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# IN-VITRO EVALUATION OF *Curcuma longa L.* (TURMERIC) LEAF EXTRACT AGAINST URINARY TRACT INFECTION (UTI) - CAUSATIVE PATHOGEN *Escherichia Coli*

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## ABSTRACT

Urinary tract infections (UTIs) caused by *Escherichia coli* remain a significant public health concern, highlighting the need for alternative antibacterial treatments. This experimental study investigated the antibacterial potential of *Curcuma longa* L. (turmeric) leaf extract against *E. coli* using an in-vitro method. Three treatment groups were applied: turmeric leaf extract (experimental), distilled water (negative control), and ciprofloxacin (positive control). Phytochemical screening revealed the presence of flavonoids, tannins, and phenols in turmeric leaves, while alkaloids and saponins were absent. Antibacterial activity was assessed by measuring the zone of inhibition (ZOI) after 24 hours of exposure. Distilled water showed no inhibition (0.00 mm²), turmeric extract produced a moderate but significant ZOI (698.13 mm²), and ciprofloxacin exhibited the highest inhibition (1245.01 mm²). One-way ANOVA revealed significant differences among the treatment groups ($p < 0.05$), and Tukey's HSD test confirmed that all pairwise comparisons were statistically significant: Ciprofloxacin vs. Turmeric ($p = 0.045$), Turmeric vs. Distilled Water ($p = 0.016$), and Ciprofloxacin vs. Distilled Water ($p = 0.001$). These results suggest that while ciprofloxacin remains the most effective, turmeric leaf extract demonstrates notable antibacterial activity against *E. coli*, supporting its potential for future formulation development, in-vivo studies, and clinical applications.

***Keywords***: *Ciprofloxacin, Curcuma longa L. leaf extract, Escherichia coli, Experimental Research, In vitro, Philippines*


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## 1.0 INTRODUCTION

Urinary tract infections (UTIs) are among the most widespread bacterial infections, significantly impacting public health systems worldwide. Each year, approximately 150 million people suffer from UTIs (Totsika et al., 2012, as cited in Gebretensaie et al., 2023), with the number of reported cases rising from 252.25 million in 1990 to 404.61 million in 2019 (Dahal, 2022). This rising trend signals a serious and growing health concern, especially as UTIs continue to be associated with high recurrence and increasing mortality, particularly in healthcare settings (Foxman, 2022). The primary causative agent is Escherichia coli (E. coli), a Gram-negative bacterium known for its adaptability and growing resistance to commonly used antibiotics.

In Asia, UTIs continue to burden health systems due to the increasing antimicrobial resistance exhibited by uropathogenic strains. A study revealed a 9.8% prevalence of UTI cases from a sample of 6,706 patients, identifying E. coli as the predominant pathogen. Alarmingly, these strains have shown significant resistance to fluoroquinolones—antibiotics often used as first-line treatment (Choe, 2018, as cited in Alhazmi et al., 2023). This resistance highlights a pressing challenge in effective UTI management across the region.

In the Philippine setting, UTIs remain a common medical condition and were ranked as the third most prevalent disease in the country in 2020 (Statista Research Department, 2023). Data from the Provincial Health Office of Pangasinan reported an increase in UTI cases from 8,807 in 2012 to 15,881 in 2022, indicating a steady rise over the past decade. The combination of rising incidence and growing antibiotic resistance illustrates the need for new strategies to manage this infection more effectively.

Despite various efforts to discover alternative treatments, there is still a lack of focused studies on the use of turmeric (Curcuma longa L.) leaf extract in combating uropathogenic E. coli. While turmeric roots have been widely explored for their therapeutic properties, minimal attention has been given to its leaves, particularly in their potential as antibacterial agents. This gap in research presents an opportunity to explore underutilized plant parts as possible solutions to antibiotic resistance, a challenge of critical importance today.

In response to this issue, the present study seeks to evaluate the in-vitro antibacterial effect of turmeric leaf extract against E. coli using ethyl alcohol as a solvent. By exploring the efficacy of this plant-based alternative, the research aims to contribute to the development of safer, accessible, and cost-effective treatment options. Ultimately, the findings may serve as a foundation for future studies on herbal formulations, in-vivo investigations, and clinical applications, particularly in addressing the growing problem of UTIs and antimicrobial resistance.

### Statement of the Problem

The purpose of this study was to conduct an in-vitro evaluation of Curcuma longa L. (Turmeric) Leaf extract against *E. coli*. Specifically, this sought to answer the following questions:

1. What are the chemical compounds found in Turmeric leaves?
2. What is the measure of the area of the Zone of Inhibition (ZOI) of bacteria after twenty-four (24) hours of exposure to: Distilled water (Negative Treatment); Ciprofloxacin (Positive Treatment); and 100% solution of turmeric leaf extract (Experimental Treatment)
3. Is there a significant difference in the measure of the Zone of Inhibition (ZOI) among the setups?

### *Hypothesis*

The null hypothesis in this study was tested at 0.05 level of significance.

$Ho_1$: There is no significant difference in the measure of the Zone of Inhibition (ZOI) among the setups.

