

## Antibacterial Effect of *Curcuma zedoaria* Extract on *Bacillus cereus* and *Staphylococcus epidermidis*

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### Abstract

**Background:** White turmeric (*Curcuma zedoaria*), a plant used traditionally for medicine, is easily obtained at a relatively cheap price in Indonesia. White turmeric contains alkaloids, phenols, saponins, glycosides, steroids, terpenoids, and other compounds; and these compounds have shown antimicrobials, antifungal, anticancer, antiallergic, antioxidant, and analgesic effects. The aim of this study was to determine the antibacterial effect of white turmeric (*Curcuma zedoaria*) rhizome extract on the growth of *Bacillus cereus* and *Staphylococcus epidermidis* bacteria.

**Methods:** This was an experimental study with a Post-test Only Control Group Design. It was carried out using the disc diffusion method with six treatments, consisting of negative control (aquadest), positive control (ciprofloxacin), and the extract of white turmeric rhizome with various concentrations. Kruskal Wallis test and one way ANOVA were used to analyze the data. The results showed a statistically significant value smaller than  $\alpha$  ( $0.021 < 0.05$ ), and the One Way ANOVA test had a statistically significant value ( $p$ ) less than  $\alpha$  ( $0.000 < 0.05$ ).

**Results:** The results of this research showed that the extract of white turmeric (*Curcuma zedoaria*) inhibited the growth of *Bacillus cereus* and *Staphylococcus epidermidis* bacteria at concentrations of 25%, 50%, 75%, and 100% as shown by the diameter inhibitory zone between 10-20 mm, compared to the positive control which had  $>20$ mm.

**Conclusions:** *Curcuma zedoaria* has an antibacterial effect against *Bacillus cereus* and *Staphylococcus epidermidis*. Further study is needed, exploring the effectivity of white turmeric in the animal models.

**Keywords:** Antibacterial, *Bacillus cereus*, rhizome extract, *Staphylococcus epidermidis*, white turmeric

### Introduction

Indonesia is a developing country, located in a tropical area where various infections occur. Therefore, its population is susceptible to various infectious diseases, caused by virus, parasite or bacteria.<sup>1</sup> One of the most prevalent disease in Indonesia is diarrhea, that is associated with a high case fatality rate (CFR).<sup>2</sup> The main cause of diarrhea are foodborne and waterborne pathogens, among others *Bacillus*, *Salmonella spp.*, and *Campylobacter jejuni*.<sup>3</sup> *Bacillus* is a genus of gram-positive, rod-shaped aerobes. *Bacillus cereus*, for example, can be found in food and can release enterotoxins that can cause food poisoning. The main clinical symptoms are vomiting and diarrhea with abdominal pain.<sup>4</sup>

Another interesting infection prevalent in Indonesia is a skin infection that causes acne. Acne often occurs on the surface of the skin in the face, neck, chest, and back. Acne appears when sebaceous glands are too active, and the pores are blocked.<sup>5</sup> In Indonesia about 95–100% of men and around 83–85% of women at the age of 16–17 years have acne.<sup>6</sup> Acne can also be caused by bacteria *Staphylococcus epidermidis*. This bacterium is part of normal flora in the skin, but it can be harmful elsewhere. If *Staphylococcus epidermidis* develops in the sebaceous glands, and the gland is blocked, it will produce substances that can irritate in the surrounding area, then swelling. Rupture of the glands may cause inflammation to the surrounding skin tissue.<sup>7</sup>

Antibiotics are usually used to treat

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infections. Incorrect use of antibiotics may result in drug resistance for pathogenic microbes, and the emergence of resistant microbes is a major cause of treatment failure. Therefore, an alternative treatment such as medicinal plants that can be utilized its active antimicrobial content.<sup>8</sup> Indonesia has high biodiversity in plants, which means that the country has various potential plants that can be developed for medicinal uses. One example of a medicinal plant that is also known as spices is *Curcuma zedoaria*. This plant can be found in many countries in Asia. In Indonesia, *Curcuma zedoaria* can be found in Mount Dempo in Sumatra, in East Java teak forests, and many other areas.<sup>9</sup> *Curcuma zedoaria* contains compounds such as alkaloids, phenols, saponins, glycosides, steroids, terpenoids, which has antimicrobials, antifungal, anticancer, antiallergic, antioxidant, and analgesic properties.<sup>10</sup>

This study aimed to determine the antibacterial effect of *Curcuma zedoaria* extract on the growth of gram-positive bacteria *Bacillus cereus* and *Staphylococcus epidermidis*, two types of bacteria often occur in Indonesia

