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INHIBITORY EFFECTIVENESS OF WHITE TURMERIC (*Curcuma zedoira*) EXTRACT AGAINST *Eschericia coli* ATCC 25922

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ABSTRACT

Background: The use of natural ingredients as antibacterial agents has been increasingly studied as an alternative in infection treatment. White turmeric (*Curcuma zedoaria*) contains active compounds such as curcuminoids, flavonoids, and essential oils, which are presumed to have antibacterial potential. However, its effectiveness against *Escherichia coli* ATCC 25922 has not been widely proven scientifically. **Objective:** To determine the effectiveness of white turmeric extract against the growth of *E. coli.* This true experimental study with a post-test only control group design used *Escherichia coli* ATCC 25922 isolates divided into five groups: negative control (DMSO), positive control (ciprofloxacin), and white turmeric extract at concentrations of 50%, 70%, and 90%. The antibacterial test was carried out using the disk diffusion method on Mueller Hinton Agar, with five replications. The inhibition zones were measured after incubation for 18–24 hours at 37°C, and the data were analyzed using the Kruskal–Wallis test. **Results:** Observations showed that inhibition zones appeared only in the positive control group (ciprofloxacin), with a mean diameter of 32.4 mm. The negative control (DMSO) and all white turmeric extract groups (50%, 70%, 90%) showed no inhibition zones (0 mm). **Conclusion:** White turmeric extract has not demonstrated effectiveness against the growth of *Escherichia coli* ATCC 25922. Further research using different methods or concetrations is recommended.

Keywords : *Curcuma zedoaria, Escherichia coli*, inhibition zone, antibacterial, disk diffusion

INTRODUCTION

Escherichia coli bacteria were generally known as Gram-negative rod-shaped bacteria, in which they belonged to the group of facultative anaerobic microorganisms, meaning they could live and reproduce under both aerobic and anaerobic conditions. As commensal microorganisms, *E. coli* played a role as normal flora in the human digestive tract without causing pathological effects.[1] However, there were pathogenic variants that were classified as *diarrheagenic E. coli* or diarrhea-causing strains, as well as *extraintestinal pathogenic E. coli* (ExPEC). Their presence could cause health problems, including diarrhea, urinary tract infections, and various other gastrointestinal diseases.[2]

The study by Ayu et al. stated that 85% of urinary tract infections (UTIs) and 50% of hospital-acquired (nosocomial) infections were caused by *E. coli.* According to data from the World Health Organization (WHO), diarrheal disease ranked as the second leading cause of death in children, especially those under five years of age. Bacteria were the second leading cause of death in several diarrheal cases in children after *rotavirus*. Globally, diarrhea caused the death of approximately 525,000 children each year, and in developing countries, *E. coli* was the main cause of acute diarrhea. The presence of E. coli indicated poor sanitation practices because it could be transmitted between individuals through fecal-oral transmission or indirectly through contaminated water, food, hands, and objects.[3,4,5]

Escherichia coli was a bacterium that could cause various infections, not only in the digestive tract but also in other body systems, including urinary tract infections, sepsis, neonatal meningitis, prostatitis, pneumonia, as well as wound and gallbladder infections.[6] The use of antibiotics as the main therapy often faced challenges due to bacterial resistance, which reduced the effectiveness of treatment. Many strains of *E. coli* had already shown resistance to various types of antibiotics, including quinolones, which were commonly used in the treatment of bacterial infections. Therefore, alternative strategies were required, such as the development of natural compound-based therapies, to inhibit or eliminate *E. coli* strains resistant to

conventional antibiotics.[7,8]

White turmeric (*Curcuma zedoaria*), which belonged to medicinal plants (TOGA), was a plant from the *Zingiberaceae* family. This plant was widely found in various regions of Indonesia, making it well known among the community. White turmeric was often used as a traditional medicine in various countries to treat several diseases, including in Indonesia. The use of white turmeric as a natural ingredient had advantages compared to synthetic materials, such as being more environmentally friendly, more affordable, and abundantly available. Its natural active compounds made white turmeric a safer alternative for treatment compared to chemical substances. White turmeric contained 60–70% carbohydrates, 8.6% protein, 5–10% fat, 3–5% curcumin compounds, and 2–7% fiber. Its phytochemical contents consisted of curcuminoids, essential oils, flavonoids, alkaloids, and saponins. The presence of various secondary metabolites in white turmeric could have antibiotic activity. The antibacterial activity of white turmeric was proven effective against bacteria such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Bacillus cereus*.[9]