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***Conceptual Framework***

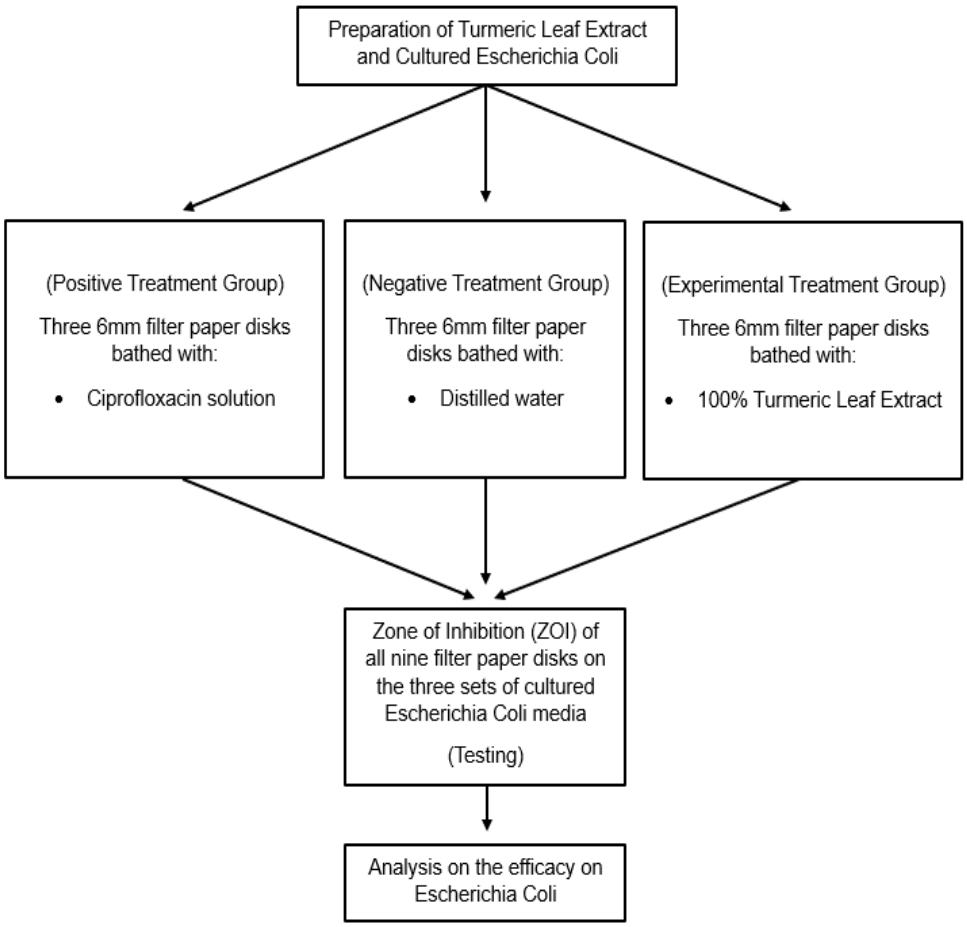
Turmeric (Curcuma longa) is known for its bioactive compound curcumin, which possesses antibacterial, anti-inflammatory, antioxidant, and anticancer properties (Jain et al., 2022). While research has primarily focused on the rhizome, studies confirm that turmeric leaves also contain curcumin (Kim et al., 2021), along with other phytochemicals such as alkaloids, flavonoids, saponins, glycosides, and tannins, which contribute to its potential antibacterial effects.

Curcumin has shown efficacy against both Gram-positive and Gram-negative bacteria by disrupting bacterial membranes, inhibiting virulence factors and biofilm formation, and inducing oxidative stress (Lin et al., 2022). These properties make it a promising alternative or adjunct to conventional antibiotics, especially amid the global issue of antibiotic misuse and resistance (WHO, 2014; Driscoll et al., 2017).

To explore this potential, this study focuses on assessing the antibacterial activity of turmeric leaf extract against Escherichia coli, the most common pathogen causing urinary tract infections (Nielubowicz & Mobley, 2010). The experiment involved preparing turmeric leaf extract and culturing E. coli. Three (3) E. coli cultures were exposed to 6mm filter paper disks treated with (1) 100% turmeric leaf extract, (2) ciprofloxacin as the positive control, and (3) distilled water as the negative control. The diameter of each zone of inhibition (ZOI) was recorded once measurable, up to a maximum of forty-eight (48) hours. Upon completion of the experiment, the antimicrobial effect of turmeric leaf against Escherichia coli was evaluated using statistical analysis.

Figure 1

*Conceptual Framework of the Study*



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### *Significance of the Study*

The findings of this study would be valuable to nursing program heads and faculty members, as they would provide insights that could strengthen the curriculum and guide policy decisions in integrating alternative therapies into nursing education. By considering these findings, they would be better equipped to ensure that instruction remains aligned with emerging evidence and relevant to the evolving needs of healthcare practice. Likewise, clinical instructors would benefit from these results, as they would serve as a foundation for imparting knowledge to their students, promoting awareness of natural remedies, and emphasizing the importance of keeping abreast of new studies and their potential clinical applications.