### Methods

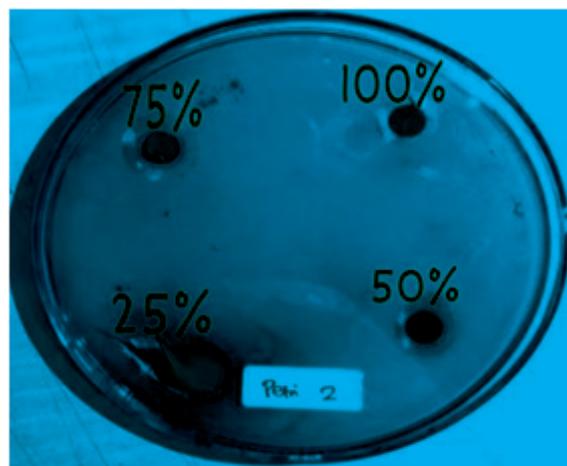
This experiment was a Posttest Only Control Group Experimental Design. This research was conducted in the Pharmacy and Toxicology Lab of the Faculty of Pharmacy, University of North Sumatra and the Microbiology Laboratory in

**Table 1 The Concentration of White Turmeric Rhizome Extract**

| White turmeric extract (gr) | Final Volume (mL) | Concentration (%) |
|-----------------------------|-------------------|-------------------|
| 2.5                         | 10                | 25                |
| 5.0                         | 10                | 50                |
| 7.5                         | 10                | 75                |
| 10.0                        | 10                | 100               |

**Table 2 Classification of Antibacterial Activity**

| Average Inhibition Zone Diameter | Category    |
|----------------------------------|-------------|
| >20 mm                           | Very Severe |
| 10–20 mm                         | Severe      |
| 5–10 mm                          | Moderate    |
| <5 mm                            | Weak        |



**Figure 1 The Inhibitory Zones Diameter Measured using Calipers after Putting 25%, 50%, 75%, and 100% Concentrations of *Curcuma zedoaria***

the Faculty of Medicine, University of Prima Indonesia in July–August 2019. The protocol research was granted ethical clearance by The Ethical Research Commission of the Universitas Prima Indonesia no. 017/KEPK/UNPRI/IX/2019.

The sample used in this study came from the white turmeric rhizome plant in the form of dried *Simplicia* powder obtained from UPT Materia Medica Batu, East Java. *Curcuma zedoaria* was washed and dried in a drying cabinet. After drying, *Curcuma zedoaria* was blended in a blender. The extract was created by the maceration method using 96% ethanol which has been distilled at ten times the weight of *Curcuma zedoaria*, 1,000 grams of *Curcuma zedoaria* powder was put into the container then 96% ethanol was added as many as 75 parts (7.5 mL). The powder was soaked for 5 days while being stirred frequently and being protected from light, and then it was filtered and cleaned. The filtered *Curcuma zedoaria* was soaked again with 96% (2.5 mL) ethanol for 2 days and then filtered again. The filtrate was combined then concentrated with a rotary evaporator until it reached the preferred fluidity.

The concentration of *Curcuma zedoaria* extract used in this study was 25%, 50%, 75%, and 100% solved in aquabidest. The weight of the solute (extract) used was calculated using the following formula:

$$\text{Presentation: } \frac{\text{solvent used}}{\text{volume of solution}} \times 100\%$$

**Table 3 The Inhibitory Zone Diameter of *Bacillus cereus***

| Experiment no. | Concentrations |       |       |       |
|----------------|----------------|-------|-------|-------|
|                | 25%            | 50%   | 75%   | 100%  |
| I              | 9.2            | 11.6  | 12.0  | 18.8  |
| II             | 10.5           | 12.7  | 14.2  | 19.2  |
| III            | 10.6           | 12.7  | 14.2  | 19.1  |
| Average        | 10.10          | 12.33 | 13.47 | 19.03 |
| Control +      | 29.6           |       |       |       |
| Control -      | 0              |       |       |       |

Note: Inhibitory zone diameter was measured in mm

About 6.8 gr Mueller Hinton Agar (MHA) was dissolved with 250 mL of distilled water into an Erlenmeyer tube, then placed on an autoclave hotplate with a temperature of 121°C for 15 minutes, then 25 mL of MHA was poured into the petri dish.<sup>13</sup> Nutrient broth (NB) powder 1 gr was put into a 500 mL beaker glass; then 150 mL of distilled water was added. Then, it was heated on a hotplate until all the compounds were dissolved and homogeneous. After that, the solution was put into an Erlenmeyer tube and then was put in the autoclave at 121°C for 45 minutes. Furthermore, it was put in a test tube and then in the refrigerator before use.<sup>14</sup>

To prepare the bacterial suspension, 1 ose bacteria were inoculated into a 25 mL NB. Bacterial culture in NB media was rocked using a shaker with a rotation speed of 120 rpm for 24 hours. The Claudy bacterial culture indicated the growth of bacteria.<sup>15</sup>

To measure the antibacterial effect the Kirby-Bauer method (disc diffusion) was used. Inhibitory effect of *Curcuma zedoaria* extract on the growth of *Bacillus cereus* and *Staphylococcus epidermidis* followed; the Mueller Hinton Agar (MHA) used 8 petri

dishes and 28 whatman paper discs. Whatman paper was made with a perforator so it was shaped into a 6 mm disk. Before the bacteria were planted on the MHA, the front of the petri dish was divided into four and labeled with stickers.<sup>16</sup> On the surface of the MHA, *Bacillus cereus* and *Staphylococcus epidermidis* were cultured using sterile cotton swabs on different petri dishes. The whatman paper discs that were soaked for  $\pm 15$  minutes with different *Curcuma zedoaria* extract were joined to the two bacterial cultures.

