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# Antibacterial Effects of Extraction and Fractionation of *Andrographis paniculata* and *Curcuma domestica* and Combination on the Growth of *Salmonella typhi*

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## Abstract

Typhoid fever is a health issue in several developing countries, including Indonesia. The primary standard treatment involves antibiotics, but improper and indiscriminate use of antibiotics leads to bacterial resistance. Due to the increasing antibiotic resistance, alternative approaches, such as using natural substances, are needed. Among these substances are *Andrographis paniculata* (Burm.f.) Wall. ex Nees and *Curcuma domestica* Valeton. This study was aimed at investigating the antibacterial activity of extracts and fractions of *A. paniculata* and *C. domestica* at various concentrations and their combinations by measuring the inhibition zones against *Salmonella typhi*. This experimental study is purely *in vitro*, using the maceration extraction method with 70% ethanol. The fractionation process employs liquid-liquid extraction using n-hexane, ethyl acetate, and water solvents. Antibacterial activity was assessed by the size of the inhibition zone from each plant at various concentrations and their combinations using the disk diffusion method. The results showed that inhibition zones were formed from the extracts and fractions of both plants at all concentrations and combinations, but no significant ($P > 0.05$) differences were found. The largest inhibition zone was observed in the n-hexane fraction at a 40% concentration for *A. paniculata* and in the ethyl acetate fraction at 20% for *C. domestica*. A 1:1 combination exhibited the largest inhibition zone compared to other combinations or single preparations.

**Keywords:** Plant extracts; n-hexane; Inhibition zone; *Salmonella typhi*

## Introduction

Typhoid fever is caused by the bacterium *Salmonella typhi*, transmitted through the fecal-oral route with an incubation period of 7 to 14 days, characterized by fever and digestive system disorders (Paul and Bandyopadhyay 2017). Environmental improvements and the use of antibiotics have reduced the morbidity and mortality rates of typhoid fever in developed countries. However, this disease remains a significant health issue in many developing countries, including Indonesia, with 9 million cases and 110,000 deaths reported annually. The risk of typhoid fever is particularly high in populations with poor access to clean water and sanitation, with the highest risk in children (WHO 2023). In Indonesia, an average incidence rate is 500 cases per 100,000 population, with a mortality rate of 0.6-5% (Balitbangkes 2018).

The standard treatment for eradicating *S. typhi* is fluoroquinolone antibiotics, although Chloramphenicol is still widely used (Parry *et al.* 2002). Indiscriminate and improper use of antibiotics can lead to bacterial resistance. Antimicrobial resistance in *S. typhi* isolates is a significant problem in Asian and African countries. Among all *S. typhi* isolates, 25.9% were resistant to Chloramphenicol, 38.8% to Ampicillin, 61.2% to Amoxicillin, 37.9% to Trimethoprim-sulfamethoxazole, 64.7% to Nalidixic acid, 15.0% to Ciprofloxacin, 45.0% to Ceftriaxone, 45% to Azithromycin and 35.5% are multidrug-resistant (Marchello *et al.* 2020). Given the increasing resistance of *S. typhi* to antibiotics, alternative treatments using natural source such as *Andrographis paniculata* (Burm.f.) Wall. ex Nees) and *Curcuma domestica* Valeton can be greatly effective.

Species of *Andrgraphis genus* contain several compounds, including andrographolide, diterpenoids, and flavonoids as common phytochemicals (Hapuarachchi *et al.* 2013). It has various pharmacological activities such as

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antihyperglycemic, antihyperlipidemic, antioxidant, hepatoprotective, antimicrobial and anti-inflammatory (Akbar 2011). A study showed that *A. paniculata* fractions exhibited antibacterial effects against Staphylococcus aureus and *Escherichia coli* (Febria *et al.* 2022). Likewise, *C. domestica* is claimed to have various therapeutic effects, containing curcuminoids, glycosides, terpenoids and flavonoids. It has antitoxic, anticancer, antibacterial, anti-inflammatory, and antioxidant properties (Verma *et al.* 2018). Studies show that ethanol extracts of *C. domestica* have inhibitory zones against several bacteria, including *S. epidermis, S. flexneri, P. aeruginosa, Lactobacillus,* and *V. cholerae* (Oghenejobo 2017). *In vitro* studies also show that extracts of *A. paniculata* and *C. domestica* and their combination can inhibit the growth of *E. coli* (Putu *et al.* 2014).