For nursing students, the study would enhance their understanding of the potential antibacterial properties of turmeric as an alternative treatment for urinary tract infections (UTIs). This knowledge would also prepare them to educate patients on diverse therapeutic options and encourage a holistic approach to care. Lastly, the study would be meaningful to future researchers, as it would provide a basis for further investigation into the antibacterial effects of turmeric, inspire deeper exploration into its impact on microbial infections, and contribute to addressing the growing problem of antibiotic-resistant strains of bacteria.

### *Scope and Limitations*

This study investigated the effectiveness of Curcuma longa L. (turmeric) leaf extract against the bacteria Escherichia coli, a gram-negative bacillus. The experiment aimed to identify the chemical compounds present in the turmeric leaf extract that possessed antibacterial properties. An in-vitro process was conducted in a microbiology laboratory. The study focused on measuring and comparing the zone of inhibition of 100% turmeric leaf extract (experimental treatment), ciprofloxacin (positive treatment), and distilled water (negative treatment) on three (3) petri dishes with cultured E. coli. The measurements were recorded after 24 hours to evaluate the efficacy of the extract.

The study was limited to its in-vitro nature, as the experiment was solely conducted in a laboratory setting, and thus could not fully evaluate how the extract would work within the human body (in vivo). Additionally, the research mainly focused on testing the effectiveness of the sample solely on Escherichia coli, without including other UTI-causative pathogens. Furthermore, there was a lack of standardization of the turmeric leaf extract, as the active chemical compounds were not quantified; therefore, variation in composition may have impacted the reliability and reproducibility of the outcomes.

### *Definition of Terms*

**Curcuma Longa L.** Turmeric, scientifically recognized as Curcuma Longa L is a plant that thrives for several years, belonging to the Zingiberaceae family of gingers. (Kumar et al, 2018). A lot of it is grown in Asia, mostly in India and China. In addition to its culinary use, turmeric has been widely utilized in traditional medicine throughout the world.

**E. Coli.** Escherichia coli (E. coli) is a bacterium classified as a gram-negative with a structure of a rod that causes various diarrheal diseases, including traveler's disease and dysentery. This bacterium is known to be the primary cause of uncomplicated UTIs like cystitis and is associated with several illnesses that are not related to the intestines, including bloodstream and intra-abdominal infections, pneumonia, and spontaneous bacterial peritonitis (Mueller et al., 2023).

**In Vitro Evaluation.** This pertains to experiments conducted on cells, tissues, or other biological elements extracted from the organisms of interest. (Jonathan, 2022). In this study, the In Vitro Evaluation is used to evaluate the Curcuma Longa L. Impact on the UTI pathogen E. coli. The Researchers can use this information to replicate the experiment and build upon the findings.

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## 2.0 METHODS

### *Research Design*

A posttest-only control group design is a study design used to compare the experimental and control groups using posttest measures only. A posttest-only control group design differs from a posttest-only design, in which all groups are treated, but no neutral comparison is made (American Psychological Association, 2018).

Figure 2 shows the Posttest-Only Control Group Design where the positive and experimental group are exposed to the treatments. However, the negative control group is only exposed to distilled water. The Post-test was conducted by gathering data from the Zone of Inhibition (ZOI), following the experimental and control groups.

Figure 2

*Model Design of the Study*


### *Subject of the Study*

The subject of this study is the Escherichia coli, a gram-negative bacillus that is a causative pathogen found to cause Urinary Tract Infection (National Library of Medicine, 2023). Escherichia coli is a broad and diverse genus of bacteria typically found in the environment, food, and people's intestines (Centers for Disease Control and Prevention, 2022). This research has three Petri dishes of cultured Escherichia coli.

This study utilized materials before, during, and after the experimental study. In the preparation of turmeric leaves, the materials used were 2000 mL distilled water, running tap water, scissors, three basins, and protective gear such as a lab gown, working gloves, face mask, and bouffant cap. In preparation for turmeric leaf extract, the materials used were scissors, blender, weighing scale, 95% Ethyl Alcohol, 1L beaker, aluminum foil, and protective gears. In the preparation of turmeric leaf filtration, the materials needed were filter paper, aluminum foil, 1L beaker, and protective gears. In rotary evaporation, the materials needed were protective gears, and rotary evaporators by assembling the main components: the rotary evaporator flask, condenser, vacuum pump, and rotary evaporator bath. In the phytochemical screening, various reagents specific to the test were used and conducted, such as Mayer's reagent (for alkaloids), Wagner's reagent (for alkaloids), Ferric chloride (for tannins), Lead acetate (for tannins), Shinoda reagent (for flavonoids), Folin-Ciocalteu reagent (for polyphenols), Ferric chloride or $FeCl_3$ (for polyphenols), Frothing test (for saponins), Haemolytic test (for saponins), and Silica gel or other adsorbents for Thin-Layer Chromatography (TLC) for separating and visualizing compounds, and protective gears.

