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# Utilization Of Lime Peel Waste (*Citrus aurantifolia* (Christm.) Swingle) As An Antibacterial Against *Salmonella typhi*

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**Abstract.** Typhoid fever is a digestive tract infection caused by Salmonella typhi. Treatment of typhoid fever can use natural ingredients such as lime peel waste. The purpose of this study was to determine the effect of the ethanol extract of lime peel waste on the growth of Salmonella typhi bacteria. The extraction method used the maceration technique, and the antibacterial activity test used the paper disc diffusion method. The results showed that the ethanol extract of lime peel waste at concentrations of 1 mg/ml, 3 mg/ml, 5 mg/ml and 7 mg/ml with inhibition zone diameters of 4.66 mm, 6.55 mm, 9.29 mm and 10.06 mm. The conclusion shows that there is an effect of lime peel ethanol extract in inhibiting the growth of Salmonella typhi bacteria in the weak to strong category.

**Keyword:** Typhoid, Lime Peel Waste, *Salmonella typhi*, *Citrus aurantifolia*

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## 1 Introduction

Typhoid fever is an infection of the digestive tract caused by gram-negative bacteria, namely *Salmonella typhi*. Typhoid fever is the second order among intestinal diseases after gastroenteritis [1]. Approximately 16-20 million cases of typhoid fever occur annually worldwide. *S. typhi* bacteria is a gram-negative bacterium that is a zoonotic intracellular pathogen that enters eukaryotic cells and causes a severe systemic disease in humans called typhoid fever which is a major global problem resulting in 200,000 deaths every year. The pathogenic bacteria *S. typhi* spreads through consumption of contaminated food and water [2]. Treatment of typhoid fever or commonly called typhus is carried out for complete healing and to minimize complications that arise in patients with typhoid fever such as intestinal bleeding due to perforation, gallbladder infection (cholecystitis), hepatitis and meningitis. The administration of the antibiotic chloramphenicol is currently the most commonly used treatment for typhoid fever [1]. However,

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irrational administration of antibiotics will lead to antibiotic resistance. For this reason, it is necessary to do other alternatives in the treatment of typhoid fever, namely the utilization of plants that are not utilized (waste) such as lime peels. Lime plant is a fruit that is often found in the community. Lime is also one of the toga plants used by the community, both for cooking spices, medicines, and fresh drinks [3]. Lime is usually processed as candy, syrup, and other refreshing drinks. Lime is also often used as a spice in cooking, cosmetics and medicine [4]. In its use, only the flesh is taken, while the skin, which is around 30% of the total weight, is discarded, while this part can still be used again. Lime peel waste still has bioactive compounds that can be utilized [5]. According to [6], explained that the leaves, fruit and lime peel contain flavonoid compounds. Lime peel contains flavonoids, vitamin C [7], which act as antioxidants, essential oils, alkaloids, saponins [8], terpenoids which act as antibacterials [9]. Based on previous research, it was explained that the ethanolic extract of orange (20 mg/ml) showed significant activity against clinical isolates of *Shigella, Salmonella typhi, Klebsiella*. Studies show that Shigella is more sensitive to extracts with an average inhibition zone of 14.90 mm, followed by *Klebsiella* (14.49 mm), *E. coli* (13.77 mm) and *S. typhi* (12.01 mm) [10]. The ethanol extract of lime peel has potential as an anticancer [11], anticholesterol [12], antioxidant [7].

## 2 Material and Methods

### Preparation Sample

Lime peel samples (*Citrus aurantifolia* (Christm.) Swingle) were obtained from the Simalingkar market in Medan, North Sumatra. As much as 5 Kg of fresh lime fruit sorted wet, then washed with running water. The peeled samples were taken from the skin, then dried in a drying cabinet. The dried samples were then crushed using a blender to obtain simplicia powder.

### Preparation of Lime Peel Ethanol Extract (*Citrus aurantifolia* (Christm.) Swingle)

The simplicia powder of lime peel (*Citrus aurantifolia* (Christm.) Swingle) was weighed 300 g and extracted using maceration method with 96% ethanol solvent. Maceration was carried out by soaking lime peel simplicia (*Citrus aurantifolia* (Christm.) Swingle) for 3 days with occasional stirring. The procedure was repeated until the macerate was colorless (clear). The results of the maceration (maserate) were thickened using a rotary evaporator to obtain a thick extract of lime peel (*Citrus aurantifolia* (Christm.) Swingle).