Previous studies found that antibiotic activity also depended on the concentration of the extract used. This difference showed the need for further testing to confirm those results. This study aimed to determine and confirm the type of white turmeric extract that provided the best antibiotic effect against *E. coli*. Thus, it was expected to obtain more consistent and accurate data, so that natural antibiotics could be further developed on a strong scientific basis. Therefore, the researchers conducted a study on the effectiveness of the inhibitory power of white turmeric (*Curcuma zedoaria*) extract against the growth activity of *Escherichia coli* using concentrations of 50%, 70%, and 90%. The concentrations of 50%, 70%, and 90% were chosen in this study, which stated that white turmeric extract had the potential to inhibit the growth of *E. coli*, with the possibility of increased effectiveness along with higher concentrations. Although several studies showed antibacterial activity, the results obtained were still varied and inconsistent.[10,11]

Therefore, this study was conducted to further evaluate the effectiveness of white turmeric extract at various concentrations against the growth of *E. coli*, as well as to confirm whether there was antibacterial activity at the applied concentrations. The selection of a 50% concentration of white turmeric extract was used to determine the minimal effect, 70% to observe the possibility of increased activity, and 90% to test the maximum effectiveness.

OBJECT AND METHOD

This study was a true experimental laboratory research with a post-test only control group design. The test organism was *Escherichia coli* ATCC 25922, obtained from the Microbiology Laboratory of the Faculty of Medicine, Udayana University. Pure colonies were subcultured on MacConkey Agar to ensure bacterial viability and then suspended in physiological NaCl solution. The turbidity of the bacterial suspension was adjusted to a 0.5 McFarland standard before inoculation.

White turmeric (*Curcuma zedoaria*) rhizomes were collected from local plantations in Banyuwangi, Indonesia. Fresh rhizomes were cleaned, peeled, sliced, air-dried, and ground into fine powder. The powder was extracted using the maceration method with 96% ethanol for 72 hours. The filtrate was evaporated at 40°C using a rotary vacuum evaporator to obtain a thick extract. The concentrated extract was then diluted with dimethyl sulfoxide (DMSO) to prepare three concentrations: 50%, 70%, and 90%.

Sterile paper disks were impregnated with 20 µL of each extract concentration and air-dried under sterile conditions. Ciprofloxacin (5 µg) was used as the positive control, while DMSO was used as the negative control. The prepared bacterial suspension was evenly spread on Mueller Hinton Agar (MHA) plates using sterile swabs. The impregnated disks were carefully placed on the inoculated agar surface and gently pressed to ensure firm contact. Each treatment, including controls, was performed in five replications.

All plates were incubated at 37°C for 18–24 hours. After incubation, the diameter of the inhibition zone around each disk was measured in millimeters using a digital caliper. The inhibition zone was defined as a clear area without visible bacterial growth. Data were tabulated as mean inhibition zone diameters for each group. Since the extract groups (50%, 70%, and 90%) consistently showed 0 mm zones, only the positive control demonstrated measurable inhibition, with a mean diameter of 32.4 mm. The results were analyzed descriptively, and statistical testing was performed using the Kruskal–Wallis test to confirm group differences.

This study was approved by the Ethics Committee of the Faculty of Medicine, Udayana University, with the number /UN14.2.2.VII.14/LT/2025.

RESULT

In this study, the antibiotic activity test was conducted with five replications to ensure that the data obtained were more consistent. Treatments with white turmeric extract against *Escherichia coli* ATCC 25922 were applied at concentrations of 50%, 70%, and 90%. Ciprofloxacin was used as the positive control to compare the inhibition of bacterial growth, while DMSO was used as the negative control to confirm that the solvent did not affect bacterial growth. The outcome measured in this study was the diameter of the inhibition zone, defined as the clear area surrounding the paper disk in each treatment. Inhibition zones were observed only in the positive control group, whereas no inhibition zones were found in the three white turmeric extract concentrations (50%, 70%, and 90%) or in the negative control.

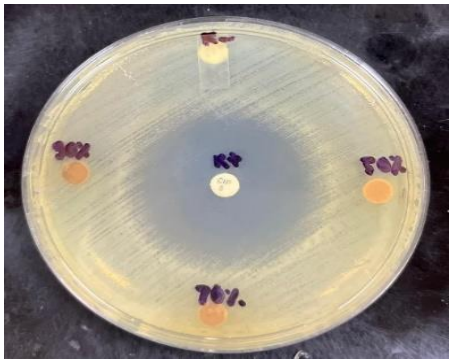

Figure 1. Inhibitory test results. The positive control (ciprofloxacin) inhibited the bacteria, whereas the white turmeric extract at concentrations of 50%, 70%, and 90%, as well as the negative control (DMSO), did not inhibit the bacteria

Table 1. Measurement Results of the Inhibition Zone Diameter of White Turmeric Extract Against the Growth of *Escherichia coli*

Treatment Type	Inhibition Zone Diameter (mm)					Mean (mm)
	1	2	3	4	5	
Control (+)	34	33	33	31	31	32,4
Control (-)	0	0	0	0	0	0
Extract 50%	0	0	0	0	0	0
Extract 70%	0	0	0	0	0	0
Extract 90%	0	0	0	0	0	0

Table 1 showed that the positive control group had a mean inhibition zone diameter of 32.4 mm. Meanwhile, the negative control group and all treatment groups of white turmeric extract (50%, 70%, and 90% concentrations) did not show any inhibition zones.

