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RESEARCH ARTICLE

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Inhibitory Activity Test of White Turmeric *(Curcuma zedoaria)* Against *Escericia coli* Bacteria

Sharfina Maulidayanti[1*], Nidhal Mijwan Nafis[1], Ing Mayfa Br Situmorang[1], Ladyka Viola Armawan[2]

[1]Teknologi Laboratorium Medis, STIKes Prima Indonesia, Jawa Barat, Indonesia
[2]Fakultas Kesehatan Masyarakat Universitas Sriwijaya, Palembang, Sumatra Selatan, Indonesia

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***Correspondent:**
Sharfina Maulidayanti
Teknologi Laboratorium Medis, STIKes Prima Indonesia, Jawa Barat, Indonesia

Email:
ajalilfina@gmail.com

Abstract

Introduction: *Escherichia coli* bacteria are microorganisms that are usually found in the digestive tract in both animals and humans. Belonging to the *Enterobacteriaceae* family, *E. coli* has a cell size of between 2.0 to 6.0 micrometers by a diameter of 1.1 to 1.5 micrometers. These bacteria are straight rods and They can be found in singular, paired, or in short chains. E. **Objective**: This study aims to evaluate the inhibition of white turmeric extract *(Curcuma zedoaria)* against the pathogenic bacteria *Escherichia coli*, which is one of the causes of gastrointestinal infections or diarrheal diseases. **Method**: White turmeric extraction was carried out using the maceration method with a 96% ethanol solvent, which was then tested on *Escherichia coli* bacteria by the Disc diffusion method, using paper discs that had been soaked in various concentrations of onion extract (15%, 25%, 50%). The inhibition zones formed around the disc are observed and measured to determine the effectiveness of inhibition. **Result**: Based on research on the inhibitory effect of white turmeric extract on the growth of *Escherichia coli* bacteria, a concentration of 15% resulted in an average diameter of 7.1 mm (resistant), 25% resulted in a diameter of 9.3 mm (resistant), and 50% resulted in a diameter of 11.3 mm (resistant) on *Escherichia coli* bacteria. **Conclusion**: The conclusion of this study is that the more effective concentration to inhibit the growth of *Escherichia coli* bacteria is at 50% concentration.

Keywords: *Curcuma zedoaria*, *Escherichia coli*, Inhibition, White turmeric

Introduction

Antimicrobial compounds are substances capable of inhibiting the growth and activity of microorganisms. These compounds can either kill or prevent microbial development by damaging the cell wall, which may lead to lysis, or by interfering with the cell wall formation in growing cells. Additionally, antimicrobials can alter the permeability of the cytoplasmic membrane, disrupt nutrient transport, cause protein denaturation, and inhibit enzymatic functions within the cell—ultimately impairing the cell's metabolic system. Antibacterial or antimicrobial agents work through various mechanisms to kill or inhibit microbial activity. One way to address microbial infections is by using antibiotics. Antibiotics are chemical compounds produced synthetically or semi-synthetically and structurally resemble natural compounds capable of eliminating other microbes. They are commonly derived from microbes, especially fungi, and possess the ability to inhibit or destroy other types of microbes(1).

Infectious diseases are often treated with antibiotics—natural or synthetic compounds that can suppress or halt biochemical processes in organisms, particularly those related to bacterial infections. Therefore, alternative solutions are needed, one of which is utilizing antimicrobial active compounds from medicinal plants (2). *Escherichia coli (E. coli)* commonly found in the digestive tract of both animals and humans. Although *E. coli* normally resides in the intestines, it can cause diarreas (3).

