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# Evaluation of the Antibacterial Activity and Effectiveness of Ciplukan Leaf Extract (Physalis angulata L.) on the Growth of Salmonella typhi Isolates

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**Abstract .** This study evaluated the antibacterial activity and effectiveness of ciplukan leaf extract (Physalis angulata L.) against Salmonella typhi, the causative agent of typhoid fever. The experiment was conducted using the disk diffusion method to measure inhibition zones at various extract concentrations. Phytochemical screening confirmed the presence of flavonoids, alkaloids, tannins, and saponins, which are associated with antibacterial properties. The extract showed the highest inhibitory effect at 40% concentration (25 mm) and the lowest at 5% concentration (18.67 mm). Paired t-test analysis yielded a significance value of 0.434, indicating no significant difference between the first and second observation days. These findings suggest that the antibacterial activity of ciplukan leaf extract is relatively stable and effective. Therefore, ciplukan leaf extract has potential as a natural antibacterial agent and a candidate for phytopharmaceutical development in the management of typhoid infection.

**Keywords**: antibacterial activity, leaf extract, phytopharmaceuticals, Physalis angulata L., Salmonella typhi.

## INTRODUCTION

Typhoid fever is a systemic infectious disease caused by Salmonella typhi and remains a major public health problem in many developing and tropical countries, including Indonesia(4). This bacterium is a Gram-negative, rod-shaped bacillus that forms black colonies on Salmonella Shigella Agar (SSA) due to hydrogen sulfide production(13). The causative agent is classified as Salmonella enterica serovar Typhi and is widely distributed in Southeast Asia, including Indonesia, Malaysia, and Thailand(3).

Transmission mainly occurs through contaminated food and beverages associated with poor environmental sanitation and inadequate personal hygiene(2). The World Health Organization, estimates that approximately 11–20 million cases of typhoid fever occur annually worldwide, with 128,000–161,000 deaths, indicating a substantial global disease burden. In Indonesia, typhoid remains one of the most frequently

reported infectious diseases in health care facilities, largely due to limited access to clean water and suboptimal clean and healthy living practices[10]. Disease control therefore depends not only on sanitation improvement but also on effective therapeutic strategies.

Antibiotics are commonly used to treat Salmonella typhi infection. However, inappropriate and excessive use has contributed to the emergence of bacterial resistance, leading to reduced treatment efficacy and increased transmission risk[2]. This global challenge highlights the urgent need to explore alternative antibacterial agents that are safer, affordable, and sustainably available, particularly from medicinal plants.

Ciplukan (Physalis angulata L.), a medicinal plant from the Solanaceae family, has traditionally been used to treat various infectious conditions[5]. Previous studies have reported that the leaf extract contains secondary metabolites such as flavonoids, alkaloids, saponins, tannins, and steroids with antibacterial, antioxidant, and antitumor activities[1,11]. Flavonoids contribute to antibacterial activity by disrupting bacterial cell walls and membrane permeability, enabling inhibitory effects against both Gram-positive and Gram-negative bacteria, although susceptibility may vary due to structural differences in the outer membrane[6,12].

Nevertheless, studies specifically evaluating the antibacterial activity and effectiveness of Physalis angulata L. leaf extract against Salmonella typhi remain limited. Therefore, this study aimed to evaluate the inhibitory activity of the leaf extract and to determine the most effective concentration using the disk diffusion method. The findings are expected to strengthen scientific evidence for the development of plant-based antibacterial alternatives and support sustainable strategies for typhoid control in endemic regions.

## METHODS

### Plant Material and Extraction

Fresh leaves of Physalis angulata L. were collected from Daya Village, Biringkanaya District, Makassar City. Only healthy, green, and uncontaminated leaves were selected. The study was conducted from July to August 2024. The plant material was macerated using 96% ethanol to obtain the concentrated extract. Phytochemical screening was performed to identify secondary metabolites, including flavonoids, alkaloids, saponins, tannins, and steroids. Extract preparation was carried out at the Pharmacy Laboratory of Almarisah Madani University, Makassar.

### Antibacterial Assay

Antibacterial activity was evaluated using the disk diffusion method on Mueller Hinton Agar. Paper disks were impregnated with extract concentrations ranging from 5% to 100%. Chloramphenicol was used as a positive control, while sterile distilled water served as a negative control. A bacterial suspension of Salmonella typhi was standardized to 0.5 McFarland and incubated at 37°C for 24–48 hours. The diameter of inhibition zones was measured to determine antibacterial activity. All microbiological testing was conducted at the Hasanuddin University Laboratory, Makassar.

### Data Analysis

Inhibition zone diameters were analyzed descriptively based on their mean values to evaluate antibacterial effectiveness.

**RESULT**

The present study was conducted in vitro in two stages, namely a phytochemical screening to identify the active compounds contained in ciplukan leaf extract and an antibacterial sensitivity test using the disc diffusion method to evaluate its inhibitory activity against bacterial growth. The results of the phytochemical test are presented in Table 1.

