Research Article

In Vitro Antibacterial Activity and Preliminary Cytotoxicity of White Turmeric (*Curcuma zedoaria*) Kombucha

Tobias Hezkel Siregar[1], Mafrikhul Muttaqin[2], & Syaefudin Suminto[1,3]*

[1] Department of Biochemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor 16680, West Java, Indonesia
[2] Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Bogor 16680, West Java, Indonesia
[3] Tropical Biopharmaca Research Center, IPB University, Bogor 16128, West Java, Indonesia

*Corresponding author: syaefudin01@apps.ipb.ac.id

ABSTRACT

White turmeric (*Curcuma zedoaria*) contains bioactive compounds with antibacterial and cytotoxic potential; however, the quantity of these compounds and their biological activity remain relatively low. Fermentation can enhance these properties by promoting microbial bioconversion. This study evaluated the total flavonoid content as well as antibacterial and cytotoxic activities of *C. zedoaria* rhizomes fermented using a Symbiotic Culture of Bacteria and Yeast (SCOBY). Antibacterial activity was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the disk diffusion method, while cytotoxic activity was analyzed using the Brine Shrimp Lethality Test (BSLT). The results showed that fermentation increased the total flavonoid content by 64% and substantially improved antibacterial performance, producing inhibition zones of 13.7±0.7 mm for *E. coli* and 12.8±0.4 mm for *S. aureus*, more than double those of the non-fermented white turmeric. Cytotoxic activity also increased, as indicated by a lower LC_{50} value (145.3 µg/ml) compared to the non-fermented white turmeric (1,381.8 µg/ml). Overall, the findings demonstrate that fermentation significantly enhances the antibacterial and cytotoxic activities of white turmeric.

Keywords: bioactivity enhancement, fermentation, functional beverage, SCOBY, white turmeric

Introduction

White turmeric (*Curcuma zedoaria*) is a traditional medicinal plant native to Southeast Asia, including India, Indonesia, and Malaysia. It contains bioactive compounds such as polyphenols, flavonoids, saponins, and steroids/triterpenoids, which exhibit antibacterial activity (Budiansyah et al., 2023). Flavonoids disrupt bacterial cell walls and inhibit protein synthesis, while steroids/triterpenoids interact with transmembrane proteins, forming strong polymeric bonds that damage bacterial porins (Donadio et al., 2021). A study on the methanol and ethyl acetate extracts of *C. zedoaria* rhizomes also revealed inhibitory activity against pathogenic bacteria, including *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* (Budiansyah et al., 2023).

Although *C. zedoaria* exhibits antibacterial potential, its bioactive compound content is relatively lower than that of other *Curcuma* species, limiting its application (Burapan et al., 2020). Fermentation offers an alternative approach to enhance bioactivity through microbial biotransformation (Herman & Herman, 2023). A well-known beverage produced by fermentation process is kombucha. It is made by a combination of bacteria and yeast, and is therefore often called SCOBY, which stands for Symbiotic Culture of Bacteria and Yeast. Traditionally, it is prepared from tea but can also be developed using plants rich in phenolic compounds. Kombucha contains organic acids, vitamins, amino acids, phenols, polyphenols, and minerals that contribute to its health-promoting properties, including antibacterial activity (Jayabalan et al., 2014). However, evaluating cytotoxicity is necessary to ensure its safety for functional food applications (Thenuwara et al., 2024).

Previous research on *C. zedoaria* kombucha has primarily focused on its physicochemical properties. Prior findings have also indicated that fermentation can increase the total phenolic content by 64% (Zubaidah et al., 2024; 2025). Despite these advances, research on the impact of fermentation on the bioactive profile of *C. zedoaria*, particularly its antibacterial and cytotoxic activities, is limited. To address this gap, the present study evaluated the inhibition zone diameters and cytotoxic LC_{50} values of *C. zedoaria* kombucha, offering insights into the role of fermentation in its biological activities and supporting its potential as a functional beverage.