E. coli is one of the most common causes of diarrheal diseases, which can spread easily through direct or indirect contact. Diarrhea is characterized by frequent watery stools—more than three times per day—and may be accompanied by blood or mucus. In Indonesia, diarrhea is considered endemic and is a potentially serious public health threat often accompanied by mortality (Ministry of Health, 2018). The highest incidence of diarrhea is found among toddlers aged 1–4 years (11.5%) and infants (9%). The age group over 75 also shows a high prevalence of 7.2%. Nationally, the prevalence of diarrhea among toddlers in Indonesia is 11%. The province with the lowest rate is Riau Islands (5.1%), while the highest is North Sumatra (14.2%). In West Java, the prevalence among toddlers reaches 12.8% (4). In Bekasi City, diarrhea cases have shown an increasing trend. In 2019, there were 27,170 cases of diarrhea, which was only 33.4% of the targeted case detection rate. Of these, 32.96% or 8,955 cases occurred in the toddler age group (West Java Provincial Health Office, 2019). Indonesia, as a tropical country, is rich in natural biodiversity. The country is estimated to have around 1,260 species of medicinal plants. Each plant contains specific secondary metabolites with unique functions. According to the World Health Organization (WHO), about 80% of the world's population relies on plant-based traditional medicines for prevention and treatment(3,5).

Turmeric (Curcuma) has long been an important part of Indonesian society, used in health, culinary, and cosmetics. One of its main active compounds is curcumin, known for its anti-tumor, antibacterial, and antioxidant properties. Curcumin, a natural yellow pigment, belongs to the polyphenol group and can cause protein denaturation and cell membrane damage. These phenolic compounds can destroy and penetrate bacterial cell walls and precipitate intracellular proteins (2). Turmeric offers various uses, such as in traditional medicine, cooking, food preservation, and coloring. The most utilized part is the rhizome, which is rich in bioactive antioxidant compounds. The yellow color of turmeric is due to curcuminoids, which have been proven to act as anti-rheumatic, anti-inflammatory, and anti-cancer agents (6). According to research by Lumbantobing et al. (2022), white turmeric *(Curcuma zedoaria)* contains secondary metabolites such as flavonoids and saponins with antibacterial potential. Their findings show that white turmeric can inhibit the growth of *E. coli* at concentrations of 25%, 50%, 75%, and 100%, with inhibition zone diameters of 7.00 mm, 8.99 mm, 11.78 mm, and 14.03 mm, respectively (7). Another study by Malahayati et al. (2021) reported that white turmeric extract inhibited *E. coli* with inhibition zone diameters of 3.25 mm, 3.55 mm, 3.94 mm, and 4.18 mm at concentrations of 0.05%, 0.1%, 0.15%, and 0.2%, respectively (6). This study aimed for further studying the inhibitory effect of white turmeric *(Curcuma zedoaria)* extract against Escherichia coli bacteria.

Methods

This study employed a descriptive experimental design to evaluate the antibacterial activity of white turmeric *(Curcuma zedoaria)* extract against *Escherichia coli*. The instruments used included standard microbiology equipment such as protective gear, glassware, micropipettes, autoclave, incubator, analytical balance, blender, and vortex mixer. The materials consisted of white turmeric extract, *E. col*i culture, 0.5 McFarland standard, sterile distilled water, paper discs, Nutrient Agar (NA) and Mueller-Hinton Agar (MHA), and 96% ethanol.

Sterilization of Tools and Materials

All tools were sterilized prior to use. Glassware was dry-sterilized in an oven at 170°C for 1 hour, while culture media were sterilized in an autoclave at 121°C for 15 minutes (8).

Plant Identification

Plant identification was performed taxonomically to confirm the species used. This process was carried out at the National Research and Innovation Agency (BRIN), Botanical Laboratory, Bogor–Cibinong, to ensure correct identification of *Curcuma zedoaria*.

Preparation of White Turmeric Extract

The extract was prepared using the maceration method. One kilogram of fresh rhizomes was cleaned, washed, sliced, and dried in an oven at ≤55°C, then ground into powder and sieved. Three hundred grams of powder were macerated in 2 L of 96% ethanol for 5 × 24 hours at room temperature, with occasional stirring. The mixture was filtered through Whatman filter paper, and the filtrate was concentrated using a rotary vacuum evaporator at 60–70°C to obtain a thick extract. Thick extract than prepared in three concentration 15%, 25% and 50% (9).

Phytochemical Screening

Preliminary phytochemical screening was conducted to detect the presence of bioactive compounds in the white turmeric extract. The tests performed included: (1) alkaloids using Mayer's and Dragendorff's reagents, indicated by the formation of a precipitate; (2) flavonoids using Mg and HCl, indicated by a red or orange color; (3) tannins with $FeCl_3$, indicated by a greenish-black or bluish-black color; (4) saponins by shaking with distilled water to observe stable foam formation; (5) steroids and terpenoids using Liebermann–Burchard reagent, indicated by a color change to green or red; and (6) phenolic compounds using $FeCl_3$, indicated by a blue or black coloration (1,10).

