addition to the fact that their chemical structures reduced the residual side effects, they were also easily found. One of these plants was turmeric. The most commonly part of the turmeric that had been used for traditional treatment was the rhizome. Turmeric rhizome had been widely known as having antimicrobial potential. In addition, the application of turmeric rhizome as a natural preservative of food products, both in fresh and processed food, in the form of essential oils and extracts, had also been widely studied [1-5].

From previous studies, antimicrobial tests were reported on the growth of *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*; it was reported that turmeric extract inhibited the growth of tested microbes [6]. In the study of turmeric leaves, the growth inhibitor effect on *Aspergillus flavus* and *Fusarium moniliforme* was tested, and it was found that turmeric leaf extract was able to inhibit the growth of the fungi [7]. Nowadays the problem of food security is considered as a global problem, so it gets the main attention in determining public health policies. Diseases originating from food contamination occur in many countries, not only in the developing countries where sanitation and hygiene conditions are generally bad, but also in developed countries. What attracted attention was that in developing countries diarrheal disease was estimated to be the leading cause of death of 2.2 million children. This disease contributed significantly to the problem of malnutrition and a suppressed immune response that was commonly experienced by children in developing countries. Diarrheal diseases that arise mainly due to foodborne and waterborne pathogens, with the cause being transferred through food, reached 70% [8-11]. *Escherichia coli*, *Shigella dysenteriae* and *Staphylococcus aureus* were the causes of diarrhea. Diarrhea makes the patients experiencing a lot of fluid loss from the body. The main cause of this disease is the microbial contamination during food processing and handling.
Lactobacillus burgaricus, including probiotics, is often used both in food, beverage, drug and pharmaceutical products and is known as lactic acid bacteria (BAL), because of its ability to produce lactic acid. The use of BAL has been known for centuries in the process of making fermented dairy products such as yogurt, kefir, yakult, and cheese [12-15]. Considering the various uses of rhizomes and turmeric leaves, results of the studies showed that turmeric rhizome and leaves was able to inhibit microbial growth. Therefore, this research was intended to explore and develop the potential of turmeric leaves as antibacterial [16-18].

2. Research Methods
The ingredients used were turmeric leaves, cultures of Staphylococcus aureus, Escherichia coli, Shigella dysenteriae, Lactobacillus acidophilus and antibiotics Tetracycline. Chemicals and media used were NA, MHA, NaCl 0.9%, aquadest, Oxoid paper, methanol, ethyl acetate, dimethylsulfoxide (DMSO) and n-hexane. The experiment was conducted using a completely randomized design with two factors, consisting of: solvent type (P): water, methanol, ethyl acetate, and concentration (K) of turmeric leaf extract: 20%, 40%, 60% and 80%. There was 12 combination of treatment (PK). The experiment consisted of five stages, those were: 1. making turmeric leaf extract, 2. preparation of tested bacteria, 3. test of turmeric leaf extract, 4. proximate analysis of turmeric leaf powder, 5. phytochemical test of turmeric leaf extract. Flowchart of the research method is shown in Figure 1.

3. Results and Discussion
3.1 Results of turmeric leaf powder proximate analysis
The initial stage of proximate analysis began with the production of turmeric leaf powder. Fresh turmeric leaves were thinly sliced and then dried using an oven at 35°C for 24 hours, then grounded and sieved using a 20 mesh sieve. The proximate analysis of turmeric leaf powder obtained results is presented in Table 1.

| Analysis      | Turmeric leaf powder (%) |
|---------------|--------------------------|
| Water         | 6.83                     |
| Ash           | 5.45                     |
| Protein       | 12.44                    |
| Fat           | 6.32                     |
| Carbohydrate  | 68.96                    |

Note: Done with 5 replications, the results were the average.
The results of the proximate analysis on the water content of turmeric leaf powder was 6.83%. This was in line with the requirements set by Materia Medika Indonesia (MMI) of <10%. Water content that exceeds 10% can be a good medium for fungi growth during storage time, so the quality of simplicia will decrease.

3.2. Phytochemical test results of turmeric leaf extract

The phytochemical test was conducted to identify the components of bioactive compounds contained in turmeric leaf extract. Components of bioactive compounds tested in this study were: alkaloids, flavonoids, glycosides, saponins, tannins and steroids/triterpenoids. The phytochemical test of turmeric leaf extract using water, methanol and ethyl acetate solvents showed the presence of several compounds as shown in Table 2.