Materials and Methods

Materials

Dried powdered rhizomes (simplicia) of *C. zedoaria* were obtained from an e-commerce shop in Jakarta, Indonesia, and the SCOBY starter culture was purchased from an e-commerce shop in Banten, Indonesia. Other materials included commercial sugar, NaOH, phenolphthalein indicator, aluminum chloride ($AlCl_3$), potassium acetate (Merck, Germany), quercetin (Sigma-Aldrich, USA), nutrient agar (Himedia, India), *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, and *Artemia salina* eggs.

Moisture Content

Moisture content of the dried powdered *C. zedoaria* was determined gravimetrically following AOAC (2007). As much as 1 g of simplicia powder of *C. zedoaria* was placed in a pre-weighed porcelain crucible. After drying in an oven at 105°C for 3 h, the sample and porcelain crucible were then weighed to achieve a constant weight. All procedures were conducted in triplicate. The moisture content was determined using following formula:

$$Moisture\ content\ (\%) = \left(\frac{(W1-W2)}{(W1-W0)}\right) \times 100$$

Where W_0 is the weight of empty porcelain crucible (g), W_1 is the weight of porcelain crucible with sample before drying (g), W_2 is the weight of porcelain crucible with sample after drying (g).

Preparation of Extract and Kombucha

Simplicia of *C. zedoaria* was extracted using the infusion method (Marliani et al., 2017). A total of 1 g of *C. zedoaria* simplicia powder was extracted in hot water (90°C) for 15 min. The extracts were filtered and adjusted with water to a final volume of 250 ml to obtain a concentration of 4,000 μg/ml. The filtrates were divided into two treatments: non-fermented and fermented kombucha. Fermentation was conducted in sterilized 300 ml glass jars under static conditions. Each jar contained 250 ml of filtrate and was supplemented with 10% (w/v) sucrose, 10% (v/v) starter culture, and one SCOBY sheet. The jars were incubated at 27°C for 14 days (Zubaidah et al., 2024). All fermentation was performed in triplicate.

Acidity (pH) and Total Titratable Acidity (TTA)

The pH was measured using a calibrated pH meter, and the TTA was determined by titration with 0.1 N NaOH using phenolphthalein as an indicator

(Miranda et al., 2016). All measurements were conducted in triplicate.

Total Flavonoid Content

The total flavonoid content (TPC) was quantified using the $AlCl_3$ method with quercetin as the standard (Putri et al., 2025; Suminto et al., 2025). Absorbance was measured at 415 nm using a microplate spectrophotometer, and results were expressed as mg quercetin equivalents (QE)/l filtrate. All analyses were performed in triplicate.

Antibacterial Activity

Antibacterial assays against *E. coli* and *S. aureus* were performed using the disc diffusion method (Bhattacharya et al., 2016; Hasanah et al., 2017). Non-fermented and fermented kombucha samples were tested at a concentration of 4,000 µg/ml, while amoxicillin (10 µg/ml) served as the positive control. Inhibition zone diameters were measured in triplicate.

Brine Shrimp Lethality Test (BSLT)

Cytotoxicity was evaluated using *A. salina* larvae, as described by Atthalia (2024). The assay was conducted in 12-well microplates, each containing 1 ml of saline solution, 10 *A. salina* larvae, and 10 µl of the test sample. The plates were incubated under incandescent light for 24 h. After incubation, the number of dead larvae in each well was recorded and compared with the total number of larvae to determine the percentage of mortality. The lethal concentration (LC_{50}) values were calculated using probit analysis. LC_{50} is defined as the concentration of sample that causes 50% mortality of *A. salina* larvae. All experiments were conducted in triplicate using saline solution as the blank control.

Data Analysis

A paired sample *t*-test was applied to analyze flavonoid content, while the Wilcoxon signed-rank test was used to compare the filtrate and freeze-dried samples. Differences in antibacterial activity were evaluated using one-way analysis of variance (ANOVA). Probit analysis was conducted to determine the LC_{50} values in the BSLT assay. All analyses were performed using Microsoft Excel 2016 and IBM SPSS Statistics 26, with a 95% confidence level and a significance threshold of $p < 0.05$.