Antibacterial Activity Test

Antibacterial activity was evaluated using the disc diffusion method. Sterile paper discs were soaked in prepared white turmeric extract at concentrations of 15%, 25%, and 50% for 15 minutes, with ceftriaxone as a positive control and sterile distilled water as a negative control. The discs were then placed on Mueller-Hinton Agar (MHA) plates inoculated with Escherichia coli and incubated at 37°C for 24 hours. The antibacterial effect was assessed by measuring the diameter of inhibition zones around the discs using a caliper.

Results

Determination Results

The sample was sent to the National Research and Innovation Agency (BRIN), Botanical Laboratory in Bogor – Cibinong, for identification of the white turmeric sample to determine its eligibility for the research phase. After approximately two weeks, the result confirmed that the sample was suitable for testing and identified as *Curcuma zedoaria*, belonging to the *Zingiberaceae* family. Determination is the process of comparing a plant species with another previously identified species. The purpose of conducting plant determination is to avoid errors in collecting the research material.

Organoleptic & Phytochemical Properties of white turmeric extract

Organoleptic observations and phytochemical screening were conducted to characterize the ethanol extract of white turmeric (*Curcuma zedoaria*). These assessments provide initial information on the extract's physical properties and the presence of secondary metabolites that may play a role in its antibacterial activity

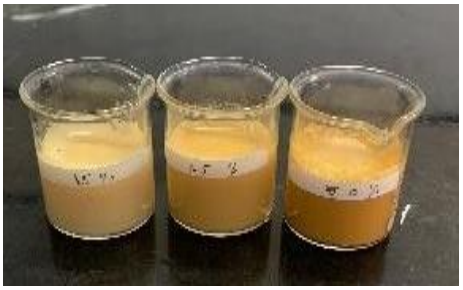

Figure 1. Organoleptic Properties of White Turmeric Extract

Table 1 Organoleptic & Phytochemical Properties of white turmeric extract

Parameter	Observation/Result
Color of solvent after maceration	Yellowish-brown
Color of extract after maceration	Reddish-brown
Flavonoids	Positive (+)
Tannins	Positive (+)
Saponins	Positive (+)

Table 1 & Figure 1 show the organoleptic and phytochemical characteristics of white turmeric extract. The maceration process produced a yellowish-brown solvent and a reddish-brown extract, indicating the presence of soluble compounds while the prepared extract dilutions (15%, 25%, and 50%) showed visual differences in color intensity and solution thickness, with higher dilutions producing lighter extract colors. Phytochemical screening confirmed the presence of flavonoids, tannins, and saponins, which are known to contribute to antibacterial activity (8).

Antibacterial Activity

Antibacterial activity test of white turmeric (*Curcuma zedoaria*) extract against *Escherichia coli* performed by disc diffusion method Gram staining aims to determine and identify the morphology of bacterial cells and to recognize these microscopic organisms as well as to differentiate gram-positive bacteria from gram-negative bacteria. *E. coli* is rod-shaped and appears red. Gram-positive bacteria have a thick peptidoglycan layer, so they will appear blue to purple. Meanwhile, gram-negative bacteria contain more lipids, so they will appear red to pink (Putri et al., 2016).

Table 2. Antibacterial activity of white turmeric (*Curcuma zedoaria*) extract against *E.coli*

Sample	Average Inhibition (mm)	Criteria
Extract 15%	6.8	Resistant
Extract 25%	11.5	Resistant
Extract 50%	12.1	Resistant
Positive Control (Ciprofloxacin)	22	Sensitive
Negative Control (Distilled Water)	0	0

Table 2 presents the antibacterial activity of white turmeric (*Curcuma zedoaria*) extract against *Escherichia coli*. The extract produced inhibition zones of 6.8 mm, 11.5 mm, and 12.1 mm at concentrations of 15%, 25%, and 50%, respectively. Although all values fall within the resistant category, the increasing inhibition with higher concentrations suggests a concentration-dependent antibacterial effect. For comparison, ciprofloxacin (positive control) produced a 22 mm inhibition zone, while the negative control (distilled water) showed no activity.