Based on this background, it is necessary to delve into the antibacterial effects of *A. paniculata* and *C. domestica* extracts and fractions, as well as their combination, against the growth of *S. typhi* in culture media.

## Materials and Methods

### Experimental details

This *in vitro* experimental study was conducted using a post-test-only control group design. The purpose was to measure the inhibition zones formed from the 70% ethanol extract, n-hexane fraction, ethyl acetate fraction, and water fraction of *A. paniculata* and *C. domestica*, as well as their combinations, against *S. typhi*. The extraction process was conducted at Balitro Bogor, fraction preparation at the Faculty of Pharmacy, Universitas Indonesia, and bacterial culture and testing at the laboratory of the Faculty of Medicine, Universitas Muhammadiyah Prof. Dr. Hamka, in August 2024.

### Plant material source

The plants used in this study were *A. paniculata* and *C. domestica*. For *A. paniculata*, all components including leaves, flowers and stems were used, for *C. domestica*, while the rhizome were also utilized. *A. paniculata* (aged 3-4 months after planting, harvested during flowering stage) and *C. domestica* rhizomes (aged 8-10 months after planting, harvested when the leaves had turned yellow and dried) were collected from the Tawang Mangu area in Central Java in July 2024. Both plants were washed with running water to remove dirt until clean, chopped and cut into small, thin pieces, and dried using an oven at 40°C until completely dry. Once dried, the plants were stored in airtight containers at room temperature until extraction.

### Extraction process

The dried plants were ground using a blender and sieved with a 60-mesh sieve to obtain a fine powder. The extraction used the maceration method with 70% ethanol as the solvent. One kg of each plant powder was macerated with 5 L of 70% ethanol in dark containers for 72 h at room temperature. The mixture was shaken every 24 h to enhance the interaction between the solvent and plant material. After 72 h, the mixture was filtered using a filter cloth and then Whatman No. 1 filter paper. The filtrate was evaporated using a rotary evaporator at 40°C to obtain a thick extract. The extract was then stored in glass bottles at 4°C for further fractionation and antibacterial testing.

### Fractionation process

A 70% ethanol extract fractionation was performed using liquid-liquid extraction (LLE) with n-hexane, ethyl acetate, and water solvents. A 10 g of the 70% ethanol extract was dissolved in 100 mL of distilled water. The solution was mixed with 100 mL of n-hexane in a separatory funnel, gently shaken, and allowed to settle for phase separation into the n-hexane and aqueous layers. The n-hexane layer was collected, and the process was repeated until the solution became clear. The n-hexane layer was evaporated, leaving the n-hexane fraction. Following the same procedure, the aqueous layer was further fractionated with 100 mL of ethyl acetate. The ethyl acetate layer was collected and evaporated to obtain the ethyl acetate fraction. The remaining aqueous layer was evaporated in a water bath until it became concentrated, forming the water fraction. Each fraction was weighed to calculate the yield relative to the initial weight. The dried fractions were stored at 4°C for antibacterial testing.

### Bacterial culture and inoculum preparation

The bacterium used in this study was *S. typhi*, a Gram-negative bacterium responsible for typhoid fever. The bacterial strain was obtained from the Microbiology Laboratory of the Faculty of Medicine, Universitas Muhammadiyah Prof. Dr. Hamka, and stored in a glycerol solution. *S. typhi* from glycerol was cultured on nutrient agar (NA) media and incubated for 24 h. A portion of the colonies was taken using a previously heated inoculation loop, and the colonies were suspended in 0.9% NaCl saline and homogenized using a vortex mixer. The turbidity of the bacterial suspension was compared to a 0.5 McFarland standard solution. Once homogenized, 0.15 mL of the suspension containing *S. typhi* colonies was transferred to NA media using a pipette and evenly spread using a sterile glass spreader. The plates were incubated at 37°C for 24 h after the test discs were placed on the media.

