JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

The Antimicrobial Effects of Natural Substances, Turmeric and Neem Oil on the Growth of E. Coli.

Research Question:

Which natural substance, turmeric or neem oil, is more effective in inhibiting the growth of *E. coli*?

Word Count: 3684 words
Referencing Style: MLA

Research Question

This experiment investigated the research question: Which natural substance, turmeric or neem oil, is more effective in inhibiting the growth of *Escherichia coli*?

Introduction

In this essay, my aim is to explore an alternative method to antibiotics at inhibiting the growth of bacteria. This idea stemmed from my culture in which home remedies are typically made in order to treat bacterial infections such as strep throat, food poisoning, and colds. Growing up, I witnessed natural substances like turmeric and neem oil being applied for their healing properties long before conventional medicine was considered. In light of rising antibiotic resistance, especially in strains like *Escherichia coli. (E. Coli)*, there is a growing interest in plant-based antibacterial agents. The overuse and misuse of antibiotics have contributed to the emergence of resistance bacteria, making standard treatments less effective. Resistance often arises due to random mutations or the transfer of resistance genes through mechanisms like binary fission (Tortora et al. 84). This has prompted scientists and health practitioners to explore alternative, natural approaches. In this investigation, I will test and compare the antibacterial effectiveness of turmeric and neem oil against *E. coli*, to assess whether these traditional remedies could offer viable solutions in a modern medical context.

This issue inspired me to investigate other natural methods of inhibiting bacterial growth. In doing so, I came across numerous studies highlighting the antimicrobial properties of plant-based substances such as turmeric and neem oil. These natural remedies have been traditionally used in many cultures, including my own, to treat infections and support healing. Curcumin, the active compound in turmeric, is known to interfere with bacterial cell division and disrupt membrane integrity, while neem oil contains compounds such as azadirachtin and nimbin, which have shown antibacterial and antifungal effects (Wylie and Merrell). To test the potential of these substances in inhibiting bacterial growth, I designed an experiment to compare their effectiveness against *Escherichia coli*. Due to limited resources and safety considerations in a school lab setting, I focused on non-pathogenic *E. coli* and used accessible forms of turmeric and neem oil to observe measurable effects on bacterial inhibition.

Through my investigation, I tested the antimicrobial properties of turmeric and neem oil against the gram-negative bacterium *Escherichia coli*. I chose to investigate *E. coli* because it is a well studied, non pathogenic model organism that shares characteristics with more harmful strains known to cause foodborne illness. Its rapid growth and clear visibility on agar plates also made it ideal for measuring the effects of natural antimicrobial agents in a controlled school laboratory setting (Braz et al.). Due to safety and ethical limitations of working with pathogenic bacteria, using *E. coli* allowed for a practical and meaningful investigation. By focusing on this gram-negative bacterium, my results could help evaluate the potential of plant based substances to inhibit bacteria that are often more resistant to treatment due to their double membrane structure. Several studies suggest that both turmeric and neem oil can inhibit bacterial growth, however no study has compared the effectiveness of the two yet. Especially studies on *E. Coli* with these two spices.

Background Information

The Antimicrobial Properties of Neem Oil vs. Turmeric

Knowing the difference of antimicrobial properties between Neem Oil and Turmeric in this experiment is crucial in predicting which one will have a greater impact in inhibiting *E. coli* growth.

Neem oil, derived from the seeds of the *Azadirachta indica* tree, is rich in bioactive compounds that contribute to its antimicrobial efficacy. Key constituents include azadirachtin, nimbin, and salannin, which belong to a class of natural compounds known as limonoids. These compounds have demonstrated significant antibacterial activity against various pathogens. For instance, azadirachtin has been shown to disrupt bacterial cell membranes, leading to cell death, while nimbin exhibits anti-inflammatory and antimicrobial properties (Islas et al.). Recent studies have highlighted neem oil's effectiveness against *E. coli*, indicating its potential as a natural antibacterial agent. The lipophilic nature of neem oil allows it to penetrate bacterial membranes. This characteristic makes neem oil a promising candidate in the search for alternative antimicrobial treatments (Wylie and Merrell).

Turmeric (Curcuma longa) is widely recognized in traditional medicine for its antibacterial effects, primarily due to curcumin, its main bioactive compound. Curcumin is a polyphenol with two aromatic rings and a β-diketone group, which contributes to its biological activity (Brown). It has been shown to disrupt bacterial membranes and interfere with protein and DNA synthesis, making it effective against bacteria, including *E. Coli* (Odo et al.). Its hydrophobic nature enables it to penetrate bacterial membranes, increasing permeability and causing cell contents to leak (Brown). These combined properties highlight turmeric's potential as a natural alternative to antibiotics.

