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Research Article

Study of Antibacterial Properties of Turmeric Extract Against *Salmonella Typhi* and *Staphylococcus Aureus*

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Abstract

The Zingiberaceae family includes 40 species, among which *Curcuma longa* or turmeric is notable. Numerous genera within this family are recognized for their therapeutic properties in tropical Asia. This research aims to assess the antibacterial potential of *Curcuma longa* along with various antibiotics. Rhizome-derived ethanol and aqueous extracts were prepared for experimentation. The antibacterial activities were investigated using the agar well diffusion assay, employing *Staphylococcus aureus* and *Salmonella typhi*, both susceptible to turmeric, as test organisms. In comparison to five standard antibiotics (azithromycin, ciprofloxacin, ofloxacin, kanamycin, and ceftriaxone), the inhibition zones of the extracts were evaluated for effectiveness. Ethanol extracts at varying concentrations exhibited significant inhibitory effects against these bacteria, particularly *Staphylococcus aureus.* Conversely, the aqueous plant extract demonstrated negligible inhibitory activity. Notably, the tested bacteria displayed resistance to the aqueous rhizome extract. The ethanol extract's inhibition zone ranged from 13mm to 18mm against gram-positive bacteria and 9mm to 14mm against gram-negative bacteria. In contrast, the aqueous extract exhibited weak inhibitory activity, indicating bacterial resistance. This study underscores turmeric's potential as an antibacterial agent containing medicinal compounds. The results may contribute to the integration of turmeric into conventional medicine for enhanced antibacterial efficacy.

Keywords: Antibacterial activity, Curcuma rhizome, agar well diffusion method, zone of inhibition

1. Introduction

Curcuma longa, a member of the ginger family known as Turmeric, has been recognized as a medicinal plant since ancient times. The ongoing challenge of bacterial resistance to drugs has prompted researchers to explore the antibacterial properties present in plants. Within the Zingiberaceae family, *Curcuma longa* stands out as a medicinal herb. Apart from being utilized for its distinctive flavoring and coloring attributes, it is renowned for its remedial properties (Naz et al. 2010). The rise of drug resistance in human diseases against commonly used antibiotics has necessitated the exploration of novel antibacterial compounds from alternative sources. Traditional medicine extensively relies on a diverse array of plants. Materials that exhibit the ability to either impede or halt the growth of microorganisms are being investigated as potential candidates for the development of new medicines to address a range of disorders. Rural areas in many developing countries are renowned for their utilization of plants in traditional medicine practices. Preliminary research has demonstrated the effects of numerous plant extracts on the growth of various bacteria (Erdogrul 2002).

The global recognition of antimicrobial resistance as a significant threat underscores the urgent need for the development of robust infection control strategies. Additionally, there is a crucial

requirement for effective antimicrobial management approaches and innovative treatments to reshape and mitigate this escalating challenge (Thabit, Crandon, and Nicolau 2015). Antimicrobial resistance (AMR) occurs when organisms can thrive and survive in the presence of medications that would typically inhibit their growth (Founou, Founou, and Essack 2017). Infections due to AMR currently result in approximately 700,000 deaths annually. Alarmingly, projections indicate that this number is anticipated to surge to 10 million by the year 2050 (O'Neill 2016). The ESKAPE pathogens, comprising *Enterococcus species, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter species*, have garnered global attention due to their ability to elude treatment and develop high levels of resistance to multiple drugs (Founou, Founou, and Essack 2018).

As bacteria evolve to become increasingly resistant to treatment, revitalizing the development pipeline of anti-infective drugs is more crucial today than ever. Furthermore, the exploration of novel methodologies for anti-infective interventions is imperative (Coon et al. 2017). Plants are a good source of natural products to attain bioactive compounds (Khan et al. 2018). Plant-based remedies constitute the cornerstone of essential human healthcare, being integral components of traditional medicinal systems worldwide (Shinde and Mulay 2015). Plants, by producing a broad spectrum of biologically active chemicals, emerge as a diverse source of pharmaceutical compounds (Nair, Thomas, and Loft 2005). Extracts derived from various parts of plants can be harnessed to address a spectrum of health issues, including but not limited to diarrhea, dysentery, cough, cold, fever, bronchitis, cholera, and various other ailments (Joshi et al. 2011). Preliminary investigations indicate that turmeric and curcumin exhibit sustained safety profiles even at elevated concentrations, showing no lethal effects. Consequently, both turmeric and curcumin possess the potential to be effective in treating a diverse range of diseases (Yadav et al. 2017). The purpose of this study was to evaluate the antibacterial activities of *Curcuma longa* and different antibiotics against *Staphylococcus aureus* and *Salmonella typhi.*

2. Materials and Methods

2.1. Sampling Site and Study Duration

The research was carried out at the Okara District Hospital, spanning from March 2021 to November 2021. The study adhered to the principles outlined in the Declaration of Helsinki. Informed consent was obtained from the patients participating in the study, granting permission for research and subsequent publication. Ethical approval for the research was acquired from the University of Okara.