Results and Discussion

Moisture Content, pH, and Total Titratable Acidity of Non-Fermented and Fermented White Turmeric

The moisture content of white turmeric rhizome simplicia was determined gravimetrically in triplicate, yielding an average value of 8.174±0.04% (Table 1). This moisture level meets the quality standard of ≤ 10% for simplicia, which is crucial for maintaining product stability and preventing microbial growth (Ministry of Health, 2014). The gravimetric method involves repeated heating until a constant weight is achieved, indicating complete water evaporation (Maciel & Steppe, 2017). Low moisture content reduces water activity (Aw), inhibiting enzymatic degradation and microbial proliferation, thus extending shelf life (Stephenus et al., 2023). Additionally, dry simplicia enhances extraction efficiency by facilitating solvent diffusion and maximizing the solubilization of bioactive compounds (Yasi et al., 2022). In this study, water-based infusion extraction was used, chosen for its safety and effectiveness in dissolving polar compounds (Balde et al., 2019).

The pH of the samples decreased significantly by 36.04% after fermentation, from 6.52±0.09 to 4.17±0.05 (Table 1), whereas the total titratable acidity (TTA) increased threefold, from $0.01 \pm 0.06 \times 10^{-2}$ to $0.03 \pm 0.03 \times 10^{-2}$. The pH remained within the safe consumption range (≤ 4.2), indicating successful fermentation (Morales et al., 2023). The inverse relationship between pH and TTA is consistent with previous studies (Kumar & Joshi, 2016), reflecting acid accumulation during fermentation. This acidification results from the synergistic activity of acetic acid bacteria (AAB) and yeast in SCOBY: yeast ferments glucose into ethanol and CO_2, and AAB oxidizes ethanol into acetic acid, contributing to the acidity and characteristic flavor (Antolak et al., 2021).

Table 1. Moisture content, pH, and total titratable acidity (TTA) of non-fermented and fermented white turmeric.

Parameter	Sample type	Mean value ± SD
Moisture content (%)	White turmeric simplicia	8.174±0.04
pH	Non-fermented	6.52±0.09
	Fermented	4.17±0.05
TTA (%)	Non-fermented	$0.01\pm0.06 \times 10^{-2}$
	Fermented	$0.03\pm0.03 \times 10^{-2}$

The decline in pH influences the growth rate of fermenting microbes and leads to structural changes in phytochemicals in kombucha. As reported by de Lima et al. (2025), the total flavonoid content of *Passiflora edulis* leaves increased following fermentation by SCOBY. A similar trend was also observed by Dwiputri and Feroniasanti (2019) in kombucha prepared from butterfly pea, where fermentation enhanced flavonoid content. The increase in flavonoid levels may be driven by microbial activity that transforms polyphenols into flavonoid derivatives (da Silva Júnior et al., 2022).

Total Flavonoid Content in Non-Fermented and Fermented White Turmeric

The total flavonoid content increased significantly from 3.529±0.053 mg QE/l extract in non-fermented white turmeric to 5.786±0.039 mg QE/l extract in fermented samples (Figure 1). This 63.96% increase was statistically significant (p = 0.02; paired-sample t-test). This enhancement is likely due to microbial biotransformation during fermentation, which can release bound flavonoids or synthesize new bioactive compounds (Rao, 2024).

Previous studies have shown that microbial activity can reshape phytochemical profiles during fermentation. For example, *Aspergillus oryzae* has been reported to break down isoflavone glycosides into their aglycone forms during the solid-state fermentation of soybean flour (da Silva Júnior et al., 2011). It is worth noting, however, that each microorganism tends to generate its own set of transformed products. Quercetin provides a good example: *Bacillus cereus* converts it into isoquercetin, whereas *Aspergillus flavus* and *Aspergillus niger* produce a completely different compound, 2-protocatechuoyl phloroglucinol carboxylic acid (Thai et al., 2014). In the present study, fermentation was performed using a mixed community of bacteria and fungi. With such a consortium, biotransformation is highly likely to occur, which is reflected in the increased flavonoid content observed after fermentation.