### Test solution preparation

A 1 g of the 70% ethanol extract, n-hexane fraction, ethyl acetate fraction, and water fraction from each plant was

diluted by adding 1 mL of DMSO and homogenized to make a 100% solution. Then, 800 µL of the 100% solution was taken into a cryotube and diluted by adding 200 µL of DMSO, making an 80% solution. From the 80% solution, 500 µL was taken into a cryotube and diluted with 500 µL of DMSO to make a 40% solution. Finally, 500 µL of the 40% solution was taken and diluted with 500 µL of DMSO to create a 20% solution.

**Antibacterial activity testing**

The antibacterial activity test was conducted using the disc diffusion method. Sterile paper discs with a diameter of 6 mm were soaked in 40 µL of the 70% ethanol extract, n-hexane fraction, ethyl acetate fraction, and water fraction of *A. paniculata* and *C. domestica* at concentrations of 100, 80, 40 and 20% for 20 to 30 min. The soaked discs were then placed on the surface of NA media that were inoculated with *S. typhi*. Chloramphenicol (30 µg/mL) was used as a positive control, and DMSO was used as a negative control. Each treatment was performed in triplicate. After disc placement, the petri dishes were incubated at 37°C for 24 h. The inhibition zones were measured using callipers and expressed in mm.

**Combination of antibacterial activity test**

The inhibition zones measured from the ethanol extract, n-hexane fraction, ethyl acetate fraction and water fraction of *A. paniculata* and *C. domestica* at the highest concentrations were then combined in various ratios: one part of *A. paniculata* to one part *C. domestica* (1:1), one part *A. paniculata* to two parts *C. domestica* (1:2), and two parts *A. paniculata* to one part *C. domestica* (2:1). These combinations were tested for antibacterial activity using the disc diffusion method. The inhibition zones from these combinations were compared to determine which combination produced the largest zone and whether there were statistically significant differences.

**Data analysis**

The inhibition zone data were analyzed using SPSS version 25. Normality tests were conducted first to ensure that the data followed a normal distribution. A One-way ANOVA and post hoc test were performed if the data were normally distributed to determine the differences between the treatments. If the data were not normally distributed, a data normalization test was performed, and if the data remained non-normal, the Kruskal-Wallis test was conducted. Statistical significance was determined at $P < 0.05$. The results were presented in tables for easier interpretation.

## Results

Table 1 shows the inhibition zones of bacterial growth with mean values and standard deviations (SD) at various concentrations (100, 80, 40 and 20%) for each preparation using the disc diffusion method. The inhibition zone reflects the antibacterial effectiveness of the tested compounds, the larger the zone, more effective the compound. Both medicinal plants demonstrated antibacterial activity against *S. typhi* at all preparations and concentrations, although the effect was significantly lower than that of Chloramphenicol (Table 1). The differences in inhibition zones among all preparations and concentrations were not statistically significant ($P > 0.05$).

For *A. paniculata*, the largest inhibition zone across all preparations and concentrations was found in the n-hexane fraction at a 40% concentration (b/v), with a zone of 8.7 ± 0.6 mm. However, at 100% (b/v) and 20% (b/v) concentrations, the largest inhibition zones were seen with the 70% ethanol extract, measuring 7.8 ± 0.6 mm and 8.5 ± 0.5 mm, respectively. At 80% (b/v) concentration, the largest zone was seen in the water fraction, measuring 8.2 ± 0.3 mm (Table 1). For *C. domestica*, the largest inhibition zone across all preparations and concentrations was observed in the ethyl acetate fraction at a 20% concentration (b/v), measuring 9.3 ± 0.6 mm. At 100, 80 and 40% concentrations, the largest inhibition zones were observed with the 70% ethanol extract, measuring 8.5 ± 1.3 mm, 8.5 ± 0.5 mm, and 8.2 ± 0.6 mm, respectively (Table 1).

Table 2 shows the antibacterial activity of *A. paniculata* and *C. domestica* combinations against *S. typhi*. Results revealed that combining both herbal plants resulted in larger inhibition zones than each plant used individually. However, the differences in inhibition zones between the three combinations were not statistically significant ($P > 0.05$). The results are displayed for the combination of 1 part *A. paniculata* with 1 part *C. domestica* (1:1), 1 part *A. paniculata* with two parts *C. domestica* (1:2), and two parts *A. paniculata* with 1 part *C. domestica* (2:1). The preparations and concentrations used were the ones that produced the highest inhibition zones for each plant: the 40% n-hexane fraction for *A. paniculata* and the 20% ethyl acetate fraction for *C. domestica*. The results showed that the 1:1 combination produced the largest inhibition zone (11.0 ± 0.5 mm), indicating a synergistic effect in inhibiting *S. typhi* growth.