Table 2. Results of phytochemical screening of turmeric leaf extract.

| Bioactive Compounds | Solvent          |
|---------------------|------------------|
|                     | Water | Methanol | Ethylacetate |
| Alkaloids           | -     | +        | +            |
| Flavonoids          | +     | +        | +            |
| Glycosides          | +     | +        | +            |
| Saponin             | +     | +        | +            |
| Tanin               | +     | +        | +            |
| Triterpenoid / Steroid | -   | -        | +            |

Notes: (+) = Contains a class of compounds
(-) = Does not contain a class of compounds

Phytochemical screening results revealed that alkaloid compounds were obtained from turmeric leaf extract using methanol and ethyl acetate solvents. Flavonoid compounds, glycosides, saponins and tannins were obtained from turmeric leaf extract with water, methanol and ethyl acetate solvents. While for steroid/triterpenoid compounds only obtained from turmeric leaf extract using ethyl acetate. Methanol and ethyl acetate are organic solvents, so alkaloids can be attracted to the solvent. Alkaloids are polar compounds. In the free form, alkaloids are weak bases that are difficult to dissolve in water but are easily soluble in organic solvents [19].

Flavonoids are generally more soluble in water or polar solvents because they have bonds with hydroxyl groups. Glycosides are compounds that contain sugar and non-sugar components. Saponins are generally in the form of glycosides so they tend to be polar. Saponins are surface active compounds that produce foam if shaken in water. This happens because saponins have polar and non-polar groups that will form micelles. When the micelle is formed the polar group will face out and the non-polar group face inside so it looks like foams. Water and methanol are polar solvents, while ethyl acetate is a semi-polar solvent, and these three solvents can attract flavonoids and glycosides contained in the material [20].

Tannins which are phenolic compounds tend to dissolve in water and tend to be polar. Water and methanol are polar solvents, while ethyl acetate is a semi-polar solvent, so that the tannin can be attracted to the three solvents. Terpenoids are fat soluble. One of the terpenoids which has the potential as an antimicrobial is triterpenoid, while steroids are fat groups and are part of the triterpenoid. So that these compounds tend to dissolve in non-polar solvents, and therefore in the steroid / triterpenoid test of turmeric leaf extract, only those using ethyl acetate solvent can attract the compound, where water and methanol are polar solvents, while ethyl acetate is a semi solvent polar. The process of material extraction occurred by following the solvent into the material cell which
caused the protoplasm to swell, and the content of the cell in the material would dissolve according to its solubility. High solubility is related to solvent polarity and polarity of the extracted material.

3.3. Effect of inhibition of turmeric leaf extract on Escherichia coli
The type of solvent had a very significant effect on the diameter of the zone of inhibition of *Escherichia coli* growth. To determine the difference in inhibition zones at each level of treatment the Least Significant Range test was carried out. The relationship between the solvent type and the diameter of *E. coli* growth inhibition zone can be seen in Figure 2.

![Figure 2. Effect of turmeric leaf extract type on *Escherichia coli* growth inhibition zone](image)

The increase of the diameter of *E. coli* growth inhibition zone with the concentration of turmeric leaf extract produced due to the higher concentration. The higher the concentration, the more bioactive compounds contained, so that the diffusion ability of antimicrobial materials was also greater and resulted in a higher diameter of inhibitory zones. The diameter of the inhibitory zone also depended on the absorption capacity of the antibacterial agent into the agar plate and the sensitivity of the bacteria to the antibacterial substance.

Factors that influenced the occurrence of inhibitory zones were the diffusion capacity of the antimicrobial materials into the media and their interaction with the tested microbes, the number of tested microbes, the growth rate of the tested microbes, and the level of microbial sensitivity to antimicrobial materials. The ability of each bacterium to fight antibacterial activity varied depending on the thickness and composition of the cell wall. *E. coli*, a gram negative bacterium, was more resistant to antimicrobials compared to gram positive bacteria. This was because *E. coli* had a lipopolysaccharide layer which was attached to the outer membrane with a hydrophobic bond. Based on previous research, it was known that the tested turmeric rhizome extract had antibacterial activity against *Salmonella typhimurium* bacteria. The results showed that the presence of metabolic compounds in the form of alkaloids, flavonoids, steroids/triterpenoids, essential oils, and tannins were proven to inhibit the growth of *Salmonella typhimurium*. Turmeric leaf extract also had an antifungal activity against *Aspergillus flavus* and *Aspergillus moniliforme*. Some studies also mentioned that turmeric extract had antifungal activity against *Alternariaporri* in vitro.

3.4. Antibacterial activity of turmeric leaf extract against Staphylococcus aureus
The inhibition zone diameter at the concentration of turmeric leaf extract of 20% was not significantly different with that of 40%, and was significantly different with of 60% and 80%. The inhibition zone diameter at the concentration of turmeric leaf extract of 40% was significantly different with that of 60%, and 80%, and the treatment of concentration of turmeric leaf extract of 60% had a significantly different effect with 80% concentration treatment. It was able to be explained that the diameter of *S.
Staphylococcus aureus growth inhibition zone increased along with the increasing of the concentration of turmeric leaf extract. With the increase in the concentration, the bioactive compounds contained in the extract also increased, so that the diffusion rate of antimicrobial materials was also higher and it resulted in a larger diameter of inhibitory zones. Different concentrations showed different effect on the resulting inhibition zones. The diameter of the inhibitory zone also depended on the absorption capacity of the antibacterial agent into the agar plate and the sensitivity of the bacteria to the antibacterial substance. The relationship between the solvent types on the diameter of the growth zone of *Staphylococcus aureus* can be seen in Figure 3.