DISCUSSION

The results of this study showed that white turmeric (*Curcuma zedoaria*) extract at concentrations of 50%, 70%, and 90% did not exhibit antibacterial activity against the growth of *Escherichia coli*. All three concentrations failed to form inhibition zones, resulting in inhibition zone diameters of 0 mm in all treatments. In contrast, the positive control group using ciprofloxacin as the standard antibiotic demonstrated significant antibacterial activity, with a mean inhibition zone diameter of 32.4 mm, indicating high effectiveness in inhibiting the growth of *E. coli*. Meanwhile, the negative control group, which used DMSO as the solvent, also showed no inhibition zones (0 mm), confirming that the solvent did not affect bacterial growth.

Curcuma zedoaria was known to contain various bioactive compounds with potential antibacterial activity, such as curcumin, curzerenone, β-turmerone, germacrone, and furanodiene. These compounds had been reported to possess antimicrobial, anti-inflammatory, and anticancer properties. The phenolic and flavonoid contents in white turmeric extract were also known to damage bacterial cell membrane integrity, cause ion and protein leakage, and interfere with DNA and RNA synthesis.[12,13] However, the effectiveness of these compounds in inhibiting bacterial growth greatly depended on factors such as extraction method, type of solvent, concentration, type of microorganism tested, and environmental conditions of the plant source. Differences in the active compound content of white turmeric from different regions were suspected to be one of the reasons why inhibition zones were not formed. A study by Astuti reported that essential oil content in white turmeric varied by region, ranging from 1.87% in Kulonprogo, 1.25% in Bantul, to 1.06% in Gunung Kidul. This variability indicated that the chemical composition of white turmeric was not uniform, and the samples used in this study might have contained very low levels of essential oils or curcumin, which were insufficient to inhibit *E. coli* growth.[14]

Previous studies provided diverse evidence regarding the antibacterial activity of white turmeric. For instance, a study reported that ethanol extract of white turmeric had a minimum inhibitory concentration (MIC) of 21% and a minimum bactericidal concentration (MBC) of 22% against *E. coli*, indicating that antibacterial effects only appeared at relatively high concentrations. Another study reported that white turmeric extract at 100% concentration produced an average

inhibition zone of 14.3 mm against *E. coli* and *Staphylococcus epidermidis*, whereas antibacterial activity was not detected at lower concentrations. This finding reinforced the notion that extract concentrations below 100% might not be strong enough to inhibit the growth of Gram-negative bacteria such as *E. coli*.[15]

A study by Tania using in vitro and in silico approaches showed that secondary metabolites such as curcumenol and germacrone in white turmeric extract had the potential to inhibit important enzymes in *E. coli* such as MurE and DNA Gyrase B. Although these results provided a theoretical basis for antibacterial potential, it needed to be recognized that in silico predictions did not necessarily reflect actual effectiveness in biological systems, particularly if the active compounds were not available in optimal concentrations in the extract.[16]

Extracts of *Curcuma zedoaria* were reported to show antibacterial activity against *E. coli* when extracted using ethanol and methanol solvents. One study by Alghannay reported that antibacterial effectiveness strongly depended on extract concentration, extraction technique, solvent type, and test conditions. In this study, although extracts were prepared using 96% ethanol and applied at high concentrations (50%–90%), no inhibition zones were observed against *E. coli*. This indicated that ethanol extraction under certain conditions might not yield effective antibacterial activity, especially against Gram-negative bacteria with complex cell wall structures like *E. coli*. The low effectiveness of active compounds extracted with ethanol was suspected to be the main cause of the absence of inhibition zones. This finding was consistent with Sasanti, who reported that the highest antibacterial activity was obtained from the n-hexane fraction, with an MIC value of 3.125 ppm against *E. coli*, while the ethanol fraction showed much lower activity. The antibacterial activity of white turmeric extract was indeed strongly influenced by the solvent used, as different solvents had different capacities to dissolve bioactive compounds such as flavonoids and phenolics, which were generally lipophilic. Alghannay further reported that methanol, ethanol, and isopropanol were more efficient in extracting such compounds, with methanol and ethanol extracts producing inhibition zones ranging from 7.33 mm to 10.67 mm at concentrations of 10–50 mg/mL. Isopropanol extracts even showed higher activity, with MIC values as low as 1.25 mg/mL.[17,18]

