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Research Article

The Concentration Variation Effect of Earring Leaves (*Acalypha Indica L.*) Extract on It's Bioactivity Ability in Inhibiting the Growth of *Salmonella Typhi* (*S. Typhi*)

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Abstract

The research has been carried out on the effect of variations in the concentration of earring leaves (*Acalypha Indica L.*) ethanol extract on the acquisition of inhibition zone values for the growth of *S. typhi* bacteria. In this study, ethanol solvent was used in the maceration and evaporation stages, then the well diffusion method was used to test its antibacterial activity. Each stage was carried out in triplo and 3 repetitions. Phytochemical test on the evaporated extract showed positive test results for flavonoid and tannin metabolite compounds. Concentration variations that used in this study were 5%, 15%, 30%, 50% and 60%, the antibiotic chloramphenicol was also used as a positive control and aquadest as a negative control. Meanwhile, the antibacterial activity test gave the result that the higher the concentration of the earring leaf extract (*Acalypha Indica L.*), the inhibition zone value also increased with the following successive values: 0mm, 11mm, 14mm, 15mm and 16mm, on the other hand chloramphenicol showed an inhibition zone of 32 mm. The research data were analyzed statistically using the One Way Anova test. Earring leaf extract (*Acalypha Indica L.*) has a significant effect of 0.000 on *S. Typhi* bacteria. However, at the smallest concentration, namely 5%, it does not have an inhibition zone diameter of 0 mm with a significant value of 1000. It can be concluded that the antibacterial activity using chloramphenicol is still higher when compared to the antibacterial activity using the ethanol extract of earring leaves (*Acalypha Indica L.*) in inhibiting growth of *S. Typhi* bacteria.

Keywords: *S. Typhi, earring leaves (Acalypha Indica L.), antibacterial activity, chloramphenicol.*

1. Introduction

Typhoid fever (typus) is a disease caused by infection with the bacterial pathogen *Salmonella Typhi* (*S. Typhi*). These bacteria can infect through the entry of contaminated food or drink into the body (Nuruzzaman, 2019). However, *S. Typhi* has the ability to change phenotypically and genotypically, making it difficult to remove from the food chain. This is exacerbated by the ability of bacteria that are resistant to heating up to 60°C for 1 hour, resistant to acidic pH so that they can survive in stomach acid conditions (Noriko, 2018).

Various efforts have been made to overcome cases of pathogenesis of *S. Typhi* bacteria, one of which is through the administration of antibiotics. However, giving increase antibiotics causes resistance from *S. Typhi* bacteria in the body. Therefore, many studies have been carried out to find other alternatives in treating *S. Typhi* infection, one of which is through the utilization of natural materials (plants) as traditional medicines.

Traditional medicine is also starting to be liked by the community because the treatment method is still simple and requires a relatively low cost (Artanti, 2020).

One of the plants that can be used as medicine is the earing plant (*Acalypha Indica L.*), was known as a medicinal plant that grows wild, usually growing in vacant land, yards, roadsides and even in gardens. *Acalypha Indica L.* has been widely used for generations as a medicine for dysentery, diarrhea, indigestion, vomiting blood, dysentery and blood urine, especially in the leaves which are efficacious for treating nosebleeds. *Acalypha Indica L.* has a bitter taste (Handayani et al, 2018). The chemical ingredients in the earrings include flavonoids, saponins, alkaloids and tannins (Nurhaini et al, 2021). Based on the chemical content and properties of the plant, it is expected that *Acalypha Indica L*. has potential as a natural antibacterial in inhibiting the activity of *S. Typhi* bacteria.

Previous research has been carried out qualitatively on the ability of *Acalypha Indica L.* to inhibit *S. Typhi* bacteria which states that *Acalypha Indica L.* extract has the potential as an antibacterial for *S. Typhi* using the boiling extraction method where the active inhibitory compound is tannin (Noriko, 2018). Based on the description above, it is very necessary to conduct research on the antibacterial activity test of the ethanol extract of the earring leaves (*Acalypha Indica L*.) against *S. Typhi* bacteria using maseration and evaporation methods.

2. Materials and Methods

2.1 Materials

The tools used in the study were Blenders, sieves, analytical balances, containers. Rotary evaporator, sieve, filter paper, glass funnel, stirring rod, aluminum foil, erlen mayer, measuring cup. Test tube, micropipette, incubator, petri dish, wire loops, bunsen burner, hot plate stirrer, label paper, erlenmeyer, autoclave, vortex, ruler, laminar air flow (LAF).

The materials used in the research were Earring leaf extract, chloramphenicol drug solution as positive control, aquadest as negative control, spiritus, Nutrient Agar (NA), Salmonella Typhi bacteria. Magnesium, concentrated hydrogen chloride (HCL), 1% ferric chloride (FeCl3) solution. 1 N NaOH, 0.1 N iodine. Simplicia of the earing leaves, 99.99% ethanol (p.a), distilled water.

