Journal of Academia Vol. 12, Issue 2 (2024) 195 – 200

ANTIBACTERIAL ACTIVITY OF TURMERIC (*Curcuma longa*) EXTRACT AGAINST *Staphylococcus aureus* AND *Escherichia coli*

Nur'alin Syahmina Hashim[1], Latifah Munirah Bakar[1]*

[1]*School of Biological Sciences, Faculty of Applied Sciences,*
Universiti Teknologi MARA (UiTM) Shah Alam, 40000 Shah Alam, Selangor, Malaysia

**Corresponding author: latifahmunirah@uitm.edu.my*

Abstract

Foodborne pathogens, such as *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*), are known to cause a wide range of illnesses and are typically found in contaminated food. *S. aureus* is a Gram-positive bacterium, while *E. coli* is a Gram-negative bacterium. This study aimed to assess the antibacterial activity of turmeric (*C. longa*) extract against these common foodborne pathogens, *S. aureus*, and *E. coli.* Turmeric extract demonstrated effective antibacterial properties against both bacteria. The foodborne pathogens were tested using the disc diffusion method, with the lowest concentration starting at 100 mg/mL, which showed a mean inhibition zone of more than 9 mm for both *S. aureus* and *E. coli.* The highest concentration tested was 500 mg/mL, which showed a mean inhibition zone of more than 10 mm for both bacteria. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) for these bacteria were found to be around 100 µg/mL. The disc diffusion method revealed that concentrations ranging from 100 mg/mL to 500 mg/mL inhibit the bacterial growth, and the percentage inhibition of diameter growth (PIDG) results confirmed significant inhibition. The PIDG of *S. aureus* was positive and increased as the concentration increased, while *E. coli* showed a negative correlation between PIDG and concentration increase. These findings support the presence of antibacterial properties in turmeric rhizome (*Curcuma longa*) and its potential as a natural means to combat foodborne pathogenic bacteria.

Keywords: Turmeric extract, foodborne pathogens, antibacterial activity, *Staphylococcus aureus*, *Escherichia coli*.

Article History:- Received: 28 June 2024; Revised: 30 June 2024; Accepted: 10 July 2024; Published: 31 October 2024

Introduction

The World Health Organization (2022) reports a staggering estimate, with around 600 million people, or one in every ten individuals worldwide, falling ill as a consequence of consuming contaminated food. This alarming statistic is further exacerbated by approximately 420,000 annual fatalities, resulting in a staggering loss of 33 million lives each year. Notably, within the realm of foodborne diseases, *S. aureus* and *E. coli* fall under the category of "foodborne diseases with diarrheal symptoms," a classification driven by the escalating prevalence of food contamination. The repercussions of foodborne illnesses extend beyond individual health, imposing significant impediments to socioeconomic development by overburdening healthcare systems and inflicting harm on national economies, tourism, and trade (World Health Organization, 2022).

According to Etter *et al.* (2020), foodborne infections are one of the world's leading health concerns. *Staphylococcal enterotoxin* (SEs) which is a significant causal agent causing food poisoning. *S. aureus* produces these exotoxins in food, which induce intoxication when consumed. Liu *et al.* (2022) mentioned that SEs released by *S. aureus* contaminate food and cause significant foodborne infections, although they are overlooked during food preparation and even cold-chain storage. Al-Mamun *et al.* (2018) stated that contamination in food comes via the food handler's nose, nasal mucosa, skin, and faeces. Those afflicted with this illness have symptoms such as nausea, stomach cramps, vomiting, and diarrhoea. Following that, the World Health Organization (2022) declared that *E. coli* strains can cause

serious food poisoning in certain circumstances. For example, Shiga toxin-producing *E. coli* (STEC).

Escherichia coli O157:H7 is a major food-borne and water-borne pathogen that causes hemorrhagic colitis and hemolytic-uremic syndrome (HUS) in humans, as well as substantial morbidity and widespread epidemics across the world.

As mentioned by Rahaman *et al.* (2020), *Curcuma longa Linn.* (*C. longa*), occasionally known as turmeric, is a member of the Zingiberaceae family and has a long history of being used to treat a variety of ailments. *C. longa* has been utilised in Unani and Ayurvedic medicine for liver blockage and jaundice, as well as topically for ulcers and inflammation. It is also used as an antiseptic in the treatment of coughs, colds, dental problems, indigestion, skin infections, blood purification, asthma, piles, bronchitis, tumours, wounds, and hepatic diseases. Curcumin, a key component of *C. longa*, is widely known for its medicinal potential in a variety of diseases as highlighted by Fuloria *et al*. (2022), turmeric has a long-standing history of utilization as herbal medicine. Despite its traditional use, there exists a conspicuous dearth of comprehensive research into the full extent of its therapeutic potential.