In the preparation of the bacteria, the materials needed were protective gears, Tryptic Soy Broth (TSB) powder or prepared liquid TSB, distilled water, autoclave for sterilization, sterile glass such as flasks or tubes, inoculating loop or sterile pipettes, Bunsen burner, incubator set, and Mueller-Hinton Agar plates. In the preparation of the ciprofloxacin solution, the materials used were protective gears, Ciprofloxacin (500mg), sterile distilled water (10ml), analytical balance, magnetic stirrer, and sterile containers or vials. In the preparation of the in-vitro process, the materials used were three Petri dishes with Mueller Hinton Agar (MHA) swabbed with *Escherichia coli* cultured in Tryptic Soy Broth (TSB), 6 mm filter paper disks with treatments, forceps, marker, ruler and protective gears. After the experimentation, the materials used were markers, rulers, and protective gear.

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***Treatments and their Applications***

The following treatments below were conducted in three replications in order to measure the diameter of each treated disk and determine the average zone of inhibition (ZOI) for the subject.

NT - applied with distilled water (negative treatment)
PT - applied with Ciprofloxacin solution (500mg/10mL)
ET – applied with Turmeric Leaf Extract

Figure 3

*Experimental Layout of the Study*

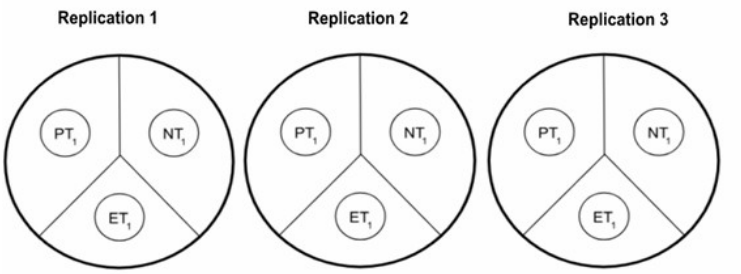


Figure 3 shows the experimental layout of the study, with the three Petri dishes of Mueller-Hinton Agar cultured with bacteria Escherichia coli. All three Petri dishes were divided into three divisions. Each Petri dish was introduced with one filter paper disk bathed in distilled water as negative treatment, one filter paper disk bathed in Ciprofloxacin solution as positive treatment, and one filter paper disk bathed in Turmeric Leaf Extract as experiment treatment, a total of three negative treatments, three positive treatments, and three experimental treatments. The Zone of Inhibition (ZOI) was measured after 24 hours with an incubation temperature of 35-37 °C after the intervention. Thus, there was only one recording.

***Experimental Procedure***

These scientific processes were followed accordingly during the conduct of the research:

***A. Pre-intervention***

Preparation of Turmeric Leaf

The researchers obtained fresh turmeric plants from the local farm of Ang's Family where plants were organically grown without any human intervention such as the use of fertilizers, pesticides, or even hormones. According to Singh et. al. (2011), unlike the commercially grown counterparts raised in controlled environments, naturally grown turmeric can lead to a broader spectrum of bioactive compounds in plants, potentially offering researchers a richer source of therapeutic or research targets.

Hence, the preparation of the Turmeric Leaf was done at home in an isolated room where prevention of contamination and safety of the process were measured. The said method in preparing the Turmeric Leaf was as follows:

a. Protective gears were used to ensure safety.
b. Materials needed were scissors, three basins, tray, running tap water, and 2L Distilled Water.
c. The petiole portion of the turmeric leaves were cut out to prevent contamination
d. They were cleaned and washed with running tap water and 3 cycled distilled waters.
e. After washing, the turmeric leaves were placed in a tray.
f. Fresh air dried the leaves in a place where sunlight was strictly prohibited.
g. After 24 hours, the leaves were totally dried.

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Preparation of Turmeric Leaf Extract

The conduct of the plant extraction was done at home through the process of Maceration. Farooq et. al. (2022) stated that maceration is an inexpensive homemade extraction technique that is used to extract essential oils and active compounds from plant materials. Therefore, this was done in an isolated room where prevention of contamination and safety of the process were measured. The said method of plant extraction was as follows:

a. Protective gears were used to ensure safety.
b. Materials needed were scissors, blender, weighing scale, 95% Ethyl Alcohol, 1L beaker, and aluminum foil.
c. The leaves were cut into 10 cm (height) and 2cm (width) before putting them in the blender.
d. The cut leaves were blended to turn into them small pieces.
e. The 500 grams of blended turmeric leaves were soaked in 650 mL of 95% ethyl alcohol in a 1L beaker.
f. The product was covered and sealed with aluminum foil and placed in the refrigerator at 4°C for 24 hours.

Preparation of Turmeric Leaf Filtration

After 24 hours of maceration, the researchers proceeded with filtration to remove solid particles of turmeric leaves and allow the desired compounds to dissolve and leach out. With the use of filter paper composed of cellulose fibers tightly woven into a matrix with microscopic pores. These pores allow liquid molecules to pass through while retaining larger solid particles on the surface due to size exclusion (Skoog et. al., 2017). Therefore, this process was done in an isolated room where prevention of contamination and safety of the process were measured. The said method of Turmeric Leaf Filtration was as follows:

a. Protective gears were used to ensure safety.
b. Materials needed were filter paper, aluminum foil, and a 1L beaker.
c. The product was filtered using filter paper at the wide mouth of the beaker.
d. The turmeric extract was slowly poured into the clean beaker.
e. For a clearer extract, the filtering process was repeated using another filter paper.
f. The filtered extract was put in a clean beaker that was covered with aluminum foil and sealed tightly.