2.2 Methods

Sampling

Collect fresh and undamaged earring leaves. Collection is done by picking from the shoots to the leaves that are not too old on the plant. It takes as much as 2 kg of earring leaves which are taken in Apitaik Village, East Lombok Regency (Selawa et al, 2018).
Sample Drying and Blending

The drying of the earrings leaves is carried out by aerating in a room with a temperature of 25°C and protected from sunlight (Luliana et al, 2016). If the earring plants have been sorted dry then in a blender to form a powder then sieved using a 30-mess sieve to obtain the same degree of fineness so that the extraction can run more optimally and transferred to a container (Yulianingtyas and Kusmartono, 2016).

Extraction of Sample

Put 200 grams of earring leaf powder into the erlenmayer then add 1 liter of ethanol p.a using a glass funnel. Extraction was carried out by maceration 3 × 24 hours in a place protected from light and occasionally stirred, after which it was filtered with filter paper (Hasnaeni, 2019). The liquid extract was then filtered using an Erlenmayer and a funnel and filter paper to separate it from the dregs. The filtrate liquid is put into a round bottom flask which is connected to a rotary vacuum evaporator until a thick extract is formed using a temperature of 40°C (Sa'adah and Nurhasnawati, 2018).

Phytochemical Test

The plant extracts that have been stored in the reaction are added with Magnesium (Mg) powder and 2-4 drops of concentrated Hydrogen Chloride (HCL). After that the tube was shaken. Plant extracts are declared positive for flavonoids if there is a red color change (Aribowo et al, 2021).

The tannin test was carried out by taking 1 ml of the extract solution, then dropping a drop of Ferric Cloride (FeCl3) solution and observing the color change. If the solution turns dark blue or blackish green, it indicates a positive tannin compound (Kurnianingsih, 2020).

Antibacterial Activity Test

Antibacterial activity testing used the well-diffusion method on Nutrient Agar (NA) media and carried out on Laminar Air Flow (LAF). Each Salmonella Typhi bacteria was cultured in Nutrient Agar (NA) media by scratching and letting it solidify completely. The entire test preparation was inserted into the wellbore until it was completely full. Incubation of the test medium was carried out for 24 hours at an incubator with a temperature of 37°C. Antibacterial activity is indicated by the formation of clear zones or inhibition zones around the wells (Edy et al, 2019)

Data analysis

Data were analyzed using the Anova test method to obtain results and research conclusions can be drawn (Sumual et al, 2021).

3. Results and Discussion

3.1 Results

Table 1. Observation Results of Inhibition Zone value of Earring Leaf Extract (*Acalypha Indica L.*), chloramphenicol and Aquadest against *S. Typhi*.

Treatment	Inhibition Zone
Extract 5%	0 mm
Extract 15%	11 mm
Extract 30%	14 mm
Extract 50%	15 mm
Extract 60%	16 mm
Chloramphenicol (control +)	32 mm
Aquadest (control -)	0 mm

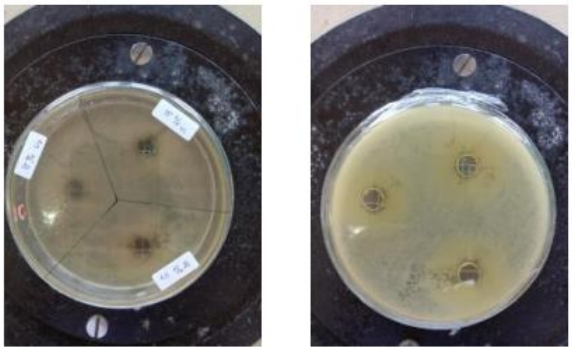

Figure 1. Observation Results of Activity Test of Earring Leaf Extract (*Acalypha Indica L.*) concentration of 15% against *S. Typhi*.

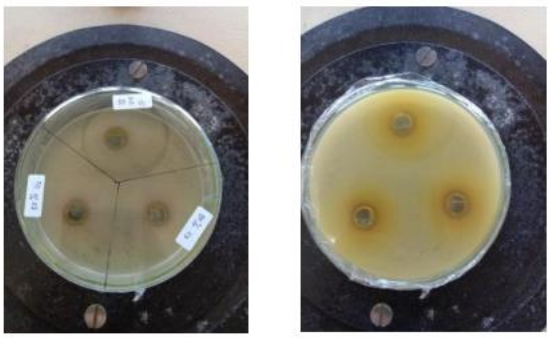

Figure 2. Observation Results of Activity Test of Earring Leaf Extract *(Acalypha Indica L.)* concentration of 30% against S. *Typhi*.

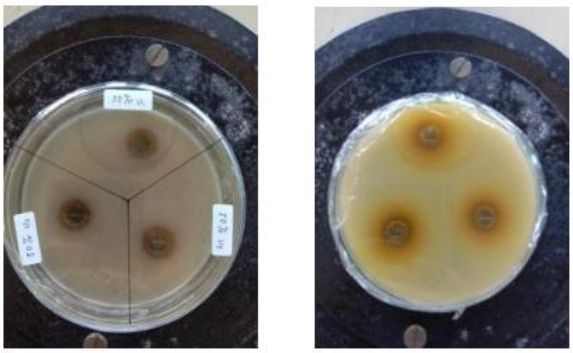

Figure 3. Observation Results of Activity Test of Earring Leaf Extract (*Acalypha Indica L.*) concentration of 50% against *S. Typhi*.