### Phytochemical *S*creening

1) Alkaloid Test

Three test tubes were prepared and 1 g of simplicia was added, then Dragendorff reagent was added, Wagner's reagent was added to the second tube, Mayer's reagent was added to the third tube and observed changes. The result is positive if the first tube (Dragendorff reagent) produces a red precipitate, the second tube (Wagner's reagent) produces a brown precipitate, and the third tube (Mayer's reagent) produces a white precipitate.

2) Flavonoid Test

Simplicia was weighed 1 g, put into a test tube and added with 20 mL of hot water until submerged, then heated and then filtered. The filtrate was added with 0.1 gram of magnesium powder, 1 mL of hydrochloric acid, 2 mL of amyl alcohol, shaken and allowed to separate. The formation of yellow, green, red or orange color indicates the presence of flavonoids.

3) Saponin Test

Simplicia was weighed 1 g, put into a test tube and added with 5 mL of hot water until all the extract was submerged and shaken vigorously for 10 seconds. There is foam after shaking as high as 1-10 cm. The foam is waited for 10 minutes and remains constant, so the extract is positive for saponin compounds.

4) Tannin Test

Simplicia was weighed 1 g, put into a test tube and added 3 mL of warm water and then filtered. The extract was tested with 1-5 drops of 1% $FeCl_3$ giving rise to a strong green, red, purple, blue or black color indicating the presence of tannin group compounds.

5) Steroid and Triterpenoid Test

Steroid and triterpenoid tests were carried out by weighing 1 g of simplicia powder, macerated with 20 mL of n-hexane for 2 hours, filtered, the filtrate was evaporated and the remainder was added with 20 drops of Liebermann Burchard reagent (anhydrous acetic acid and concentrated sulfuric acid). Samples containing steroid group compounds will change color to bluish green. While the triterpenoid group compounds will change color to form a brown or violet ring.

**Preparation of Lime Peel (*Citrus aurantifolia* (Christm.) Swingle) Ethanol Extract**

The ethanol extract of lime peel (*Citrus aurantifolia* (Christm.) Swingle) was prepared with various concentrations as 7 mg/ml, 5 mg/ml, 3 mg/ml, 1 mg/ml, DMSO as a negative control and chloramphenicol as a positive control. Then disc paper was inserted into each extract and soaked for ± 3 minutes.

**Determination of Antibacterial Activity**

Antibacterial activity was determined using the scratch plate method. As much as 20 mL of MHA media that had been sterilized was put into a sterile petri dish, then allowed to solidify. Take one ose of bacterial suspension (*Salmonella typhi*), then streak it on the media evenly. Then the paper discs were placed with concentrations (7 mg/ml, 5 mg/ml, 3 mg/ml, 1 mg/ml, chloramphenicol, and DMSO) on solid MHA media in a petri dish. Then all the cultures were incubated at 37$^{o}$C for 24 hours. Antibacterial activity test was carried out with three repetitions. Observations were made by measuring the diameter of the inhibition zone formed around the disc paper using a vernier caliper.

## 3. Results and discussion

*Results of Phytochemical Screening*

The results of the phytochemical screening of simplicia and ethanol extract of Swingle's lime (*Citrus aurantifolia* (Christm.) peel) can be seen in Table 1 below.

Table 1. Results of Phytochemical Screening of Simplicia and Lime Peel Extract (*Citrus aurantifolia* (Christm.) Swingle.

| No. | Secondary Metabolites | Results | |
|---|---|---|---|
| | | Simplicia | Extract |
| 1. | Alkaloid | + | + |
| 2. | Flavonoid | + | + |
| 3. | Saponin | + | + |
| 4. | Tannin | + | + |
| 5. | Steroid/Triterpenoid | + | + |

Table 1 explains that the secondary metabolites found in simplicia and lime peel extract show the presence of alkaloids, flavonoids, saponins, tannins and steroids/triterpenoids. According to [13], explained that lime peel contains metabolic compounds such as alkaloids, saponins, and lots of flavonoids which have the potential to be anti-inflammatory, antifungal, antibacterial, antidiabetic and wound healing. The compounds contained in lime peel, namely essential oils, tannins, saponins, phenols, and alkaloids, have antibacterial properties so they can be used as natural antibacterials. Previous research concluded that lime peel extract was able to inhibit the growth of several clinical isolates of bacteria, such as *Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis*, and *Klebsiella pneumoniae* [14].

