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ANTIBACTERIAL ACTIVITY OF TIN LEAVES (*Ficus carica* L.) ETHANOL EXTRACT AGAINST THE BACTERIA *Salmonella typhi*

Fitratul Wahyuni[1]; Aried Eriadi[2*]; Dwi Dinni Aulia Bakhtra[2]; Sugiat Bagus Hermawan[3]; Sanezea Effendy[4]; Vina Neldi[1]

[1]Departemen Farmakologi dan Farmasi Klinis, Sekolah Tinggi Ilmu Farmasi Padang, Padang, Indonesia
[2]Departemen Biologi Farmasi, Sekolah Tinggi Ilmu Farmasi Padang (STIFARM Padang)
[3]S-1 Farmasi, Sekolah Tinggi Ilmu Farmasi Padang, Padang, Indonesia
[4]Departemen Teknologi Farmasi, Sekolah Tinggi Ilmu Farmasi Padang, Padang, Indonesia
*email: aried.eriadi@gmail.com; phone: +62 813 7429 9993

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Abstract

Tin (*Ficus carica* L.) is a plant from the *Moraceae* family that contains phytochemicals including alkaloids, flavonoids, saponins, phenols, steroids, tannins. The purpose of this research was to determine the antibacterial activity of ethanol extract of tin leaves against *Salmonella typhi* bacteria by using the disc diffusion method. Disc paper containing antimicrobials is placed on a medium that has been planted with microorganisms that will diffuse into the media, the clear area indicates an inhibition of microorganism growth. The concentrations of ethanol extract tin leaves used were 10% and 5%. The strength of antibacterial activity can be seen through the parameter of inhibitory strength. Inhibition area 20 mm or more (very strong), 10-20 mm (strong), 5-10 mm (medium) and 5 mm (less). The results showed that the ethanol extract of tin leaves had strong antibacterial activity.

Keywords: *Ficus carica L.; Antibacteria; Salmonella thypii*

1. Introduction

Salmonella typhi is the bacteria that causes typhoid fever. This bacteria is gram negative which is motile and has the ability to infect humans or animals if ingested. *Salmonella typhi* bacterial infection is a cause of morbidity and mortality throughout the world (Jawetz et al., 2005). The endemic areas of this disease are Africa, Asia and Latin America. Typhoid fever is a global health problem where it is estimated that 16 million cases occur and 600,000 patients die. Transmission of typhoid fever is via the oral-face route where the intermediary is contaminated food and water (Sedric et al., 2015).

The use of antibiotics is not always based on the results of the culture of the germs that cause infection. Thus increasing the use of antibiotics without clear regulations ultimately leads to irrational use of antibiotics (Adekunle et al., 2010). Apart from that, uncontrolled use of antibiotics tends to increase the resistance of previously sensitive bacteria (Refdanita et al., (2004). This encourages the importance of finding sources of antibiotic drugs, especially those from plants. One plant that is known to be beneficial for health and is often

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used as traditional medicine is the tin plant. The tin plant which has the scientific name (*Ficus carica* L.) belongs to the Moraceae family which grows widely in tropical and sub-tropical areas. The tin plant has been widely cultivated because it is believed to cure various diseases.

In the holy book Al-Qur'an there is also a special letter that states about the tin plant (Al-Qur'an-Surat At-Tin verses 1-3). Allah SWT mentions that the tin plant in a separate letter in the Al-Quran definitely has great benefits contained in it which can be utilized by mankind (Department of Religion of the Republic of Indonesia, 2015). One part of the tin plant that can be used as traditional medicine is the leaves. Tin leaves contain phytochemical compounds, including alkaloids and saponins, which are useful as a diuretic and improve protein and fat metabolism (Allahyari et al, 2014).

2. Method

2.1 Tools

Dark bottle, filter paper, funnel (Pyrex), Erlenmeyer (Pyrex), set of rotary evaporator tools (Hahnvapors), measuring cup (Pyrex), test tube (Pyrex), test tube rack, spatula, dropper pipette, separating funnel (Pyrex), analytical balance (Precisa), vial, petri dish (AnormAx), autoclave, incubator, Laminar Air Flow (Model VL 150), micro pipette (Transferpette), hot plate (cimarec), loop needle, stir bar, paper disc (Advantec), tweezers, calipers, cotton, aluminum foil, gauze, parchment.