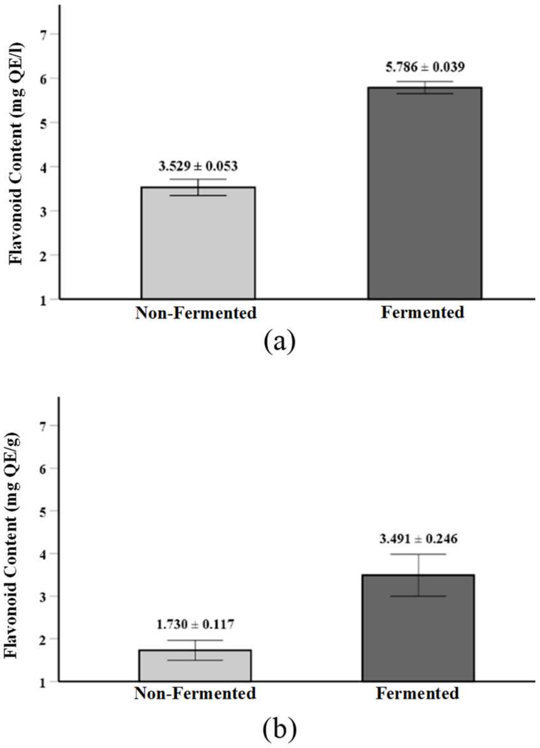


Fig. 1. Total flavonoid content of non-fermented and fermented white turmeric. (a) filtrated sample, (b) freeze-dried sample.

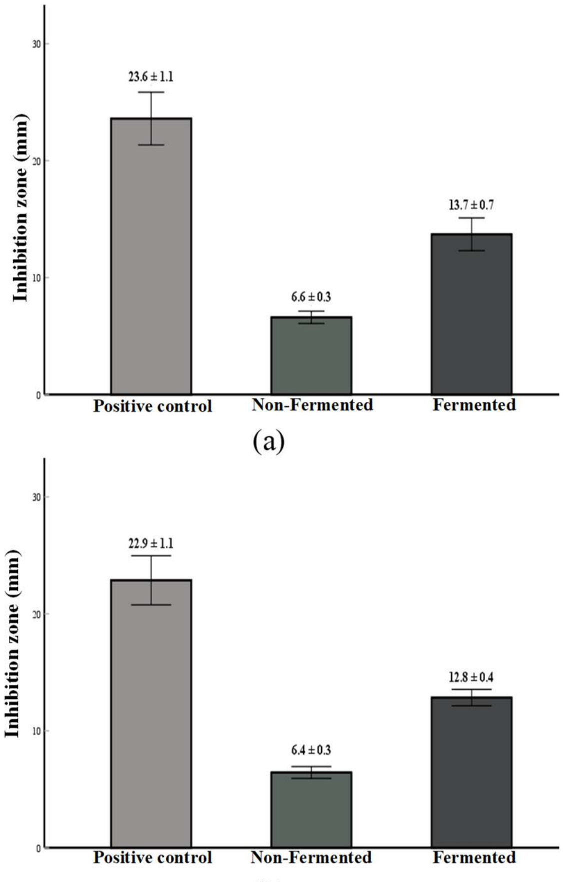
Compared to literature values (Papayrata et al., 2024; Rao, 2024), the flavonoid content here is lower, possibly due to differences in extraction methods and solvent polarity. Modern extraction techniques like ultrasound-assisted extraction and solvents such as methanol or acetone generally yield higher flavonoid concentrations than water-based infusions (Chtibi et al., 2023; Tourabi et al., 2023). Plant tissue age and environmental factors also influence phytochemical content (Mohammadi et al., 2021; Valares et al., 2016). In addition, the types of microbes and/or fermentation techniques used in the experiment may also affect the chemical composition and bioactivities. For example, solid-state fermentation with *A. oryzae* decreased the total flavonoid content while increasing the total phenolic content in *Justicia gendarussa* (Suminto et al., 2024; 2025).