Rotary Evaporation

The filtered extract was sent to a private institute in Davao Region for Rotary Evaporation. As cited in Chemistry Libretexts (2021), rotary evaporation is the process of thinly spreading a solvent over a vessel's interior at low pressure and high temperature. Using this technique makes it easier to quickly remove extra solvent from less volatile compounds. Thus, this procedure was conducted by the medical technologist of the said institute. The said method of Rotary Evaporation was as follows:

a. Protective gears were used to ensure safety.
b. Set up the rotary evaporator by assembling the main components: the rotary evaporator flask, condenser, vacuum pump, and rotary evaporator bath.
c. Ensured that the condenser was connected to a water source for efficient cooling. The cooling water helped in condensing the evaporated solvent vapor back into liquid form.
d. Connected the vacuum pump to the rotary evaporator. The solvent's boiling point was lowered by the vacuum, making it easier to remove at lower temperatures.
e. Placed the Turmeric Leaf extract in the rotary evaporator flask, making sure not to overfill the flask to allow for efficient evaporation.
f. Immersed the rotary evaporator flask into a heated water bath.
g. Started the rotation of the flask. The rotary motion increased the surface area of the extract exposed to the vacuum and heating, promoting faster and more uniform evaporation.
h. Adjusted the rotation speed, bath temperature, and vacuum level based on the properties of the solvent and the desired evaporation rate.
i. Carefully monitored the process to avoid bumping or splashing of the extract.
j. The solvent vapor traveled through the condenser, where it condensed back into liquid form.
k. Monitored the evaporation process and stopped it when the desired concentration or volume reduction was achieved.
l. Collected the desired concentration and put it in a clean container completely sealed.

m. After completion, disconnected the apparatus and cleaned all components thoroughly.
n. Disposed all the collected solvents appropriately.

Phytochemical Screening

According to Purkait et. al. (2022), various plant parts, such as the roots, bark, leaves, flowers, fruit, and seeds, contain bioactive phytochemicals. This analytic process involves the detection of diverse phytochemicals such as alkaloids, flavonoids, tannins, polyphenols, and saponins that may contribute to the plant's medicinal properties. Thus, this procedure was also conducted by the medical technologist of the said private institute in Davao Region. The said method of Phytochemical Screening was as follows:

a. Protective gears were used to ensure safety.
b. Various reagents specific to the test were used and conducted, such as Mayer's reagent (for alkaloids), Wagner's reagent (for alkaloids), Ferric chloride (for tannins), Lead acetate (for tannins), Shinoda reagent (for flavonoids), Folin Ciocalteu reagent (for polyphenols), Ferric chloride or FeCI3 (for polyphenols), Frothing test (for saponins), Haemolytic test (for saponins), and Silica gel or other adsorbents for Thin-Layer Chromatography (TLC) for separating and visualizing compounds.
c. Performed qualitative tests to estimate the concentration of specific phytochemicals if required.
d. Applied the Turmeric Plant extract to a thin layer of adsorbent material on a plate
e. Subjected the plate to a mobile phase (solvent), allowing compounds to separate based on their affinity for the adsorbent.
f. Visualized separated compounds using appropriate visualization techniques.
g. Confirmed the presence of compounds and recorded the results.
h. Collected the desired concentration and put it in a clean container completely sealed ready for intervention.

Preparation of Bacteria

The researchers reached out to medical technologist of one of the private hospitals in Tagum City, Davao del Norte, to purchase Escherichia Coli bacteria. Thus, the said private hospital was the same hospital conducting the in-vitro process. Therefore, there was no need for transportation. Tryptic Soy Broth was used in this method, it is a highly nutritious medium used for the cultivation of aerobes, anaerobes, fungi, and some bacteria (Murray et. al., 1999). Thus, the said method of preparing the bacteria was assisted by the medical technologist and was as follows:

a. Protective gears were used to ensure safety.
b. Two vials of Escherichia Coli were purchased.
c. Materials needed were Tryptic Soy Broth (TSB) powder or prepared liquid TSB, distilled water, autoclave for sterilization, sterile glass such as flasks or tubes, inoculating loop or sterile pipettes, Bunsen burner, incubator set, and Mueller Hinton Agar plates.
d. Prepared TSB in accordance with the manufacturer's instructions if using the powder or if using liquid TSB, ensured it is sterile.
e. Autoclaved the TSB to sterilize it. Autoclaving involved subjecting the medium to high-pressure steam at a temperature of 121°C for about 15 minutes.
f. Allowed the autoclaved TSB to cool at 30-33°C for bacterial growth.
g. Transferred the cooled TSB to the prepared three Petri dishes with Mueller Hinton Agar (MHA)
h. Used a sterile inoculating loop or pipette, and introduced the E. Coli bacteria into the TSB. This was done by transferring a small amount of bacterial culture into the broth.
i. Gently mixed the inoculated TSB to distribute the E. Coli bacteria evenly. Sealed the containers with caps or closures to prevent contamination.
j. Placed the sealed Petri dishes in an incubator set to a temperature of 35-37°C for 24 hours allowing the bacteria to grow and multiply.
k. Periodically checked the TSB cultured for signs of bacterial growth, Turbidity or cloudiness in the broth indicated bacterial growth.