2.2 Materials

Tin leaves (*Ficus carica* L.), ethanol 70% (Bratachem), distilled water (Bratachem), ethanol 96% (Bratachem), n-hexane (Bratachem), ethyl acetate (Brataco), butanol (Bratachem), methanol (Bratachem), dimethylsulfoxide (DMSO) (Merck), Nutrient Agar Medium (Merck), chloramphenicol (Mehta), dilute hydrochloric acid (Merck), potassium iodide (Merck), potassium bromide (Merck), aluminum chloride (Merck), acetic acid, iron (III) chloride (Merck), bromine solvent, NaCl, concentrated hydrochloric acid (Merck), oxalic acid, BaCl2.2H2O, concentrated sulfuric acid (Merck), Potassium Iodide (KI) (Merck), mercuric chloride (HgCl) (Merck), *Salmonella typhi* bacteria from the University Microbiology Laboratory Andalas (UNAND) Padang, West Sumatra.

2.3 Procedure

2.3.1 Plant Material

Samples were taken from a tin plant cultivation site in Tangkerang Labuai, Pekanbaru. The sample chosen was fresh green tin leaves.

2.3.2 Identification Plant

Identification of the plants taken are whole plants from roots to tin leaves. Identification of tin leaves was carried out at the ANDA Herbarium, Biology Department, Faculty of Mathematics and Natural Sciences (FMIPA) Andalas University (UNAND) Padang, West Sumatra.

2.3.3 Extraction

In this research, making simplicia went through several stages are sample collection, wet sorting, washing, chopping, drying, dry sorting, packing and storage. The extract is made from dried simplicia powder by maceration using ethanol 70% solvent. Put one part of dry simplicia powder into the macerator, add 10 parts of solvent. Soak for the first 6 hours, stirring occasionally, then let it be quiet for 18 hours. The filtration process was repeated at least three times with the same type and amount of solvent. Collect all the macerate, then evaporate it using a rotary evaporator until a thick extract is obtained. The yield obtained was calculated, namely the weight percentage (w/w) between the yield and the weight of chopped tin leaf simplicia (*Ficus carica* L.) used with a weigher.

2.4 Characterization of Extract

2.4.1 Drying shrinkage measurement

Take 2 grams of simplicia in a shallow weighing bottle with a lid which has previously been heated to the specified temperature and tare. Flatten the material in the weighing bottle by shaking the bottle, until it forms a layer approximately 5 to 10 mm thick, put it in the drying room, open the lid, dry at the specified temperature

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until the weight remains constant. Before ready to dry, let the bottle cool in a closed condition in a desiccator to room temperature.

2.4.2 Total Ash Content Measurement

Take 2 grams of the ground test material, place it in a silica crucible that has been incandescent and set aside. Light it slowly until the charcoal has cooled and weigh it. If this method does not remove the charcoal, add hot water and stir, strain through ash-free filter paper. Place the filter paper and the rest of the filter in the same crucible. Put the filtrate into a crucible, evaporate and ignite until the weight remains constant weight. The total ash content is calculated from the weight of the material that has been dried in air

2.4.3 Acid Insoluble Ash Content Measurement

Boil the ash obtained from determining the total ash content with 25 ml of dilute hydrochloric acid for 5 minutes. Collect the part that does not dissolve in acid, filter through ash-free filter paper, wash with hot water, ignite in a crucible until constant weight. Calculate the acid insoluble content of the air-dried material.

2.4.4 Ethanol Soluble Essence Content Measurement

Take 5 grams of powder after air drying. Place in a stoppered flask, add 100 ml of ethanol P. Shake repeatedly for 6 hours, leave for 18 hours. Filter quickly to avoid evaporation of ethanol, evaporate 20 ml of filtrate until dry in a shallow, flat-bottomed cup that has been heated to 105 ºC and set aside, heat the remainder to temperature 105 ºC to constant weight. Calculate the content in % of ethanol soluble essence.