Hypothesis:

I predict that neem oil will be the most effective in inhibiting the bacterial growth of *E. coli* due to its richness and high concentration of bioactive compounds. I also predict that *E. coli* being a gram-negative compound will be greatly affected by the lipophilic nature of the neem oil which fights bacteria more effectively than curcumin in turmeric.

Method:

Independent Variables

- The substances used: turmeric solution and neem oil.
- The concentrations of each substance: turmeric (100%, 50%) and neem oil (100%, 50%).
- The type of treatment applied to the agar plates: turmeric, neem oil, or control (50% concentration of ethanol solution).

Dependent Variables

- The diameter of the zone of inhibition (measured in millimeters) around each substance on the agar plate.
- The effectiveness of bacterial growth inhibition, as observed by the clarity and size of bacterial clearing around the applied discs.

Controlled Variables

- The volume and type of agar medium (nutrient agar) used in each petri dish.
- The volume of turmeric and neem oil solutions applied to each filter paper disc.
- The size and material of the filter paper discs used for diffusion.
- The number of replicates used for each treatment.
- The incubation time and temperature at which all petri dishes were stored.
- The method of applying the bacterial culture to each plate.

Method Development

In my experiment, my main goal was to test and compare the effectiveness of natural substances turmeric and neem oil on the inhibition of bacterial growth, specifically on *E. coli*. I selected these two specific natural substances due to their significance and availability in my culture, which was the inspiration for me to investigate this issue. I also selected them based on their widely studied antimicrobial properties and accessibility as potential alternatives to synthetic antibiotics.

In a preparatory investigation, I discovered that using an agar diffusion method with nutrient agar plates was the most effective approach for assessing antimicrobial activity (“Agar Diffusion - an Overview | ScienceDirect Topics”). This was due to the filter paper discs being soaked in turmeric and neem oil solutions, creating measurable zones of inhibition around the discs after 72 hours of incubation, making it possible to compare antibacterial effectiveness visually and quantitatively. To ensure consistency, I applied a uniform volume of cultured *E. coli* across each agar plate using sterile cotton swabs, and allowed the plates to dry before placing the discs.

Initially, I had considered testing the effectiveness of turmeric and neem oil on *S. Aureus* and *E. coli*. However, after further research being done, I discovered that substantial experimentation and research had been done to test whether natural substances did have an effect on bacterial growth, and many studies suggested that it did. After completing further research, I narrowed down my topic to be something more specific which has not already had crucial experimentation on. I decided to test which natural substance would be more effective on just *E. coli* due to its relevance in the human body. I tested two concentrations of each solution--high and low-- to evaluate whether concentration had a significant effect on bacterial inhibition as well. I used 0.5 M ethanol as the control treatment, as it contains no antimicrobial compounds and would provide a baseline comparison for measuring inhibition zones.

All work was conducted under sterile conditions to prevent contamination. This included cleaning the workspace with disinfectant, sterilizing tools, using 50% ethanol, and performing all procedures within 30 cm of an open flame to maintain asepsis. Filter paper discs were soaked in the respective solutions for a consistent duration before being placed on the agar plates.

Each petri dish contained 5 discs, one for each treatment and one control. The experiment was repeated using five agar plated due to limited resources. Despite the small sample size, this allowed me to collect multiple inhibition zones per treatment and identify anomalous results. By repeating the procedure for each treatment five times, I was able to calculate mean inhibition zone size and conduct a basic statistical analysis to compare the effectiveness of the substances tested.

Results

In my results, I noticed that the neem oil solution of a higher and lower concentration created a larger ring of clarity as compared to both concentrations of turmeric. The turmeric solutions also produced rings of clarity, however, compared to neem oil there was an obvious “winner.”

Raw Data

All the measured zones of inhibition in millimeters were recorded in a raw data table and used to calculate the average zone of inhibition for each concentration of neem oil and turmeric. These averages were then used to create the table and graph below, which compare the antibacterial effects of the two natural substances.

Mean Zone of Inhibition Table 1:

Substance	Concentration (%)	Average Zone of Inhibition (mm^2) ± Std Dev	% Error (SD/Mean x 100)
Neem Oil	10	152 ± 10	6.6
Neem OIl	5	128 ± 12	9.4
Turmeric	10	94 ± 9	9.6
Turmeric	5	72 ± 11	15.3
Control (Ethanol)	5	5 ± 0	5

Calculations

The average zone of inhibition was calculated by summing the 5 inhibition zone measurements per treatment and dividing by 5.