## Discussion

Data indicated that *A. paniculata* and *C. domestica* possess substantial antibacterial activity against *S. typhi*, although their effectiveness was significantly lower than that of the positive control (Chloramphenicol). This suggests that the extracts and fractions of these plants contain bioactive compounds capable of inhibiting *S. typhi* growth, albeit to a lesser extent than conventional antibiotics. This aligns with previous studies demonstrating the antibacterial potential of these plants

**Table 1:** Antibacterial activity of *A. paniculata* and *C. domestica* in various preparations and concentrations against *S. typhi* by disc diffusion method

| Herbal | Herbal preparations | Inhibition zone in concentration[a] (mm) | | | |
|---|---|---|---|---|---|
| | | 100% (b/v) | 80% (b/v) | 40% (b/v) | 20% (b/v) |
| *A. paniculata* | Ethanol extract 70% | 7.8±0.6 | 8.0±0.0 | 8.0±0.5 | 8.5±0.5 |
| | n-hexane fraction | 7.0±0.9 | 7.0±0.9 | 8.7±0.6 | 7.7±0.8 |
| | Ethyl acetate fraction | 6.8±0.6 | 8.0±0.0 | 7.3±0.6 | 8.0±0.9 |
| | Water fraction | 7.3±0.7 | 8.2±0.3 | 7.7±1.2 | 7.0±0.9 |
| | Positive control [b] | 29.0±1.7 | 29.0±1.7 | 29.3±1.2 | 27.3±3.1 |
| | Negative control [c] | 0.0 | 0.0 | 0.0 | 0.0 |
| *C. domestica* | Ethanol extract 70% | 8.5±1.3 | 8.5±0.5 | 8.2±0.6 | 8.7±0.3 |
| | n-Hexane fraction | 7.7±0.6 | 7.5±0.0 | 8.0±1.0 | 9.0±1.0 |
| | Ethyl acetate fraction | 7.5±1.3 | 7.3±0.8 | 8.2±0.3 | 9.3±0.6 |
| | Water fraction | 7.3±0.6 | 6.8±0.3 | 7.0±0.5 | 7.3±1.0 |
| | Positive control [b] | 30.0±0.0 | 30.0±0.0 | 30.0±0.0 | 31.3±3.8 |
| | Negative control [c] | 0.0 | 0.0 | 0.0 | 0.0 |

[a]Data showing the mean ± SD. The experiments were carried out in triplicate
[b]Chloramphenicol
[c]DMSO

**Table 2:** Antibacterial activity of *A. paniculata* and *C. domestica* combination which has the highest zone of inhibition against *S. typhi* by disc diffusion method

| Herbal combinations | Inhibition zone of herbal combinations with various comparisons[a] (mm) | | |
|---|---|---|---|
| | 1 (A) : 1 (C) | 1 (A) : 2 (C) | 2 (A) : 1 (C) |
| *A. paniculata* (A) and *C. domestica* (C) | 11.0±0.5 | 10.2±0.3 | 9.3±1.0 |
| Positive control [b] | 28.3±0.6 | 28.3±0.6 | 28.3±0.6 |
| Negative control [c] | 0.0 | 0.0 | 0.0 |

[a]The data showed the mean ± Std. Deviation. The experiments were carried out in triplicate
[b]Chloramphenicol
[c]DMSO

(Nasution *et al*. 2019; Setiyawati *et al*. 2022).

For *A. paniculata*, the largest inhibition zone was found in the n-hexane fraction. The components soluble in n-hexane are non-polar compounds, such as essential oils (andrographin, terpenoid derivatives), steroids (phytosterols), and fats. These components contribute significantly to antibacterial activity, which showed that compounds soluble in n-hexane (alkaloids, phenols, and steroids) exhibit inhibition zones against *S. typhi* (Abraham 2019). The antibacterial mechanism of terpenoid compounds soluble in n-hexane may include cell membrane disruption by altering ion channels ($Na^+$, $K^+$, $Ca^{2+}$ or $Cl^-$), increasing permeability, and leading to the release of vital intracellular components, as well as inhibiting target enzymes (Guimarães et al. 2019).