Preparation of Ciprofloxacin Solution

Ciprofloxacin belongs to the fluoroquinolone family. It's frequently used to treat bacterial infections, such as urinary tract infections or UTIs (Thai et. al., 2023). In experimental procedures, the following is a general procedure for preparing a Ciprofloxacin solution:

a. Protective gears were used to ensure safety.
b. Materials needed were Ciprofloxacin (500mg), sterile distilled water (10ml), analytical balance,

magnetic stirrer, and sterile containers or vials.
c. Used an analytical balance to accurately weigh the 500mg of Ciprofloxacin.
d. In a sterile container with distilled water, added the weighted Ciprofloxacin 500mg
e. Stirred the solution gently to aid dissolution.
f. Stored the prepared Ciprofloxacin solution and readied for intervention.

***B. Intervention***

In-vitro Process

In-vitro culture is a method used in experiments to evaluate plant cell growth and development or tissues under controlled conditions using a nutritive culture medium (Norouzi et. al., 2022). This process was the actual test to determine the effectiveness of Turmeric Leaf Extract against Escherichia Coli, and this was conducted at the microbiology laboratory in a private hospital in Tagum City, Davao del Norte. The said method of the In-vitro Process was as follows:

a. Protective gears were used to ensure safety.
b. Materials needed were three Petri dishes with Mueller Hinton Agar (MHA) swabbed with Escherichia Coli cultured in Tryptic Soy Broth (TSB), 6 mm filter paper disks with treatments, forceps, ruler, and marker.
c. Three Mueller-Hinton Agar plates were divided equally into three parts using a marker and ruler.
d. The three equally divided parts were labeled as N.T. for Negative Treatment, P.T. for Positive Treatment, and E.T. for Experimental Treatment.
e. The 6 mm diameter circular-shaped filter paper disks were soaked in the different treatments.
f. Forceps were pre-heated and left to stand for a while.
g. Using a forceps, one 6 mm filter paper disk bathed in the distilled water was placed in the first Mueller-Hinton Agar with the label NT.
h. Procedure g was repeated on the other two Mueller-Hinton Agar with the label NT.
i. Forceps were pre-heated and left to stand for a while.
j. Using a forceps, one 6 mm filter paper disk bathed in the Ciprofloxacin solution was placed in the first Mueller-Hinton Agar with the label PT.
k. Procedure j was repeated on the other two Mueller-Hinton Agar with the label PT.
l. Forceps were pre-heated and left to stand for a while.
m. Using a forceps, one 6 mm filter paper disk bathed in Turmeric Leaf Extract was placed in the first Mueller-Hinton Agar with the label ET.
n. Procedure m was repeated on the other two Mueller-Hinton Agar with the label ET.
o. All three Agar plates were placed in the incubation period for 24 hours with a temperature of 35-37
°C.

***C. Post-intervention***

a. After 24 hours of intervention, the data were measured by the area of its Zone of Inhibition (ZOI) in millimeters using a ruler. The researchers sought the assistance of a Registered Medical Technologist to assist with the disposal of materials, treatments, and bacteria following the data collection.

## *Analysis and Interpretation*

The data were analyzed using the following statistical tools, which served to quantify results and determine relationships between variables.

**Mean**. Mean is the primary statistical measure that is defined as the average value attained. It is a calculated central value of a number set (Coleman, 2016). In this study, this statistical treatment was used to determine the average results of the measured area of the Zone of Inhibition (ZOI) in every group over several hours approximately from 0-48 hours with time intervals of 0, 2, 4, 6, 8, 10, 12, to 24 hours up to 48 hours after the intervention.

**Post Hoc Test.** The significance of group differences in mean pairs is ascertained using the Tukey's Honest Significant Difference (HSD) test. Following a one-way ANOVA, when the F-test reveals a significant difference between certain groups under investigation, Tukey HSD is frequently used. As therefore, the Post Hoc Test examines how different group means differ while controlling the experiment-wise error rate (Frost, 2023). It was utilized in this investigation to ascertain whether there was a notable distinction between the experimental group and the control groups.

### Ethical Considerations

The researchers adhered to safeguards to ensure compliance with ethical standards during laboratory procedures. Five key dimensions were observed, namely social value, risks, benefits and safety, transparency, adequacy of facilities, and qualifications of the researchers. The study had social value as it explored the potential of turmeric as an accessible and sustainable remedy for Urinary Tract Infections (UTIs), offering alternatives beyond conventional antibiotics. Risks were minimized through the implementation of safety protocols in facilities that complied with laboratory standards, under the supervision of qualified personnel. Transparency was upheld by ensuring that findings were unbiased, truthful, and free from manipulation, thereby guaranteeing the reliability of the results. The facilities used were adequate, with dependable resources and access to internet and library materials that strengthened data analysis and interpretation. Finally, the researchers, as nursing students, possessed the necessary skills such as critical thinking, attention to detail, collaboration, and time management, and were guided by a research adviser whose expertise ensured the validity, reliability, and overall success of the study.