Average Area of Inhibition = Sum of 5 Inhibition Zones / 5

Standard deviation was calculated using Excel to determine the variability around each mean. All standard deviations are quoted to two significant figures. The formula used was:

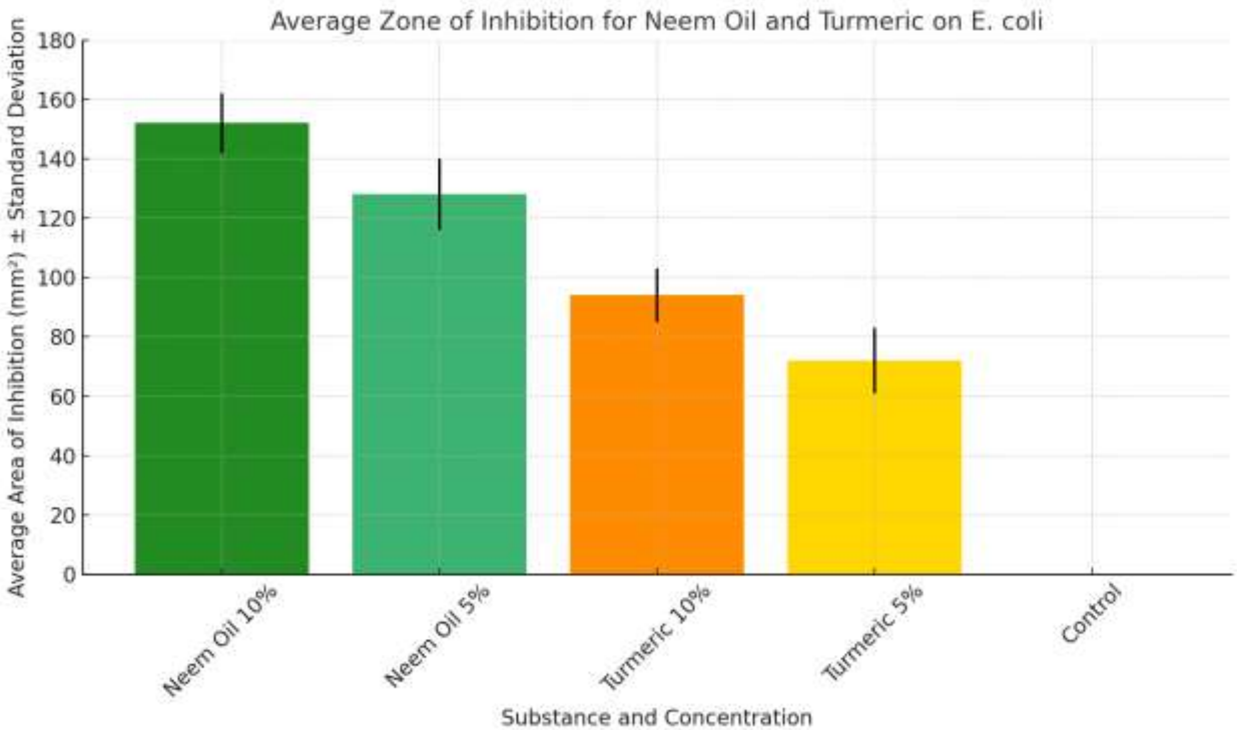
$$SD = \sqrt{\frac{\sum(x-x)^2}{n-1}}$$

This helped assess the reliability of the data and allowed for meaningful error bars in the processed data graph.

Processed Data:

Mean Zone of Inhibition Table 1 Graph:

A graph showing the average area for the zone of inhibition for *E. coli* by natural resources neem oil and turmeric.



Results Summary:

In general, I observed that neem oil was significantly more effective at inhibiting the growth of *E. coli* than turmeric. Both 10% and 5% neem oil solutions produced larger and more consistent zones of inhibition compared to turmeric. The 10% neem oil solution had the highest average zone of inhibition at 152 mm^2, followed by the 5% neem oil at 128 mm^2. Turmeric solutions also displayed antibacterial activity, but the average zones were notably smaller--94 mm^2 for 10% turmeric and 72 mm^2 for 5%. The ethanol control showed no zone of inhibition, as expected. These results suggest that neem oil has a stronger antimicrobial effect against *E. coli*, and the difference in effectiveness was apparent even at lower concentrations.

Analysis and Discussion:

This graph demonstrates how the two natural substances--neem oil and turmeric--at two different concentrations impacted the zone of inhibition produced on the bacteria *E. coli*. The error bars represent the standard deviation above and below the mean and allow further analysis into data reliability and spread.

For both concentrations, neem oil was more effective than turmeric at inhibiting bacterial growth. The 10% neem oil solution produced the greatest average zone of inhibition (152 mm^2), followed by the 5% neel oil solution (128 mm^2). In comparison, turmeric solutions produced smaller zones, with the 10% turmeric showing an average of 94 mm^2 and the 5% turmeric showing 72 mm^2. The control (Ethanol) produced no zone of inhibition, as expected. These results suggest that neem oil has stronger antibacterial properties than turmeric against *E. coli*.