2.2. Study Population

Data was gathered from a cohort of 50 typhoid patients in District Okara, with stool samples testing positive for Salmonella typhi bacteria in three cases. The age range of the patients was between 15 and 45 years. Fecal and stool samples were individually collected in aseptic plastic containers and promptly transported to the laboratory on ice. Bacteriological investigations were conducted immediately upon delivery (Aziz et al. 2018). Eye swabs were obtained from five cataract patients specifically for the presence of *Staphylococcus aureus*. The samples collected from these cataract patients were stored at a temperature of 4°C in a refrigerator.

2.3. Bacterial Isolation

To identify various pathogenic bacteria, all specimens underwent conventional microbiologic and biochemical assays. The isolates were cultured in nutrient broth for enrichment at 37°C for 20 hours (Getanda et al. 2017). Each stool sample was then streaked on Shigella agar (Becton–Dickinson Co., Sparks, MD, USA) for Salmonella species and incubated at 37 °C for 16–24 hours. For Salmonella isolation, enrichment of the obtained samples in buffered peptone water (1:10 dilution) was performed, followed by

Figure 1: The figure shows ¼ inch pieces of *C. longa,* left for air drying.

incubation for 18 hours at 37 °C, adhering to ISO 6579, 2002 (Aziz et al. 2018).

In an Eppendorf tube, a decimal dilution of the sample was made in 0.1 percent peptone water. Plate count agar (PCA) was divided into four sections and labeled individually to cater to different sample types. Four sequential dilutions within the range of 10^-1 to 10^-6 were prepared. Three drops of each dilution, totaling 10 µl, were separately inoculated onto each section of the plate count agar and incubated for 24 hours at 37°C to allow the formation of single colonies. After incubation, colony counts were determined from three drops of a specific solution, with the average colony count of those three drops reported as 3-30/10 µl. The overall bacterial count was expressed as colony-forming units (CFU) per gram or milliliter of the sample, and Salmonella species were enumerated using this method.

Staphylococcus aureus was isolated and identified using mannitol salt agar, a selective medium recommended by Chapman for isolating potentially pathogenic Staphylococci. Mannitol salt agar was employed to isolate *Staphylococcus aureus* bacteria, as it promotes the growth of specific bacteria while inhibiting the expansion of others (Shields and Tsang 2006).

2.4. Bacterial Confirmation

Gram stain and catalase tests were employed to identify colonies displaying typical morphological characteristics indicative of Salmonella and Staphylococcus bacteria (Ewing 1986, Foster 1996). Gram staining was conducted on *Salmonella typhi* to confirm the characteristics of these bacteria. The appearance of pink-colored, rod-shaped bacteria on the slide indicated *Salmonella typhi* (Taylor and Unakal 2021). However, *Staphylococcus aureus* was purple or violet, round-shaped, and in clusters (Taylor and Unakal 2021).

2.5. Preparation of Rhizome Extract of *Curcuma Longa*

The rhizomes of *Curcuma longa* were subjected to a cleaning process to eliminate mud, soil, sand, and other solid impurities. This was accomplished by washing with distilled water. Following this, the rhizomes were air-dried by cutting them into small pieces approximately 1/4 inch in size and allowing them to air-dry for two days, as depicted in Figure 1. Subsequently, the dried samples underwent additional drying in a hot air oven for 24 hours at 50°C. The resulting pieces were ground into a powder and passed through a strainer with a formal netting size of 2mm diameter. The powdered sample was then successively extracted with water and ethanol using a Soxhlet apparatus. The obtained extract was evaporated to dryness using a vacuum evaporator. The evaporated extract was vacuum-dried and stored in airtight containers until required.

For the preparation of stock solutions, concentrations were established by dissolving 40mg to 120mg of powdered turmeric in 1 ml of

Table 1: Antibacterial activity of *Curcuma longa* against *Staphylococcus aureus*.