Moreover, harnessing the power of medicinal plants for treatment proves to be more economical than relying on modern synthetic drugs. The affordability of plant-based remedies surpasses that of their synthetic counterparts. Additionally, opting for plant-derived antibiotics enhances safety in medical applications, offering a reassuring alternative to potentially riskier synthetic options. This study correlates with the journal by Vaou *et al*., (2021) stated that the antimicrobial compounds found within these botanical wonders exhibit diverse mechanisms that can hinder the proliferation of bacteria, fungi, viruses, and protozoa. Furthermore, these natural antibiotics target microbes through mechanisms, offering potential solutions against resistant strains.

Material and methods

Collection of samples

The fresh turmeric rhizomes were collected from a local market based in Shah Alam and were delivered to the Microbiology Laboratory, UiTM Shah Alam. The samples underwent a cleaning process involving rinsing with distilled water and disinfection using 70% ethanol. The rhizomes were subjected to drying in an oven at a temperature of 60 ° C for a period of 48 to 72 hours to facilitate preservation. The dried rhizome was subsequently ground into a fine powder using a grinder and precisely measured to obtain 30 g.

Plant extraction

A 30 g of dried powdered turmeric was soaked in 300 mL of ethanol for 48 hours and kept in the refrigerator at 6 ° C. The soaked material was filtered using Whatman filter paper (No. 4) and concentrated using Rotary evaporator at 50 ° C to obtain the ethanolic turmeric extract.

Microorganisms' preparation

The foodborne pathogens, *S. aureus* and *E. coli* were provided by the Microbiology Laboratory, School of Biology, UiTM Shah Alam. The bacterial strains were grown on the nutrient agar and incubated for 24 hours at 37 ° C to promote bacterial growth.

Inoculum preparation

These bacterial colonies were transferred into tubes of saline water and vigorously stirred to ensure even dispersal until the liquid slightly turned cloudy. The tubes were then vortexed using a vortex mixer to achieve a homogeneous bacterial suspension. The turbidity of each bacterial suspension was adjusted using a spectrophotometer at 600 nm wavelength to be equivalent to 1.5×10^8 CFU/mL at an optical density (OD) at 0.144.

Disc diffusion method

Gram staining and biochemical tests were employed to determine and confirm the identity of the provided pure bacterial culture. The Mueller-Hinton agar (MHA) plates were labelled and segmented

into four sections. Prepared suspensions were applied to the plates using sterile swab sticks. The 20g of turmeric extract was diluted in 1 mL of 95% ethanol, resulting in five different concentrations for the experiment: 100, 200, 300, 400, and 500 mg/mL. Sterile paper discs, 6mm in diameter and made from Whatman No. 1, were soaked in 100 µL of turmeric extract and subsequently dried at 100 ° C for two hours. These dried paper discs were then placed onto the surface of the inoculated MHA plates. The plates were incubated at 37 ° C for 24 hours. Streptomycin was used as the positive control, while the negative control consisted of 95% ethanol, the solvent. Each sample was tested in triplicate.

Minimum Inhibitory Concentrations (MIC)

The determination of the minimum inhibitory concentration (MIC) of turmeric extract for foodborne pathogens was conducted through a broth micro-dilution assay. Both *S. aureus* and *E. coli* displayed significant inhibitory activity in the disc diffusion test and were assessed for MIC using a sterile U-shaped plate with 96 wells. The turmeric extract was diluted in sterile Mueller-Hinton broth (MHB) medium, and 100 µL of the extract was added to each well of the microtiter plate. To each well, 100 µL of the test microorganisms' inoculum, with an approximate concentration of 1.5×10^8 CFU/mL, was introduced, resulting in a final volume of 200 µL in each well. Streptomycin was employed as the positive control, and 95% ethanol acted as the negative control.

Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) was defined as the lowest concentration that thoroughly inhibited bacterial growth. Viable cells from wells that showed no microbial growth were enumerated on MHA using a standard viable plate count after a 24-hour incubation period at 37 ° C overnight. The MBC was observed and recorded as the lowest concentration of turmeric extracts that resulted in a reduction of 99.9% in bacterial growth relative to the growth control. The experiments were carried out in triplicate, and the results were expressed as the mean value ± standard error of the inhibition zone.