2.4.5 Preliminary phytochemical screening of the extracts

Phytochemical analysis of the ethanol extract of *Ficus carica* leaves was performed using standard procedures to determine the active constituents present in the extracts. Tests for alkaloids, saponins, phenols, tannins, anthraquinones, terpenoids, flavonoids and steroids were performed following the methods developed before

2.4.6 Antibacterial activity test

The tools used are first washed clean and dried. All procedures are carried out using aseptic techniques. A total of 15 ml of Nutrient Agar (NA) was put into a sterile petri dish, then 100 µl of bacterial suspension was added. Then it was homogenized by shaking the petri dish containing the media and then media allowed to solidify. Place the disc previously dipped in each test solution with a concentration of 10% and 5% w/v. Then stick it on the surface of the solidified agar. As a negative control, DMSO 10 µl was used and as a positive control chloramphenicol 30 µg/ml was used. This treatment was repeated 3 times. Then the petri dish was incubated with bacteria in an incubator for 24 hours at 37ºC and the antibacterial activity was determined by measuring the diameter of the inhibitory area formed using a caliper. The provisions for the strength of the inhibitory area are as follows obstacle area of 20 mm or more (very strong), resistance area of 10-20 mm (strong), resistance area of 5-10 mm (medium) and resistance area of 5 mm (less), said no effect.

3. Result and Discussion

The percent yields of the ethanol extracts of *Ficus carica* L. leaves was 15,50%. The ethanol extracts of *Ficus carica* L. leaves tested for phytochemical compounds. The results of phytochemical screening show that the ethanol extract of fig leaves contains flavonoids, saponins and phenols, steroids, tannins (table 1)

Table 1. Phytochemical properties

Analyze	Reagents	Result	Explanation
Flavonoid	2 drops $FeCl_3$	+	Forms a blackish green color
Alkaloid	2 drops Mayer	-	Does not form white precipitate
Saponin	2 ml water (shake and let it be quiete 10 min)	+	Forms foam
Phenol	$FeCl_3$	+	Forms a blackish green color
Steroid	3 drops HCl + H_2SO_4	+	Forms a green colour
Tanin	$FeCl_3$	+	Forms a greenish brown color

Testing of antibacterial activity against Salmonella typhi was carried out using the diffusion method using paper discs. The diffusion method was chosen because this method can clearly observe the presence or absence of bacterial growth so that it makes it easier to observe the test bacteria. The clear zone around the disc indicates the presence of bacterial growth inhibitory activity (Biermer, 1973).

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Positive control used in the test is the antibiotic chloramphenicol. According to Katzung (2004), chloramphenicol is a broad-spectrum bacteriostatic antibiotic that is active against gram-positive and gram-negative aerobic and anaerobic organisms. The results of the positive control treatment showed that there was an inhibitory effect on Salmonella typhi bacteria which had an inhibitory diameter of 30 mm. This shows that the antibiotic chloramphenicol has great inhibitory power on *Salmonella typhpi* bacteria by inhibiting bacterial protein synthesis in the peptidyl transferase enzyme which acts as a catalyst to form peptide bonds in the bacterial protein synthesis process (Jefri & Suwandi, 2017).

The results of research on tin leaves extract (*Ficus carica* L.) using the disc diffusion method had inhibitory activity, the inhibitory power of tin leaves ethanol extract (*Ficus carica* L.) produced using a concentration of 5% showed an average inhibitory diameter of 12.5 mm (strong) while at 10% it shows an average diameter of resistance of 14.5 mm (strong) (Tabel 2) (figure 1)

Table 2. Antibacterial activity of Tin leaves extract

Sample	Concentration	Inhibition zone (mm)			Average inhibition zone (mm)
		1	2	3	
Ethanol extract tin leaves	5 %	9	7	9	12,5
	10 %	9,5	8,5	11	14,5
Positive control (chloramphenicol)	30 µg/ml	35	33	34	34
Negative control (DMSO)	10 µg/ml	0	0	0	0