Organism	Aqueous Extract			Ethanol Extract		
	Conc(mg)	**ZOI(mm)**	**Result**	**Conc(mg)**	**ZOI(mm)**	**Results**
Staphylococcus aureus	120	3	Resistant	120	18	Susceptible
	80	3	Resistant	80	14	Susceptible
	40	1	Resistant	40	13	Susceptible

each solvent, namely water and ethanol. The turmeric extract was created by blending 1g of powdered turmeric with 1ml of an aqueous solution and 1ml of 100% ethanolic solution.

2.6. Screening of Extract for Antibacterial Activity

To assess the antibacterial activity of *Curcuma longa* extract, the agar well diffusion method was employed. In this method, ciprofloxacin, ofloxacin, kanamycin, azithromycin, and ceftriaxone served as positive controls in the test, while water and ethanol were used as negative controls. This approach involves creating wells in an agar medium, into which the test substances (*Curcuma longa* extract and controls) are introduced. The diffusion of these substances into the agar allows for the evaluation of their antibacterial effects against the tested microorganisms.

2.7. Antibacterial Susceptibility

The Kirby-Bauer disc diffusion assay, endorsed by the National Committee for Clinical Laboratory Standards, was employed to assess the antibiotic sensitivity of each isolate. This assay involves measuring the inhibition zone of the bacterial expansion around the antibiotic disc, allowing for a rapid determination of the in vitro efficacy of the antibiotic. Mueller-Hinton agar medium was used for the disc diffusion tests. The susceptibility testing with disc diffusion was initially described by (Bauer 1966). The test organism was evenly distributed on the surface of Mueller-Hinton agar, and paper discs containing specified doses of antibacterial drugs were subsequently applied. The plate was then incubated at 35°C for 16-18 hours. After incubation, the diameter of the clear zone where the organism did not grow around the paper disc containing the antimicrobial agent was measured to determine the zone of inhibition (Wayne 2006). In accordance with the Clinical and Laboratory Standards Institute (CLSI) interpretation criteria, the zone of inhibition is utilized to categorize an organism as sensitive, intermediate, or resistant to antimicrobial drugs. This classification is based on established thresholds for each antibiotic, helping guide clinicians in determining the most effective treatment options (Alexander, Warnick, and Wiedmann 2009). The antibiotic discs employed in the study included ciprofloxacin (CIP) at a concentration of 5µg/disc, (Shariar and Kabir 2010), Additionally, ofloxacin (O) at 5µg and kanamycin (KAN) at 30µg were used for both bacteria to assess susceptibility and resistance patterns. Two different bacteria were inoculated onto separate nutrient agar medium (NAM) plates. Circular wells with a diameter of 6mm were created using an aseptic drill, positioned 2mm from the boundary of each plate. *Curcuma longa* extract dissolved in distilled water and ethanol was introduced into these wells. Antibiotic discs served as positive controls, while the extraction solvents, water, and ethanol were used as negative controls. The plates were allowed to stand for 1 hour to facilitate the diffusion of the extract from the walls. Subsequently, the plates were incubated at 37°C for 12-48 hours, and results were recorded after every 24 hours to observe the effects on bacterial growth and inhibition.

Table 2: Antibacterial activity of *Curcuma longa* against *Salmonella typhi*.

Organism	Aqueous Extract			Ethanol Extract		
	Conc(mg)	ZOI (mm)	Results	Conc (mg)	ZOI (mm)	Results
Salmonella typhi	120	2	Resistant	120	14	susceptible
	80	1	Resistant	80	11	susceptible
	40	1	Resistant	40	9	susceptible

3. Results

The ethanol extract of *Curcuma longa* rhizome demonstrated superior inhibitory effects compared to the aqueous extract against the tested pathogenic microorganisms. Specifically, the aqueous extract exhibited a very low zone of inhibition against gram-positive bacteria, ranging from 1mm to 3mm. There was a higher zone of inhibition for the aqueous extract at a concentration of 120mg/ml (3mm) compared to concentrations of 80mg/ml (3mm) and 40mg/ml (1mm). These findings suggest low susceptibility of the microorganisms to aqueous extract indicating low effectiveness (Table 1). Results also revealed that Ciprofloxacin exhibited a larger zone of inhibition (22mm) compared to Ofloxacin (20mm) and Kanamycin (20mm). In contrast, the ethanol extracts demonstrated zones of inhibition ranging from 13mm to 18mm. Notably, the highest zone of inhibition (18mm) was observed at a concentration of 120mg/ml, and as the concentration increased, the zone of inhibition also increased. Upon comparing the results (Table 1), it became evident that *Staphylococcus aureus* was highly susceptible to the ethanolic extract of *Curcuma longa.* This suggests that the ethanol extracts of *Curcuma longa* may provide a promising alternative for combating *Staphylococcus aureus*, showing comparable inhibitory effects to the antibiotics in the study.