Percentage Inhibition of Diameter Growth (PIDG)

The assessment of the percentage inhibition of diameter growth (PIDG) was conducted as part of the antimicrobial susceptibility test. The susceptibility of turmeric extract against *S. aureus* and *E. coli* was examined, with Streptomycin serving as the positive control. The size of the inhibition zone observed was inversely related to the MIC values, meaning that a larger zone indicated a lower concentration of the antimicrobial drug needed to inhibit the growth of the bacteria (Latifah-Munirah *et al*., 2015; Benkova *et al*., 2020).

$$PIDGs\ (\%) = \frac{Diameter\ of\ sample\ (mm)\ -\ Diameter\ of\ control\ (mm)}{Diameter\ of\ control\ (mm)} \times 100$$

Statistical Analysis

All data were expressed as the mean ± standard deviation from three determinations, each performed in triplicate, using SPSS software (version 17.0). The data were analysed using one-way analysis of variance (ANOVA), followed by post-hoc testing using the least significant difference test to compare between groups. A p-value of less than 0.05 (p-values < 0.05) was considered statistically significant.

Result and Discussion

Extraction of ethanolic crude paste *C. longa*

Extraction constitutes a critical initial stage in purifying and obtaining bioactive elements from plant extracts. According to Pham *et al.* (2019), the primary objective of the extraction process is to achieve the maximum yield, comprising a high concentration of the target active compounds. The extraction process involves several essential steps, such as collection, cleaning, drying, and extraction of turmeric rhizomes, offering various advantages. Proper sterilization procedures ensure the preservation of high-quality turmeric, leading to accurate and reliable results. Table 1 shows the yield of ethanolic crude

extract obtained after solvent and freeze-dried extractions, respectively.

Table 1. The percentage yield of turmeric extract

	Sample powder weight (g)	Crude extract weight (g)	Percentage yield (%)
Turmeric extract	30	10	33.33

Antimicrobial susceptibility test

The susceptibility test involves preparing and standardizing the turmeric extract and then applying it to standardized cultures of *S. aureus* and *E. coli.* The test determines the extract's antimicrobial properties by observing zones of inhibition or minimum inhibitory concentrations. The results provide insights into the potential of turmeric extract as a natural antibacterial agent, with implications for combating infections caused by these foodborne pathogens.

Disc diffusion method

The Kirby-Bauer disc diffusion method was used to evaluate the susceptibility and the resistance of pathogenic bacteria to various antibiotic agents. Table 2 displays the disc diffusion method results for turmeric *C. longa* ethanolic extract antibacterial efficacy against the foodborne pathogens was evaluated at five different concentrations. The ethanolic extract inhibited the Gram-positive bacteria, *S. aureus* with the diameter of the inhibition zone in the range of (11.33 ± 0.58 mm) and (18.33 ± 0.58 mm). Meanwhile, Gram-negative bacteria were inhibited by *C. longa* ethanolic extract with the diameter of inhibition zone in the range of (9.00 ± 0.00 mm) and (13.67 ± 0.58 mm). The results of the disc diffusion reveal that as the concentration of turmeric extract increased, the size of the zone of inhibition against *S. aureus* and *E. coli* also increased. These findings imply that higher concentrations of turmeric extract exhibit a stronger antimicrobial effect against *S. aureus* compared to *E. coli.* Besides that, both foodborne pathogens, *S. aureus* and *E. coli* were found to be more sensitive to *C. longa* crude ethanolic extract due to its statistical analysis which was considered statistically significant ($p < 0.05$).

Table 2. Antibacterial activity of turmeric ethanolic extracts against *Staphylococcus aureus* and *Escherichia coli* on disc diffusion method.

Microorganisms	Diameter of inhibition zone (mm)				
	Concentration (mg/mL)	1st	2nd	3rd	Mean ± SD
Staphylococcus aureus	100	10	11	12	11.33 ± 0.58
	200	10	9	16	12.33 ± 0.58
	300	14	13	15	14.00 ± 1.00
	400	16	14	18	16.00 ± 1.00
	500	18	13	24	18.33 ± 0.58
	Positive	16	15	16	15.67 ± 0.58
	Negative	NI	NI	NI	NI
Escherichia coli	100	9	9	9	9.00 ± 0.00
	200	10	11	10	10.33 ± 0.58
	300	10	12	11	11.00 ± 1.00
	400	13	11	12	12.00 ± 1.00
	500	12	14	15	13.67 ± 0.58
	Positive	30	29	31	30.00 ± 1.00
	Negative	NI	NI	NI	NI

Keys: SD – Standard Deviation, NI – No Inhibition

Determination of MIC and MBC values

The MIC and MBC values for ethanolic crude extracts of *C. longa* against both foodborne pathogens are shown in Table 3. Following the study, the ethanolic extract's MIC and MBC values against *S. aureus* were 12.5 µg/mL and 50 µg/mL, respectively. Meanwhile, the MIC and MBC values of *C. longa* ethanolic extract against *E. coli* were 25.0 µg/mL and 100.0 µg/mL, respectively. It was hypothesized that the extraction of bioactive substances from the *C. longa* was impacted by the solvent's polarity as

mentioned by Martinez-Correa *et al*. (2017) stated that the usage of ethanol as a solvent enhances the extraction of phenolics and curcumin where both are the most active extracts in *C. longa.* As can be seen in table 3 below, the ratio of MBC/MIC was less than or equal to 4 which hypothesized that this current study showed ethanolic extract have bactericidal action against *S. aureus* and *E. coli.* According to Jalil *et. al* (2022), antimicrobial compounds are considered bacteriostatic agents when the MBC/MIC ratio is greater than 4, and the bactericidal agents when it is 4 or below.