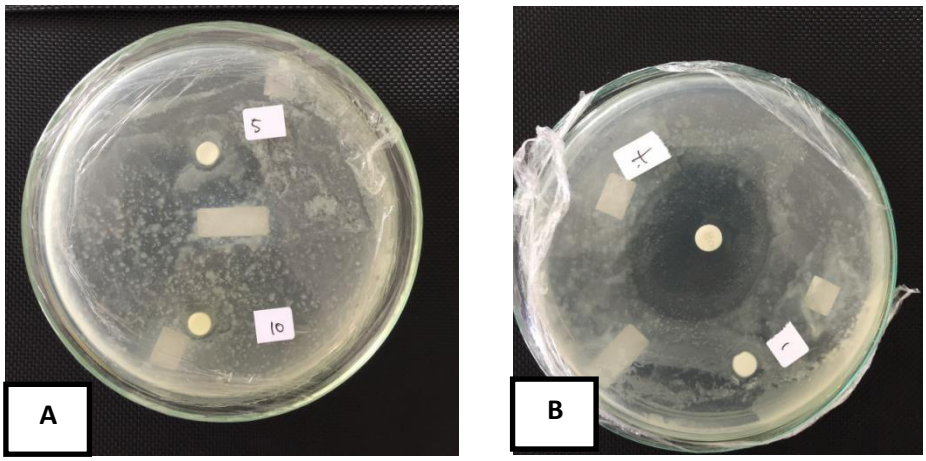

Figure 1. Antibacterial test of tin leaves extract with concentrations of 5%, 10%; (A) positive control and negative control; (B)

The results of this study show that tin leaves extract (*Ficus carica* L.) has antibacterial activity so that it inhibits the growth of *Salmonella typhi* bacteria. Secondary metabolite compounds that are thought to act as inhibitors of bacterial growth in tin leaves are flavonoids, phenol saponins, tannins, steroids (Eolia & Syahputra, 2019).

The mechanism of action of flavonoids as antibacterials can inhibit cell membrane growth and bacterial energy metabolism. When inhibiting cell membrane function, flavonoids form complex compounds with extracellular proteins which can damage the cell membrane of *Salmonella typhi* bacteria, followed by the release of the bacteria's intracellular compounds. Flavonoids can inhibit energy metabolism by inhibiting the use of oxygen by bacteria. Energy is needed by bacteria for the biosynthesis of macromolecules, so that if metabolism is hampered then the bacterial molecules cannot develop into complex molecules. Apart from that, flavonoids also contain phenolic compounds which can interfere with the growth of *Salmonella typhi* bacteria. Phenol is an alcohol compound that is acidic so it has the ability to denature proteins and damage bacterial cell membranes (Sapara et al., 2019).

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Tannins have antibacterial action which is related to their ability to deactivate bacterial adhesion, inhibit enzyme action, and inhibit protein transport in the cell envelope. The mechanism of action of tannin as an antibacterial agent is, among other things, through the destruction of bacterial cell membranes due to tannin toxicity and the formation of metal ion complex bonds from tannin which play a role in bacterial toxicity (Rahman et al., 2017). Steroids work as antibacterials by interacting with cell phopholipids which are permeable to lipophilic compounds, causing membrane integrity to decrease and cell membrane morphology to change, causing cells to become brittle and lysed, so that bacterial walls do not form completely (Madduluri et al., 2013).

The mechanism of saponin's action as an antibacterial is that it can cause leakage of proteins and enzymes from inside cells. Saponin can be antibacterial because the active substance is similar to detergent, as a result saponin will reduce the surface tension of bacterial cell walls and damage membrane permeability. Damage to the cell membrane greatly disrupts the survival of bacteria. (Harbone, J. B., 2006).

Saponin diffuses through the outer membrane and vulnerable cell walls then increases the cytoplasmic membrane thereby disrupting and reducing the stability of the cell membrane. This causes the cytoplasm to leak out of the cell resulting in cell death (Cavalieri et al., 2005).

4. Conclusion

From the results of the research that has been carried out, it can be concluded that the ethanol extract of tin leaves (*Ficus carica* L.) has antibacterial activity against Salmonella typhi bacteria.

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