Further analysis on the freeze-dried samples showed a non-significant decrease in flavonoid content compared to filtrated samples ($p > 0.05$), with reductions of 50.98% (non-fermented) and 39.66% (fermented). This decrease may have result from flavonoid degradation due to oxygen exposure and sublimation during freeze-drying (Oprica et al., 2019; Semenov et al., 2015).

Antibacterial Activity of Non-Fermented and Fermented White Turmeric

The antibacterial activity against *E. coli* and *S. aureus* was assessed using the disc diffusion method. Fermented samples (4,000 µg/ml) showed significantly larger inhibition zones (13.7±0.7 mm for *E. coli* and 12.8±0.4 mm for *S. aureus*) than non-fermented samples (4,000 µg/ml) (6.6±0.3 mm and 6.4±0.3 mm, respectively) (Figure 2). One-way ANOVA confirmed significant differences ($p < 0.001$). The increased antibacterial activity correlates with higher flavonoid content and other antimicrobial metabolites produced during fermentation (Safitri & Irdawati, 2020).

Further analysis using the disc diffusion assay showed a clear difference between the two treatments. The fermented white turmeric inhibited bacterial growth even at a relatively low concentration of 500 µg/ml (8× dilution), whereas the non-fermented samples did not produce any inhibition zones (Tables 2 and 3). Taking together, these results indicate that SCOBY fermentation noticeably strengthens the bactericidal properties of white turmeric.



(a)

(b)

Fig. 2. Inhibition zone of non-fermented and fermented white turmeric against bacteria *Escherichia coli* (a) and *Staphylococcus aureus* (b).

Flavonoids exert antibacterial effects by disrupting bacterial membranes, increasing permeability, inducing reactive oxygen species (ROS) production, and inhibiting enzymes such as DNA gyrase and ATP synthase (Jomova et al., 2025; Khan et al., 2018). Other fermentation products, such as acetic acid, lactic acid, gluconic acid, and ethanol, contribute to lowering the pH and damaging bacterial membranes (Bhattacharya et al., 2016;

Jayabalan et al., 2014; Papadimitriou et al., 2016).

Table 2. Antibacterial activity of non-fermented and fermented white turmeric against *Escherichia coli*.

Sample	Dilution factor (×)	Inhibition zone diameter (mm)
Non-fermentation	40.00	0
	20.00	0
	8.00	0
	4.00	0
	2.67	0
	2.00	0
	1.60	0
Fermentation (kombucha)	40.00	0
	20.00	0
	8.00	6.8±0.2
	4.00	7.1±0.5
	2.67	7.6±0.6
	2.00	8.4±0.3
	1.60	8.9±0.3

Table 3. Antibacterial activity of non-fermented and fermented white turmeric against *Staphylococcus aureus*.

Sample	Dilution factor (×)	Inhibition zone diameter (mm)
Non-fermentation	40.00	0
	20.00	0
	8.00	0
	4.00	0
	2.67	0
	2.00	0
	1.60	0
Fermentation (kombucha)	40.00	0
	20.00	0
	8.00	6.9±0.1
	4.00	7.3±0.1
	2.67	8.0±0.2
	2.00	8.5±0.4
	1.60	9.2±0.2

The tested strains differ structurally: *E. coli* (Gram-negative) has an outer membrane with lipopolysaccharides acting as a barrier, but lipophilic flavonoids and acetic acid can penetrate via porins (Jubair et al., 2022; Rachmawati et al., 2021). *S. aureus* (Gram-positive) lacks an outer membrane, making it more susceptible to membrane-targeting compounds (Prasety et al., 2019).