Table 3. Determination of MIC values of the crude extracts via broth micro-dilution assay.

Microorganisms	**Ethanolic extract**		
	MIC (µg/mL)	**MBC (µg/mL)**	**Ratio (MBC/MIC)**
Staphylococcus aureus	12.5	50	4 (bactericidal)
Escherichia coli	25.0	100	4 (bactericidal)

Determination Percentage of Diameter Growth (PIDG)

Both *S. aureus* and *E. coli* showed sensitivity to all concentrations of turmeric extracts, as evidenced by the presence of inhibition zones after incubation. According to the current study, the ethanolic extract's MIC and MBC values against *S. aureus* were 12.5 µg/mL and 50 µg/mL, respectively. Meanwhile, the MIC and MBC values of *C. longa* ethanolic extract against *E. coli* were 25.0 µg/mL and 100.0 µg/mL, respectively. Table 4 shows the PIDG values for the positive control, Streptomycin against the concentrations of *C. longa* ethanolic extract on *S. aureus* and *E. coli.* In this study, the PIDG values for Streptomycin against the five different concentrations *S. aureus* were between range of -37.50% and 12.50%. Meanwhile, the PIDG values for Streptomycin against the five different concentrations *E. coli* were between range of -70.00% and -56.67%. The PIDG values for Streptomycin against both pathogens showing increasing in number. This demonstrates a decline in the inhibition of diameter growth as the concentration of the turmeric extract increased However, starting at concentration 400 mg/mL and 500 mg/mL, the PIDG value was positive, indicating that the antibacterial activity of turmeric extract at this concentration was even stronger than the positive control in restraining the growth of *S. aureus.* In contrast, the PIDG values against *E. coli* showed negative PIDG values when compared to the positive control. This implies that the antibacterial activity of turmeric extract at these concentrations was relatively weaker than the positive control in inhibiting the growth of *E. coli*.

Table 4. Percentage Inhibition of Diameter Growth (PIDG) values for Streptomycin against the concentrations of turmeric extract on *S. aureus* and *E. coli.*

Microorganisms	**Concentration (mg/mL)**	**PIDG (%)**
Staphylococcus aureus	100	-37.50
	200	-33.33
	300	-12.50
	400	0.00
	500	12.50
Escherichia coli	100	-70.00
	200	-66.67
	300	-63.33
	400	-60.00
	500	-56.67

Conclusion

The findings in this study discovered a potential antibacterial activity of the turmeric extract against foodborne-pathogen bacteria by exhibiting bactericidal effects. Turmeric extract demonstrates antibacterial activity against *S. aureus* and *E. coli*. The extraction yield by ethanol was 33%. The antibacterial activity of turmeric extracts against food-borne pathogen bacteria. was observed via disc diffusion assay where the zone of inhibition's sizes for turmeric extracts ranges from 13 to 18 mm in diameter. Following the study, the ethanolic extract's MIC and MBC values against *S. aureus* were 12.5

µg/mL and 50 µg/mL, respectively. Meanwhile, the MIC and MBC values of *C. longa* ethanolic extract against *E. coli* were 25.0 µg/mL and 100.0 µg/mL, respectively. The percentage inhibition of diameter growth (PIDG) results indicates the extent of bacterial growth inhibition by turmeric extract. This can be proven by the PIDG reading for *S. aureus* exceeding from negative amount to positive amount increasing the concentrations as the extract disc formed a larger zone of inhibition compared to the PIDG reading for *E. coli*. The PIDG reading for *E. coli* becomes all negative but increases the concentrations due to the larger zone of inhibition of antibiotic disc that extract disc. These findings support that turmeric extract possesses promising antibacterial properties against *S. aureus* and *E. coli*.

Acknowledgement/Funding

The authors would like to thank Universiti Teknologi MARA (UiTM) Malaysia for research funding and facilities. The authors would like to convey our appreciation to those who were engaged, either directly or indirectly, to contribute to the completion of this project.

Author Contribution

All authors contribute equally.

Conflict of Interest

Authors have declared that there is no conflict of interest.

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