![Figure 3](image.png)

**Figure 3.** The relationship between concentration of turmeric leaf extract on the diameter of the growth inhibitory zone of *Staphylococcus aureus*

*Staphylococcus aureus* is a gram positive bacterium. Generally gram positive bacteria are more sensitive to antibacterial compounds compared to gram-negative bacteria because the cell walls of gram-positive bacteria have no lipopolysaccharide layer so that antimicrobial compounds that are hydrophilic or hydrophobic can pass through the cell wall of gram positive bacteria through a mechanism of passive diffusion then interact directly with peptidoglycan in growing cells bacteria and causing cell death.

3.5. Antibacterial activity of turmeric leaf extract against Shigella dysenteriae

Each type of solvent had different inhibitory activity on the growth of *Shigella dysenteriae* as shown in Figure 4.

![Figure 4](image.png)

**Figure 4.** Relationship of concentration of turmeric leaf extract and the diameter of growth inhibition zone of *Shigella dysenteriae*
The use of solvents in extracting food ingredients affected the test results of the bioactive compounds of the food. The diameter of the growth inhibition zone of *S. dysenteriae* affected by turmeric leaf extract produced using water, methanol and ethyl acetate solvents depended on the nature of each solvent itself in absorbing/attracting antibacterial compounds. Only extract using ethyl acetate solvents was able to absorb steroid/triterpenoid compounds, so there was a significantly different effect between water and methanol solvent treatment. Polar compounds are more soluble in polar solvents and non-polar compounds are more soluble in non-polar solvents. Polar compounds will be extracted in polar solvents and non-polar compounds in non-polar compounds. Ethyl acetate is a semi-polar solvent so it is able to dissolve polar and non-polar compounds.

Water and methanol are polar solvents, but in the results of the alkaloid screening test, water solvent was unable to attract alkaloid compounds because in the free form alkaloids are weak bases that are difficult to dissolve in water and are easily soluble in organic solvents. The use of 30 µg/ml tetracycline antibiotics aimed to compare the inhibitory ability of turmeric leaf extract with tetracycline antibiotics to tested bacteria. Tetracycline has an inhibitory power of 11.0 mm against *S. dysenteriae* bacteria. Analysis of variance showed that the concentration of turmeric leaf extract had a highly significant effect on the diameter of *S. dysenteriae* growth inhibition zones.

### 3.6. Antibacterial activity of turmeric leaf extract against *Lactobacillus acidophilus*

The interaction of the type of solvent and concentration of turmeric leaf extract was not able to provide inhibitory power to the growth of *Lactobacillus acidophilus*. It was found that the bacteria were still able to grow after being given turmeric leaf extract using water, methanol and ethyl acetate solvents, both with concentrations of 20%, 40%, 60%, and 80%. It was able to be explained that allegedly turmeric leaf extract contained oligosaccharide compounds which are a good growth media for *Lactobacillus*.

Functional oligosaccharides are short polysaccharides with unique chemical structures that cannot be digested by human digestive enzymes. So, like dietary fiber, oligosaccharides will eventually reach the large intestine. Thus, this compound is a good medium for the growth of Bifidobacteria in the large intestine (colon) so that oligosaccharides are called prebiotics. Oligosaccharides have low molecular weight so they are easily dissolved in water. Food fiber has a physical effect by increasing viscosity; it is mild laxative because it increases the volume of feces by binding water so as to prevent constipation. Oligosaccharides are not physically active, but have a biological effect, which facilitates the fermentation of beneficial microbes in the colon. Oligosaccharides increase the adsorption of calcium, zinc and magnesium minerals, whereas food fiber actually inhibits it. Some foods naturally contain oligosaccharides. For example, fructo oligosaccharides can be found in onions, garlic and asparagus. Soybeans contain soybean oligosaccharide. The use of 30 µg/ml tetracycline antibiotics aimed to compare the inhibitory ability of turmeric leaf extract with tetracycline antibiotics to the tested bacteria. Tetracycline had a 11.7 mm inhibitory power against *L. acidophilus* bacteria.

### 4. Conclusion

Extract of turmeric leaves dissolved in ethyl acetate contained alkaloid, flavonoid, glycoside, saponin and triterpenoid /steroid compounds, while those dissolved in contained flavonoid compounds, glycosides and saponins and those dissolved in methanol solvents contained alkaloids, flavonoids, glycosides and saponins. Turmeric leaf extract was one of the good media for the growth of *Lactobacillus acidophilus* bacteria.

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Acknowledgements
The author expressed his gratitude to the lecturers Master of Food Science Universitas Sumatera Utara.