In this study, DMSO was not used as the main solvent for maceration but only as a supporting solvent to prepare extract concentrations before antibacterial testing. The use of DMSO at low concentrations (±1%) had been reported not to significantly affect *E. coli* growth, thus ruling out DMSO as the cause of the absence of inhibition zones. Therefore, the lack of inhibition zones was more likely due to the low content of active compounds in the white turmeric extract rather than the DMSO solvent. This finding indicated that antibacterial activity was not solely dependent on high extract concentrations but also on the suitability of solvents, extraction methods, and the ability of active compounds to diffuse effectively in the test medium. Consequently, the negative results in this study were likely due to extraction and solvent limitations rather than the absence of antibacterial potential in *Curcuma zedoaria*.[19]

However, in this study, the extraction method and solvent used were clearly described, with maceration performed using 96% ethanol to obtain the active compounds and DMSO employed to dissolve the extract before antibacterial testing. Despite this, the results still showed that the extract did not demonstrate antibacterial activity against *E. coli*. This was further supported by the fact that *E. coli* was a Gram-negative bacterium with a complex lipopolysaccharide (LPS) cell wall structure, which made it more resistant to antibacterial agents, especially those derived from plants (Tortora et al., 2018). The low permeability of the *E. coli* cell wall to polar compounds caused most plant-derived active compounds to have difficulty penetrating the outer membrane to reach their biological targets.[20]

Several technical aspects of the experimental design might also have influenced the results, such as the test method used (disk diffusion or well diffusion), incubation duration, and the volume of extract applied. Uneven diffusion of antibacterial compounds in the agar medium or the use of solvents incompatible with the medium characteristics could have hindered the optimal spread of active compounds, resulting in the absence of inhibition zones even though the compounds theoretically had antibacterial potential. This condition was in line with CLSI (2024), which stated that the disk diffusion method was qualitative and highly dependent on the ability of compounds to diffuse in the test medium. Large molecular size or certain chemical properties might impede diffusion, preventing visible inhibition zones despite potential antibacterial activity.[19]

Recommendations for further research included the use of more effective organic solvents to improve extraction efficiency of white turmeric bioactive compounds. Selecting more suitable solvents was expected to yield extracts with higher and more stable concentrations of active compounds. Fractionation of bioactive compounds should also be considered to isolate and identify key components such as curzerenone and germacrone, which were reported to have dominant antibacterial activity. By fractionating, researchers could more specifically evaluate the effects of each compound against the target bacteria. Increasing extract concentrations above 90% was also a relevant consideration, since some previous studies indicated that the antibacterial activity of white turmeric only became evident at very high concentrations. More precise measurement methods such as Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests using broth microdilution should also be employed, so that antibacterial activity could be quantified more accurately. Additionally, testing against Gram-positive bacteria should be conducted to determine the broader antibacterial spectrum of white turmeric extract. This was supported by previous studies suggesting that white turmeric extract tended to be more effective against Gram-positive bacteria compared to Gram-negative bacteria.[15]

Apart from these factors, this study also had several other

limitations that needed consideration. Variations in raw material quality, such as rhizome age, harvesting location, and storage conditions, could have influenced the active compound content, thereby limiting test consistency. The extraction duration and method used, namely maceration for 72 hours, might not have been optimal compared to methods such as Soxhlet or ultrasonic extraction. Furthermore, the volume of extract applied to the test medium might not have been sufficient to form measurable inhibition zones, particularly given the lipophilic nature of the compounds, which made diffusion in agar difficult. The stability of active compounds could also have decreased during drying, storage, or heating prior to extraction. Environmental factors such as medium pH, incubation temperature, and bacterial density might also have affected the results, making it necessary to interpret antibacterial activity with these aspects in mind. Therefore, the negative results obtained in this study were more reflective of methodological and experimental limitations rather than a complete rejection of the antibacterial potential of white turmeric.[19,20]

In conclusion, this study contributed important insights into the effectiveness of white turmeric (*Curcuma zedoaria*) extract against the growth of *Escherichia coli*. It also opened opportunities for further development in formulation and optimization of extraction methods, so that the bioactive compounds in *Curcuma zedoaria* could be maximally utilized as antibacterial agents in the future.

CONCLUSIONS AND SUGGESTIONS

This study concluded that white turmeric (*Curcuma zedoaria*) extract at concentrations of 50%, 70%, and 90% did not demonstrate antibacterial activity against *Escherichia coli*, as no inhibition zones were observed, while ciprofloxacin as the positive control showed significant inhibitory effects.

Further investigations are recommended by employing higher concentrations, alternative extraction methods, or different solvents, as well as testing against other bacterial strains, to provide a more comprehensive understanding of the antibacterial potential of *Curcuma zedoaria*.

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