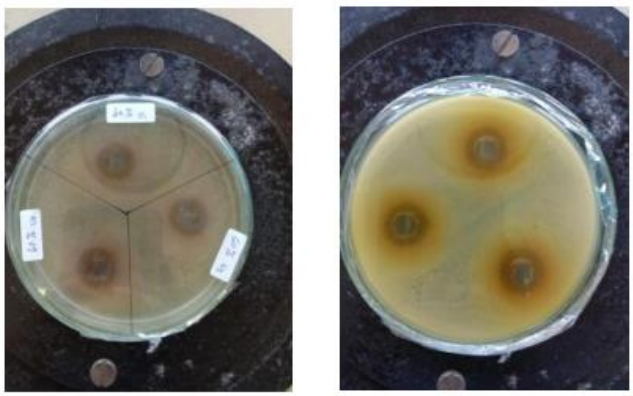

Figure 4. Observation Results of Activity Test of Earring Leaf Extract (*Acalypha Indica L.*) concentration of 60% against *S. Typhi*

3.2 Discussion

In this activity test study using the well-diffusion method (holes). What is done on the Laminar Air Flaw (LAF) table is carried out by preparing the necessary tools and materials from a petri dish that already contains Nutrient Agar (NA) which is solid and has been sterilized in an autoclave. After that, the bacterial suspension is taken in a test tube using a sterile cotton bud and then streaked in the Nutrient Agar (NA) medium in a zigzag or evenly distributed manner on the surface of the media, repeated (Sumiati, 2018).

Nutrient Agar (NA) solid media that had been planted with bacteria were made as many as 3 wells in each petri dish with a diameter of 6 mm, then filled with earring leaf extract (*Acalypha Indica L.*) with each concentration between them (5%, 15%, 30%, 50% and 60%) into the wellbore with 100-micron liters each using a micropipette. In this study, using a positive control of chloramphenicol and aquadest as a negative control. Then incubated for 1x24 hours at 37°C, then the inhibition zone was measured using a ruler. the results can be seen in table 1 above (Yunita et al, 2020).

Based on the results of the table above with a total of 3 repetitions of each treatment. Where each treatment has a different diameter of the inhibition zone at each concentration. In the negative control (Aquadest) and a concentration of 5% has an

inhibition zone diameter of 0 mm with a weak category (inhibition zone ≤ 5mm). The concentration of 15% has an inhibition zone diameter of 11 mm with a strong category (inhibition zone 10-19mm). The concentration of 30% has an inhibition zone diameter of 14 mm with a strong category. The concentration of 50% has a concentration of 15 mm with a strong category. The concentration of 60% has an inhibition zone diameter of 16 mm with a strong category. Whereas the positive control (chloramphenicol) had an inhibition zone diameter of 32 mm with a very strong category (inhibition zone ≥ 20mm). The antibacterial activity using chloramphenicol was still higher when compared to the antibacterial activity using the ethanol extract of the earing leaves (*Acalypha Indica L.*) in inhibiting the growth of *S. Typhi* bacteria, because the average inhibition zone in the positive control treatment was still higher and different compared to the average. - Average of inhibition zones in the treatment of earring leaf extract (*Acalypha Indica L.*). Therefore, the higher the concentration of the leaf extract of the earrings (Acalypha Indica L), the more the diameter of the inhibition zone against *S. Typhi* bacteria increases (Kurama, 2020).

Based on the results of the One Way Anova test of the leaf extract of the earrings (*Acalypha Indica L.*) it has a significant value of 0.000, meaning that there is an effect of the antibacterial activity of the ethanol extract of the earring leaf (*Acalypha Indica L.*) on *S. Thypi* bacteria. Because the value <0.05, the mean value between treatment groups of earring leaf extract (*Acalypha Indica L.*) was significantly different. To find out which treatment groups had significant differences, a Post-Hoc Analysis was then carried out (Yulianingtyas and Kusmartono, 2018).

Conclusion

Variations in the concentration of the extract of the earrings (*Acalypha Indica L.*) leaves have an inhibitory effect on *S. Typhi* bacteria, where the higher the concentration, the greater the inhibition of *S. Typhi* bacteria. The inhibitory power of each concentration, namely at a concentration of 5%, has an inhibition zone diameter of 0 mm with a weak category. The concentration of 15% has an inhibition zone diameter of 11 mm with a strong category. The concentration of 30% has an inhibition zone diameter of 14 mm with a strong category. The concentration of 50% has a concentration of 15 mm with a strong category. The concentration of 60% has an inhibition zone diameter of 16 mm with a strong category. based on the 5 variations of these concentrations, no one has been able to approach the value of the inhibition zone of chloramphenicol as a positive control antibiotic, so that the leaf extract of the earrings (*Acalypha Indica L.*) cannot be recommended as an antibiotic.

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