The ethanol extracts of *Curcuma longa* exhibited a zone of inhibition ranging from 9mm to 14mm, against *Salmonella typhi*, with the highest inhibition zone observed at a concentration of 120mg/ml. A 14mm zone of inhibition indicates susceptibility to the ethanol extract. In contrast, different concentrations of aqueous extracts of *Curcuma longa* demonstrated very low activity against *Salmonella typhi*, with a zone of inhibition ranging from 1mm to 2mm (Table 2).

The antibacterial susceptibility test results revealed that *Salmonella typhi* exhibited high susceptibility against ciprofloxacin, azithromycin, and ceftriaxone. The zones of inhibition against *Salmonella typhi* were notably large, with ciprofloxacin showing a 22mm inhibition zone, azithromycin with a 21mm inhibition zone, and ceftriaxone with a 20mm inhibition zone. All these antibiotics utilized against gram-negative bacteria *Salmonella typhi* demonstrated high susceptibility.

4. Discussion

In the present study, ethanol extract of *Curcuma longa* demonstrated a significant inhibition zone against *Staphylococcus aureus* (gram-positive) at 18mm and *Salmonella typhi* (gram-negative) at 14mm. In contrast, the aqueous extract exhibited a very low zone of inhibition, ranging from 1mm to 3mm. These findings suggest that the ethanol extract of *Curcuma longa* is more effective in inhibiting the growth of both gram-positive and gram-negative bacteria compared to the aqueous extract.

It has been reported previously that ethanol extract of turmeric, at various concentrations, was effective against five bacteria: *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae,* and *Klebsiella pneumonia*. In contrast, both hot and cold-water extracts exhibited no activity against these bacteria. Methanol extract,

on the other hand, demonstrated moderate activity. Consistent with our study, these results indicate the prominence of ethanol extracts in displaying antibacterial efficacy across a spectrum of bacterial strains (Abbasi, Shah, and Science 2015).

In another investigation, the antibacterial susceptibility test results for five strains of pathogens (*Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Bacillus subtilis*) showed varying zones of inhibition. Against *Staphylococcus aureus,* the zone of inhibition ranged from 7mm to 30mm. With aqueous extracts of turmeric, the zone of inhibition ranged from 7mm to 25mm against *Salmonella typhi*. For *Pseudomonas aeruginosa,* the zone of inhibition ranged from 13mm to 23mm, and for *Bacillus subtilis*, it ranged from 10mm to 24mm (Adudu et al.). The findings indicating a very low zone of inhibition with aqueous extract in this study differ slightly from the results reported in the present study. These variations could be attributed to differences in experimental conditions, concentrations, extraction methods, or the specific turmeric samples used. It underscores the importance of considering various factors that may influence the outcomes of antibacterial susceptibility when comparing results across studies.

The results of the present study or more consistent with the reports published by Jamal and colleagues. In that report, Jamal and colleagues have shown that ethanol extract of *Curcuma longa* possesses inhibitory effects, with zones of inhibition measuring 25.2mm for *S. aureus*, 26mm for *S. epidermidis*, 22.2mm for *E. coli*, and 18.5mm for *B. subtilis*. No zone of inhibition was observed with the aqueous extract of *Curcuma longa* or by the inorganic components of this plant (Jamal et al. 2013). This consistency across studies highlights the possibility of ethanol as an extraction solvent in bringing out the antibacterial properties of *Curcuma longa* against the tested bacterial strains.

5. Conclusion

We conclude that *Curcuma longa* may serve as an alternative to synthetic drugs based on the observed antibacterial properties. The study highlights distinct results between ethanol and aqueous extracts of *Curcuma longa.* Specifically, the ethanol extract exhibited significant antibacterial actions, while the aqueous extract showed weaker activities. The effective antibacterial activities of the ethanol extract may be attributed to the presence of various curcumin-like compounds.

Conflict of Interest

The authors declare that they have no competing interests.

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Study Approval

The study was approved by the ethical review committee, the University of Okara, Okara, Pakistan.

Consent Forms

NA.

Authors Contribution

YN and SS conceptualized the study and wrote the final manuscript. IS, SR, and FT helped in the analysis and writing the first draft, did the experimental analysis, and YN supervised the whole project and wrote the final manuscript.

Data Availability

All the relevant data of this manuscript is available with the authors.

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