## 3.0 RESULTS AND DISCUSSION

### Chemical Compounds Found in Turmeric Leaves

Table 1 presented the compounds found in the sample because of the phytochemical screening of the extract from turmeric leaves. The analysis's findings indicated that the turmeric leaf extract contained the bioactive compounds flavonoids, tannins, and phenols. Whereas alkaloids and saponins were not detected in the sample.

Table 1. *Chemical Compounds Found in Turmeric Leaves*

| Phytochemical Test | Turmeric Leaf Extract |
|---|---|
| Flavonoids | + |
| Alkaloids (Dragendorff's test) | - |
| Saponins | - |
| Tannins | + |
| Phenols | + |

Remarks: *(+) Present; (-) Not detected/Absent*

These compounds have similar antibacterial properties by breaking down the bacterial cells leading to cell death and inhibiting cell growth (Rasyadi, 2021). Aside from that, through in-vitro processes, the active compounds flavonoids, tannins, and phenols were also found to have a strong oxidant quality (Anyaoku et al., 2023). Hence, this links to the Turmeric's antimicrobial effect against bacteria.

The results aligned with the findings of the study conducted by Ilham (2018; as cited in Dohude et al., 2023) that the phytochemical screening revealed the presence of some bioactive compounds such as alkaloids, ethyl acetate, flavonoids, glycosides, saponins, tannins, and triterpenoids. Also, the study of El-Sayed et al. (2023) revealed the presence of alkaloids (0.73%), saponins (0.49%), tannins (1.10%), sterols (0.04%), phenols (0.09), and flavonoids (0.42%).

Nevertheless, alkaloids and saponins were not found in the sample, which was consistent with the conclusion made by Harsha et al. (2013; cited in Salma et al., 2022) in their study that the turmeric extracts utilized did not detect any alkaloids. Additionally, a study carried out in India also revealed that there were no saponins in the findings of the phytochemical screening (Pawar et al., 2014; as cited in Salma et al., 2022). Therefore, based on the previous studies, the differences in the results of qualitative phytochemical screening of a turmeric extract may have been due to varying factors such as the extraction process and specific parts of the plant used, which may have contributed to the concentration of phytoconstituents (Grover et al., 2021; Permatananda et al., 2020; Dohude et al., 2023).

### Measure of the Zone of Inhibition after Twenty-Four (24) Hours of Exposure to Treatments

Table 2 showed the area of the zone of inhibition of the groups treated with distilled water, ciprofloxacin solution, and the turmeric leaf extract after twenty-four (24) hours of intervention. With an average diameter of 1245.010 mm², it showed that the area of the zone of inhibition was greatest in the group treated with ciprofloxacin, which served as the positive control. Following this was the experimental group, the one treated with Turmeric leaf extract which had an average diameter of 698. 127 mm². Whereas the other groups demonstrated positive

outcomes, the negative control showed no result, as indicated by a mean measurement of 0.00 mm². These findings highlight the comparative effectiveness of Ciprofloxacin and Turmeric leaf extract in inhibiting bacterial growth E. Coli, underscoring the potential of Turmeric leaf extract as a natural antibacterial agent worthy of investigation for therapeutic applications.

Table 2. *Measure of the Zone of Inhibition after Twenty-Four (24) Hours of Exposure to Treatments*

| Treatment (Set Up) | Zone of Inhibition ($mm^2$) | | | |
|---|---|---|---|---|
| | Replicate 1 | Replicate 2 | Replicate 3 | Average |
| Positive Control *(Ciprofloxacin)* | 1417.71 | 1186.92 | 1130.4 | 1245.010 |
| Negative Control *(distilled water)* | 0 | 0 | 0 | 0 |
| Experimental *(Turmeric leaf extract)* | 847.8 | 932.58 | 314 | 698.127 |

This is supported by the study conducted by Odo et al. (2023), on the analysis of the antibacterial effects of turmeric against particular bacterias including E. coli wherein the findings showed that turmeric extract exhibited a zone of inhibition of 7mm. The p value of 0.22 indicated statistical significance. Moreover, a study by Afriyie et al. (2018; as cited in Shariati et al., 2022) showed that ciprofloxacin demonstrated higher efficacy compared to levofloxacin against uropathogens isolated at their quasi-government hospital with a sensitivity rate of 30 (69.8%) on Escherichia coli. Furthermore, the result affirmed the findings of Tchesnokova (2023), who asserted that ciprofloxacin is the most used treatment for urinary tract infections (UTIs). The empirical antibiotic therapy data aligned with Tchesnokova's assertion, showing that ciprofloxacin was the most frequently prescribed antibiotic, accounting for 54%.

**Significant Difference in the Measures of the Zone of Inhibition**

Table 3 highlighted the significant difference in the measures of the zone of inhibition between the groups. The computation revealed a computed p-value of 0.001, which meant that the null hypothesis was rejected. Therefore, there was a significant difference in the zone of inhibition between the groups treated with distilled water, ciprofloxacin, and turmeric extract.