Discussion

Medicinal plants are increasingly being considered as alternative treatments besides using synthetic drugs. One medicinal plant that is starting to be cultivated is white turmeric (*Curcuma zedoaria*). Although the exact mechanism of white turmeric's action is not yet fully understood, it is believed that the substances contained in white turmeric—from the tip of the leaf to the tip of the root—have healing properties. The compounds found in it include saponins, flavonoids, tannins, essential oils, and curcumin, which have antioxidant and antibacterial properties (4,10).

Naturally, plants contain compounds called phytochemicals that have positive effects on health, contributing to the prevention and defense against various diseases. Phenolic compounds such as flavonoids, saponins, tannins, organic acids, and vitamins are part of phytochemicals (11,12).

This study tested the inhibitory effect of white turmeric extract (*Curcuma zedoaria*) against *Escherichia coli* bacteria. The method used for the inhibition test was the disc diffusion method, which involves placing discs on a growth medium so that the extract directly contacts the medium to assess the extract's inhibitory power, which can be seen from the formation of an inhibition zone or clear zone around the paper disc. Table 5.2 shows the results of measuring the diameter of the inhibition zone, which indicates the extent of *Escherichia coli* growth with different concentrations of white turmeric extract. The larger the inhibition zone, the higher the inhibitory activity of the extract against bacterial growth. This research aligns with the study by Lumbantobing et al. (2022), who conducted antibacterial activity tests of papaya leaf extract (*Carica papaya*) and white turmeric extract (*Curcuma zedoaria*) against *Escherichia coli* and *Staphylococcus epidermidis* at white turmeric extract concentrations of 25%, 50%, 75%, and 100%, with inhibition zone diameters of 7.00 mm, 8.99 mm, 11.78 mm, and 14.03 mm respectively (7). It also corresponds with the study by Malahayati et al. (2021), which characterized curcumin extracts from white turmeric (*Kaemferia rotunda L.*) and yellow turmeric (*Curcuma domestica Val.*) at concentrations of 0.05%, 0.1%, 0.15%, and 0.2%, producing inhibition zones of 3.25 mm, 3.55 mm, 3.94 mm, and 4.18 mm respectively (6). According to the researchers related to these studies, the antibacterial activity test of papaya leaf extract (*Carica papaya*) and white turmeric extract (*Curcuma zedoaria*) against *Escherichia coli* and *Staphylococcus epidermidis* showed that white turmeric extract at 100% concentration was able to inhibit the growth of *Escherichia coli*, as indicated by an inhibition zone diameter of 14.03 mm around the disc mixed with the white turmeric extract. The growth of *Escherichia coli* can be inhibited by white turmeric extract due to the antibacterial activity of compounds such as flavonoids, which cause damage to the permeability of the bacterial cell wall, microsomes, and lysosomes as a result of interactions between flavonoids and bacterial DNA. The hydroxyl groups present in the flavonoid structure cause changes in organic components and nutrient transport, ultimately resulting in toxic effects on the bacteria (7). Based on the inhibition test results of the extract against *Escherichia coli* at concentrations of 15%, 25%, and 50%, the average inhibition zones from three repetitions were 6.8 mm (15%), 11.5 mm (25%), and 12.1 mm (50%). The results show that the higher the extract concentration, the stronger the inhibitory activity against bacterial growth, and the larger the diameter of the inhibition zone.

Conclusion

White turmeric *(Curcuma zedoaria)* extract exhibits significant antibacterial activity against Escherichia coli, as evidenced by the formation of inhibition zones in the disc diffusion assay. The study demonstrated that higher concentrations of the extract result in stronger inhibitory effects, with the largest inhibition zones observed at 50% concentration. This antibacterial activity is likely attributed to bioactive compounds in white turmeric, including flavonoids, saponins, tannins, essential oils, and curcumin, which interfere with bacterial cell structures and metabolic processes. These findings support the potential of white turmeric as a natural alternative for controlling bacterial growth and highlight its relevance in medicinal plant research.

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Conflict of Interest

The authors declare that there's no conflict of interest regarding this article.

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