**Antibacterial Activity Results**

The results of the antibacterial activity test can be seen from the diameter of the inhibition zone on the growth of *Salmonella typhi* bacteria. The wider the diameter of the inhibition zone, the more effective the ethanol extract of Swingle's lime peel (*Citrus aurantifolia* (Christm.) as an antibacterial.

Table 2. Results of Inhibition Zone Diameter of Ethanol Extract of Lime (*Citrus aurantifolia* (Christm.) Swingle) Peel Against *Salmonella typhi* Bacteria

| Concentration | Inhibition Zone Diameter (mm) | | | Average | Category |
|---|---|---|---|---|---|
| | R1 | R2 | R3 | | |
| 7 mg/ml | 10,78 | 11,22 | 11,19 | 10,06 | Strong |
| 5 mg/ml | 7,11 | 11,34 | 9,44 | 9,29 | Moderate |
| 3 mg/ml | 6,12 | 6,89 | 6,65 | 6,55 | Moderate |
| 1 mg/ml | 0 | 6,87 | 7,12 | 4,66 | Weak |
| Chloramphenicol | 34,75 | 32,19 | 33,66 | 33,36 | Very Strong |
| DMSO | 0 | 0 | 0 | 0 | Weak |

Based on Table 2 it can be seen that the diameter of the inhibition zone of the ethanol extract of lime peel at each concentration has an average value sequentially at concentrations of 1 mg/ml, 3 mg/ml, 5 mg/ml and 7 mg/ml is 4.66 mm, 6.55 mm, 9.29 mm, and 10.06 mm with

weak to strong categories. The formation of a wider diameter of the inhibition zone along with the high concentration of the ethanol extract of lime peel is closely related to the presence of phytochemical compounds present in the extract, such as the presence of alkaloids, flavonoids, tannins, saponins and steroids/triterpenoids.

The mechanism of action of alkaloids works by interfering with the synthesis of nucleic acids and proteins, modifying the permeability of the bacterial cell membrane, destroying the cell membrane and bacterial cell wall and interfering with the metabolism of the bacterial cell. Alkaloids are also able to inhibit nucleic acid synthesis and protein synthesis by inhibiting the DNA replication process causing bacteria to be unable to carry out cell division [14]. Alkaloids have the ability to interfere with the constituent components of peptidoglycan in bacterial cells, so that the cell wall layer is not formed completely and causes cell death [15].

Flavonoid compounds are a class of active phenolic compounds. Where phenolic compounds cause denaturation of proteins found in the cell walls so that they can damage the composition and change the permeability mechanisms of microsomes, lysosomes and cell walls [16]. Flavonoid compounds are also able to penetrate the peptidoglycan of bacterial cells which causes the cell layer not to form completely. Flavonoids are able to form complex compounds with extracellular proteins causing damage to the bacterial cell membrane. Another mechanism by which flavonoids inhibit the work of the DNA gyrase enzyme causes the process of protein synthesis to be inhibited and bacterial cells are unable to replicate [14]. According to [15], the mechanism of action of flavonoids as antibacterials is by inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy metabolism.

The mechanism of action of saponins by changing the permeability of the cell membrane causes the release of enzymes from inside the bacterial cell. Saponins are also able to bind to lipopolysaccharides in the bacterial cell wall which causes the surface tension of the bacterial cell wall to decrease. The decrease in surface tension causes an increase in the permeability of the bacterial cell wall. So that the bacterial cell will lysis and die. Saponins also destroy the components that make up cells, causing bacteria to be unable to replicate and cells to lyse [14]. Saponins are substances that can interact with bacterial cells, so the bacterial cell walls will lyse and break. Tannin compounds can inhibit bacterial growth by coagulating the bacterial protoplasm [16]. Lime peel contains active ingredients that are thought to provide antibacterial effects such as tannins. Tannins can affect the permeability of the cytoplasmic membrane [1].

## 4. Conclusions

The results of the study concluded that there was an effect of lime peel ethanol extract in inhibiting the growth of *Salmonella typhi* bacteria in the weak to strong category. The results of the phytochemical screening showed the presence of alkaloids, flavonoids, tannins, saponins, and steroids/triterpenoids.

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