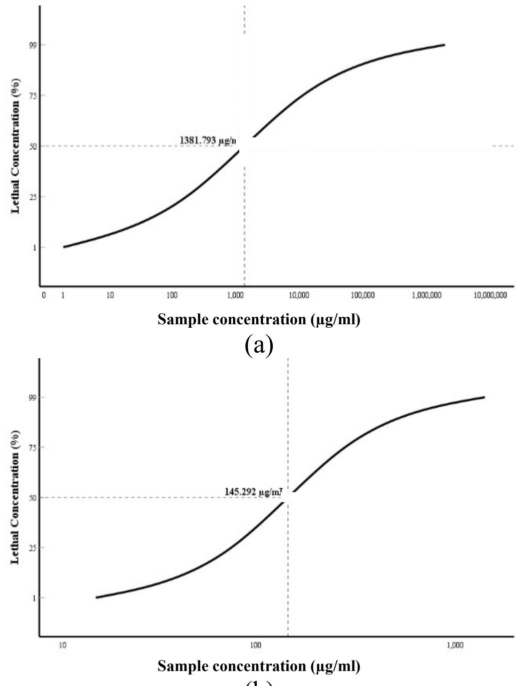
Cytotoxic Activity

The cytotoxicity assay showed increased mortality of *A. salina* larvae with increasing concentrations of both fermented and non-fermented white turmeric. Probit analysis yielded LC_{50} values of 145.292 µg/ml (27.53× dilution) for fermented samples and 1,381.793 µg/ml (2.894× dilution) for non-fermented samples (Figure 3). The 89.48% decrease in LC_{50} after fermentation indicates enhanced cytotoxicity, consistent with previous studies (Syahbirin et al., 2024; Uswatun & Wijayanti, 2020).

Flavonoid aglycones, which have higher lipid solubility, may penetrate cell membranes more effectively and interact with cellular and mitochondrial structures to induce apoptosis via ROS generation (Slika et al., 2022). Acetic acid produced during fermentation can also penetrate larval membranes, dissociate intracellularly, lower pH, inhibit enzymes, and induce oxidative stress, leading to apoptosis (Kurokawa et al., 2022).

The probit method effectively estimates toxicity parameters by transforming data into a normal distribution, allowing for accurate LC_{50} and LC_{95} calculations (Ryzhenko & Kavetsk, 2017; Shevchuk et al., 2023).

According to Meyer's toxicity index, fermented white turmeric exhibits moderate toxicity (LC_{50} = 100–500 µg/ml), while non-fermented samples are non-toxic (LC_{50} > 1,000 µg/ml) (Hamidi et al., 2014). BSLT serves as a sensitive preliminary assay for cytotoxicity testing in more complex biological systems (Filipe et al., 2022). As BSLT serves only as an initial toxicity screen, these results should be interpreted cautiously, particularly because this study used kombucha directly rather than a concentrated extract. These findings indicate that further cytotoxicity assessments are warranted, and fractionation steps may be needed to ensure the safety of functional beverages.



(a)

(b)

Fig. 3. Cytotoxic activity values (LC_{50}) for non-fermented (a) and fermented (b) white turmeric.

Conclusion

Fermentation of white turmeric (*C. zedoaria*) with SCOBY culture noticeably improved its ability to inhibit the growth of *E. coli* and *S. aureus*. Additionally, the cytotoxicity increased from non-toxic to moderate toxicity following fermentation. These stronger antibacterial and cytotoxic responses corresponded with higher flavonoid content and increased acidity as fermentation progressed. However, the scope of the conclusion is restricted to the inhibition zones and LC_{50} values obtained under the tested conditions. These findings suggest the potential use of fermented white turmeric as a natural antimicrobial component in food and nutraceutical products. Further profiling of its active metabolites (e.g., via liquid chromatography-mass spectrometry or high-performance liquid chromatography) and a more detailed safety assessment are essential before considering its application in humans.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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