In the case of *C. domestica*, the largest inhibition zone was observed in the ethyl acetate fraction. The components soluble in ethyl acetate are semi-polar compounds, including curcuminoids (curcumin, demethoxycurcumin, bisdemethoxycurcumin), essential oils (turmerone, zingiberene), and xanthorrhizol (Naz *et al*. 2011). One antibacterial mechanism of *C. domestica* compounds is by inhibiting DNA gyrase. DNA gyrase plays a role in regulating DNA in cells. This enzyme requires energy from ATP hydrolysis to catalyze negative supercoiling. The B subunit of DNA gyrase carries out ATP hydrolysis. Therefore, when DNA gyrase is inhibited by compounds in *C. domestica*, DNA synthesis is disrupted, ultimately causing bacterial cell death (Herdiawan *et al.* 2018).

The differences in antibacterial activity observed across various preparations (70% ethanol extract, n-hexane fraction, ethyl acetate fraction, and water fraction) and concentrations can be attributed to several factors, including phytochemical composition, interactions between compounds, solubility and diffusion, as well as the phenomenon of hormesis. In terms of phytochemical composition, each preparation contains different mixtures of compounds that influence its antibacterial activity. For instance, non-polar fractions (such as n-hexane) may be richer in terpenoid compounds. In contrast, polar fractions (like water) will likely contain higher amounts of glycosides and flavonoids (Sasidharan *et al.* 2011). Interactions between compounds can also vary at different concentrations, leading to synergistic, additive, or antagonistic effects (Chou 2010). The solubility of compounds in agar medium and their ability to diffuse influence the size of the inhibition zones observed (Balouiri *et al.* 2016). The phenomenon of hormesis, where lower doses of certain compounds produce more beneficial effects than higher doses, further explains why some preparations exhibit higher antibacterial activity at lower concentrations (Calabrese and Mattson 2017).

Results further showed that combining *A. paniculata* and *C. domestica* resulted in larger inhibition zones than using each plant individually. This synergistic effect can be

explained on the basis of several mechanisms. First, dual targeting, where compounds from both plants act on different bacterial defense mechanisms, enhances overall effectiveness (Wagner 2011). Second, increased bacterial membrane permeability, where a compound from one plant increases membrane permeability, allowing compounds from the other plant to enter the bacterial cell more easily (Hemaiswarya *et al.* 2008). Third, inhibition of resistance mechanisms, where compounds from one plant may reduce bacterial resistance, thereby enhancing the antibacterial effectiveness of compounds from the other plant (Li *et al*. 2023). Fourth, modulation of quorum sensing, where some phytochemicals have been shown to disrupt bacterial cell-to-cell communication (quorum sensing), making the bacteria more vulnerable to other antibacterial compounds (Kalia 2013).

This research provides a basis for developing new plant-based antibacterial agents to combat *S. typhi*. However, further research is needed to optimize, understand, and ultimately develop safe and effective antibacterial therapies based on combinations of *A. paniculata* and *C. domestica*. Standardization of preparation processes and a deeper understanding of the interactions between these compounds will be crucial for developing herbal medicines. A multidisciplinary approach involving phytochemistry, microbiology, pharmacology, and clinical research will be essential in realizing these herbal combinations' potential as alternatives to conventional antibiotics for treating *S. typhi* infections.

## Conclusion

Extraction and fractionation of *A. paniculata* and *C. domestica* at all concentrations and their combinations inhibited the growth of *S. typhi*. The 40% n-hexane fraction of *A. paniculata* and the 20% ethyl acetate fraction of *C. domestica* produced the largest inhibition zones. The combination of *A. paniculata* and *C. domestica* produced even larger inhibition zone than using *A. paniculata* or *C. domestica* alone. The 1:1 combination of *A. paniculata* and *C. domestica* produced the largest inhibition zone compared to other combinations.

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## Author Contributions

All authors contributed equally to this work and consented to its publication.

## Conflict of Interest

All authors declare no conflict of interest.

## Data Availability

The data presented in this study are available to the corresponding author upon reasonable request.

## Ethics Approval

Ethical approval applies to this manuscript.

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