Table 3. *Significant Difference in the Measures of the Zone of Inhibition*

| (I) Treatment | (J) Treatment | Mean Difference (I-J) | p-value | Remarks |
|---|---|---|---|---|
| Positive Control | Negative Control | 1245.010 | 0.001 | Significant |
| | Experimental | 546.883 | 0.045 | Significant |
| Experimental | Negative Control | 698.127 | 0.016 | Significant |

This further proved that ciprofloxacin effectively combats both Gram-positive and Gram-negative bacteria such as E. coli, efficiently treating various bacterial illnesses as an antibiotic therapy (Shariati, 2022; Agubata et al., 2023). Although, Turmeric leaf extract also exhibited antimicrobial properties (Zafar, 2020; cited in Dohude et al., 2023), due to the presence of following phytochemicals: polyphenols, flavonoids, tannins, alkaloids, curcumin, and saponins, which have characteristics effective against different pathogens by permeating and attacking the cell membranes and reducing its proliferation (Di Lorenzo et al., 2021; Rasyadi, 2021; Yan et al., 2021; Silva et al., 2023; Mieres-Castro & Mora Poblete, 2023).

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On the other hand, Table 4 presented the results of Post Hoc Comparison using Tukey HSD Test for identifying the control groups that made a significant difference in the measures of the zone of inhibition. The results revealed that between the positive and negative control, the mean difference between the two treatments was 1245.010 mm², with a computed p-value of 0.001. Meanwhile, for the positive control and experimental control, the mean difference between the treatments was 546.883 mm², with a computed p-value of 0.045, revealing a significant difference between the positive and experimental control. For the experimental and negative control, the mean difference between the two was 698.127 mm², with a p-value of 0.016, revealing a significant difference between the two treatments. Moreover, the findings indicated that the positive control, ciprofloxacin which was the commonly prescribed medication for urinary tract infections, was a more effective treatment with a mean difference of 1245.010 mm² compared to the experimental control, turmeric leaf extract, which had a mean difference of 698.127 mm².

Table 4. *Post Hoc Comparison Using Tukey HSD Test for the Measures of the Zone of Inhibition*

| Source of Variation | Sum of Squares | df | Mean Square | F | p-value | Remarks |
|---|---|---|---|---|---|---|
| Between Groups | 2336512.133 | 2 | 1168256.062 | 25.84 | 0.001 | Significant |
| Within Groups | 271258.958 | 6 | 45209.826 | | | |
| Total | 2607771.082 | 8 | | | | |

The outcome of the measures of the zone of inhibition of the treatments using the Tukey HSD Test aligned with the result of a study conducted by Diansyah et al. (2021), wherein the 12.5 % Curcuma longa extract exhibited a larger diameter of the growth of inhibition zone compared to other treatments against a bacterium with a result of 11.3867 ± 0.93611 mm. Although the control groups used in the study of Diansyah et al. (2021) both inhibited the growth of the bacteria, the larger diameter shows the effectiveness of the antibacterial activity of the C. longa.

## Conclusions

Based on the results of the study, the following conclusions were drawn regarding the antibacterial properties of turmeric leaf extract against *Escherichia coli*.

1. The turmeric leaves were found to contain flavonoids, tannins, and phenols.
2. After 24 hours of exposure, the measurements of the zones of inhibition revealed that Ciprofloxacin (positive treatment) produced the largest zone. The turmeric leaf extract (experimental treatment) showed a smaller yet notable zone of inhibition, while distilled water (negative treatment) exhibited no inhibition.
3. A significant difference was observed in the zones of inhibition among the positive, experimental, and negative treatments.

## Recommendations

Based on the findings of this study, the following recommendations are proposed to ensure the safe and effective development of Curcuma longa L. (turmeric) leaf extract as a potential treatment for urinary tract infections (UTIs):

1. Regulatory Authorities should review and evaluate comprehensive findings on turmeric leaf extract to consider its approval as a potential alternative treatment for UTIs.
2. Future Researchers should conduct in-vitro cytotoxicity tests on human cell lines to ensure safety, develop a stable and standardized formulation of the turmeric leaf extract, perform in vivo studies on animal models to assess pharmacokinetics, pharmacodynamics, and safety, and, if results are promising, carry out phased clinical trials to determine its safety and efficacy in humans.

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## 5.0 COMPETING INTERESTS

Authors have declared that no competing interests exist.

## 6.0 AUTHORS' CONTRIBUTIONS

Researcher 1 drafted and edited the initial manuscript, and literature searches, Researcher 3 designed the study and primarily led the conduct of the experimental procedure, statistical computation of the results of the Zone of Inhibition (ZOI). Researchers 2 and 4 were responsible for external communication and inquiries. All researchers have equally contributed in crafting the parts of the manuscript, and it was thoroughly reviewed, and approved.

## 7.0 CONSENT

No person is involved in the conduct of this study hence, consent is not applicable.

## 8.0 ETHICAL APPROVAL

This paper was submitted and reviewed by the St. Mary's College of Tagum Research Ethics Committee, and secured a certificate of exemption from review. Therefore, the study may be implemented without undergoing an expedited or full review as it does not involve human participants.

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