Table 1. Phytochemical Test Results

| No | Compound | Results |
|---|---|---|
| 1 | Flavonoid | + |
| 2 | Alkaloid | + |
| 3 | Tanin | + |
| 4 | Saponin | + |
| 5 | Steroid | - |

Based on Table 1, the phytochemical test results for ciplukan leaf extract show positive results for flavonoids (+), alkaloids (+), tannins (+), saponins (+), and negative results for steroids (-). Furthermore, the results of sensitivity tests of ciplukan leaf extract against *Salmonella typhi* at various concentrations are presented in Table 2.

Table 2. Average Plate Results for Day One and Day Two

| No | Concentration | First Day | Second Day |
|---|---|---|---|
| 1 | 5% | 18,67 mm | 17,77 mm |
| 2 | 10% | 17,70 mm | 21,13 mm |
| 3 | 30% | 21,03 mm | 22,33 mm |
| 4 | 40% | 25,00 mm | 21,83 mm |
| 5 | 80% | 23,27 mm | 15,47 mm |
| 6 | 100% | 29,90 mm | 17,57 mm |
| 7 | + | 32,92 mm | 37,70 mm |
| 8 | - | 12,68 mm | 13,70 mm |

Based on Table 2, it can be seen that the average values of the triplo plate on the first and second days correspond to each concentration. At a concentration of 5%, the values were 18.67 mm on the first day and 17.77 mm on the second day. At a concentration of 10%, the values were 17.70 mm on the first day and 21.13 mm on the second day. At a concentration of 30%, the values were 21.03 mm on the first day and 22.33 mm on the second day. At a concentration of 40%, the first day was 25.00 mm and the second day was 21.83 mm. At a concentration of 80%, the first day was 23.27 mm and the second day was 15.47 mm. At a concentration of 100%, the first day was 29.90 mm and the second day was 17.57 mm. In the positive control, the measurement on the first day was 32.92 mm and on the second day 37.70 mm. In the negative control, the measurement on the first day was 12.68 mm and on the second day 13.70 mm. From these results, the values were unstable between the first day, which had a high average value, and the second day, which had a low average value, and vice versa.

Next, to determine the consistency of inhibitory power over time, statistical analysis was performed using a paired t-test. The results of the analysis are presented in Table 3.

Tabel 3. Hasil perbandingan hari pertama dan kedua menggunakan SPSS

| Statistical Test | Significance Value (p) | Description |
|---|---|---|
| Paired t-test | 0,434 | Not significantly different |

Table 3 shows a result of 0.434 > 0.05, which means there is no difference in the inhibitory power values between the first and second days.

## DISCUSSION

### Phytochemical screening

Phytochemical screening revealed that ciplukan leaf extract (Physalis angulata L.) contains flavonoids, alkaloids, tannins, and saponins. These secondary metabolites are widely reported to possess antibacterial properties through different mechanisms. Flavonoids and tannins can damage bacterial cell walls and inactivate proteins, whereas alkaloids and saponins disrupt metabolic processes and increase membrane permeability, leading to bacterial cell dysfunction[9,7]. Similar findings have also been reported, confirming that Physalis angulata L. leaf extract contains bioactive compounds responsible for its antibacterial activity[8].

### Antibacterial Activity

The sensitivity test indicated that the inhibitory effect increased with extract concentration and reached an optimum response at 40%, while the minimum inhibitory effect was observed at 5%. This trend suggests a synergistic interaction among secondary metabolites in suppressing bacterial growth. However, a decrease in activity was observed at higher concentrations, which may be attributed to solvent saturation or degradation of active compounds in the medium, resulting in reduced diffusion or bioavailability[14]. These findings support the concept that increasing concentration does not always correspond to proportional enhancement of antibacterial activity.

### Statistical Analysis

Paired t-test analysis showed no significant difference in antibacterial activity between the first and second observation days, indicating that the inhibitory effect of the extract was relatively stable during the experimental period. This result is consistent with the study reported previously[2].

Overall, these findings highlight the potential of ciplukan leaf extract as a natural antibacterial source against Salmonella typhi. This potential is further supported by previous reports[1]. In addition, other studies have shown that endophytic fungi associated with Physalis angulata L., such as Fusarium sp. and Aspergillus sp., exhibited antibacterial and antioxidant activities, indicating that the Physalis angulata ecosystem is a rich source of bioactive compounds[15]. This finding indirectly supports the antibacterial potential of ciplukan as a medicinal plant.

## CONCLUSION

The results of this study demonstrated that ciplukan leaf extract (Physalis angulata L.) exhibits antibacterial activity against Salmonella typhi. This activity is attributed to the presence of secondary metabolites, including flavonoids, alkaloids, tannins, and saponins, which contribute to bacterial growth suppression. All tested extract concentrations showed inhibitory effects, although the magnitude of activity

varied among concentrations. Statistical analysis indicated no significant difference in antibacterial activity across observation times, suggesting that the inhibitory effect was relatively stable.These findings indicate that ciplukan leaf extract has potential as a candidate for phytopharmaceutical development as a natural antibacterial agent against Salmonella typhi. Nevertheless, further studies involving toxicity assessment and in vivo evaluation are required to confirm its safety, efficacy, and clinical applicability.

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