As a positive control, 2 Ciprofloxacin discs were used and as a negative control whatman paper discs were used, immersed in sterile distilled water for  $\pm 15$  minutes. The paper discs were placed on the surface of the MHA with the help of sterile tweezers with a little pressure so that whatman paper discs adhered well. Paper discs were incubated at 37°C for 48 hours. Inhibitory zones or clear zones that formed on the paper discs were measured with the classification of an antibacterial activity as shown in Table 2. The average of inhibition zone diameter was categorized as very severe when the diameter  $>20$  mm; severe for diameter 10–20 mm, moderate for

**Table 4 The Inhibitory Zone Diameter of *Staphylococcus epidermidis***

| Experiment no. | Concentrations |       |      |       |
|----------------|----------------|-------|------|-------|
|                | 25%            | 50%   | 75%  | 100%  |
| I              | 11.5           | 12.3  | 16.3 | 20.3  |
| II             | 11.3           | 14    | 15.2 | 19.6  |
| III            | 12.1           | 13.4  | 16.5 | 20.2  |
| Average        | 11.63          | 13.23 | 16.  | 20.03 |
| Control +      | 27.7           |       |      |       |
| Control -      | 0              |       |      |       |

Note: Inhibitory zone diameter was measured in mm

diameter 5–10 mm, and weak for diameter <5 mm.

The antibacterial effectiveness of *Curcuma zedoaria* extract against *Bacillus cereus* bacteria was observed using a sensitivity test indicated by the presence of inhibition zones or clear zones around disc paper. The inhibition zone diameter was measured using calipers after putting 25%, 50%, 75%, and 100% concentrations of *Curcuma zedoaria* as shown in Figure 1.

## Results

The diameter of inhibition zone of *Bacillus cereus* was examined in triplo, measured using calipers after putting 25%, 50%, 75%, and 100% concentrations of *Curcuma zedoaria*, and the average of diameter was 10.10 mm, 12.33 mm, 13.47 mm, and 19.3 mm, respectively. The positive control showed a strong inhibitory zone (29.6 mm) that was more than 20 mm, and the negative control was confirmed to have no inhibitory zone (Table 3).

The antibacterial effectiveness of *Curcuma zedoaria* extract against *Staphylococcus epidermidis* was depicted in Table 4. *Curcuma zedoaria* had an antibacterial property against the bacteria *Staphylococcus epidermidis* at concentrations of 25%, 50%, 75%, and 100%, with the average inhibitory zone was 11.63 mm, 13.23 mm, 16.00 mm, and 20.03 mm, respectively. The positive control showed a strong inhibitory zone (27.7 mm) that was more than 20 mm, and the negative control was confirmed to have no inhibitory zone (Table 4).

## Discussion

*Curcuma zedoaria* in our study has confirmed to have antibacterial properties against *Bacillus cereus* and *Staphylococcus epidermidis*. The effectiveness of *Curcuma zedoaria* extract is indicated by the inhibitory zone. The average of the inhibitory zone of *Bacillus cereus* and *Staphylococcus epidermidis* at various concentrations is between 10–20 mm. Especially at 100% concentration, the diameter of inhibition zone is about 20 mm, indicating that *Curcuma zedoaria* is a strong inhibitor.<sup>21</sup>

*Bacillus cereus* and *Staphylococcus epidermidis* are gram-positive bacteria that have cell wall structures with more peptidoglycan and the hydrophilic nature that makes it more polar. *Curcuma zedoaria* can penetrate the cell wall more easily because it contains

polar flavonoid compounds.<sup>17</sup> Flavonoids contain phenol which can also disrupt and damage the microbial membrane.<sup>18</sup> This can damage the cytoplasmic membrane of which can inhibit bacterial growth and follow by bacterial death.<sup>19</sup> *Curcuma zedoaria* also contains triterpenoid compounds. This can reduce the permeability of the cell membranes of the bacteria by damaging the purines, and again this can inhibit bacterial growth or cause death.<sup>18</sup>

The limitation of this study is that the components of *Curcuma zedoaria* are not measured. Therefore, future study is needed to reveal the specific benefit of white tumeric extract.

To conclude, *Curcuma zedoaria* has an antibacterial effect against *Bacillus cereus* as well as *Staphylococcus epidermidis*. Further study is needed to explore the effectivity of *Curcuma zedoaria* or white turmeric in the animal models.

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