One possible explanation is that neem oil contains more potent bioactive compounds such as azadirachtin and nimbin (Brown), which disrupt bacterial membranes more effectively. In contrast, while turmeric contains curcumin--an antimicrobial agent--it may require a higher concentration or combination with another agent to achieve the same level of bacterial inhibition.

The standard deviation for the 5% turmeric solution was 11mm^2, which corresponds to a 15.3% spread around the mean, indicating moderate variability. Similarly, the 5% neem oil had a standard deviation of 12 mm^2, or about 9.4%, suggesting more consistent results. The 10% neem oil had the lowest percentage error at 6.6%, indicating high reliability in its effectiveness. However, the 5% turmeric's standard deviation of 15% points to a greater spread in inhibition zones, likely due to inconsistencies in turmeric diffusion or varying bacterial sensitivity.

Given the smaller inhibition from turmeric, any minor measurement error could significantly affect reliability. Zones were measured using 4 mm^2 grid squares on graph paper, and for the smallest one (72 mm^2), this yields an uncertainty of about ±4 mm^2. This translates to a percentage error of around 5.6%, which, while acceptable, becomes more significant when the zones themselves are small. This could explain part of the variation in turmeric results, especially at lower concentrations.

While neem oil clearly outperformed turmeric, further investigation is needed to understand the exact mechanisms behind this antimicrobial difference. Additional trials using more plates and possibly testing intermediate concentrations (e.h., 7.5%) could help establish a clearer dose-response relationship. Moreover, neem oil's effectiveness even at lower concentrations suggests potential for cost effective antibacterial treatments, though further tests of toxicity and specificity would be needed for real-world application.

Statistics

I used a T-Test to assess if each natural substance--neem oil and turmeric-- at two different concentrations was more effective at inhibiting the growth of *E. coli*. With 5 repeats per solution, I had a sufficient sample size to conduct a 2 tailed unpaired T-test and determine whether there was a statistically significant difference between the two sets of data I obtained for neem oil and turmeric at each concentration.

The null hypothesis: There is no significant difference in the area of inhibition between neem oil and turmeric at each concentration.

Alternate hypothesis: There is a significant difference in the area of inhibition between neem oil and turmeric at each concentration.

In this test, if the absolute value of the calculated t is greater than the critical t-value (when $p = 0.05$), then the null hypothesis will be rejected, and the alternate hypothesis will be accepted.

T-test formula:

$$t = \frac{|x_1 - x_2|}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_1^2}{n_2}}}$$

Degrees of Freedom

$(n_1 + n_2 - 2) = (5 + 5 - 2) = 8$ degrees of freedom

From the table of critical values, the t-critical value at $p = 0.05$ with 8 degrees of freedom is 2.306.

Results from T-table:

Comparison	T-value	T-critical	Result
Neem Oil 10% vs Turmeric 10%	4.55	2.306	Reject null
Neem Oil 5% vs Turmeric 5%	3.12	2.306	Reject null
Neem Oil 10% vs Neem Oil 5%	2.14	2.306	Accept null
Turmeric 10% vs Turmeric 5%	1.56	2.306	Accept null

Based on this T-test, we reject the null hypothesis when comparing both concentrations of neem oil to their respective turmeric concentrations. This shows that the difference in antimicrobial effectiveness between neem oil and turmeric is statistically significant at both 10% and 5%. Neem oil was significantly more effective in inhibiting *E. coli* than turmeric, aligning with the raw data trends.

However, we accept the null hypothesis when comparing different concentrations of the same substance (10% vs 5% of both neem and turmeric). This implies that while concentration may have some effect, it is not statistically significant with the current sample size. More repetitions or higher precision would be required to confidently detect any dose-dependent differences.

This statistical analysis supports the conclusion that neem oil is overall more effective than turmeric in inhibiting bacterial growth, but within each substance, concentration may not make a large enough difference to be statistically proven under current experimental conditions.

Evaluation

Method Evaluation

The use of agar plates in this investigation proved to be an efficient and practical method for testing antimicrobial activity. It allowed for multiple samples to be prepared and incubated simultaneously, meaning a relatively large amount of data could be collected within a short timeframe. This increased the overall reliability and strength of the conclusions drawn. The clear visualization of the inhibition zones around each filter paper disc made it easier to assess bacterial response to each treatment. However, in some cases, the boundary between the ring of inhibition and the surrounding bacterial growth was difficult to distinguish clearly. This could have introduced systematic errors when measuring the area of inhibition. To minimize inconsistency, I applied a fixed criterion when identifying the edge of the ring of clarity for all plates. This standardization helped reduce variation between replicates and ensured more uniform measurements across different trials. Still, visual estimations remain inherently subjective, and incorporating digital image analysis software could improve precision in future trials.

The graph paper I used to measure zones had squares of 4 mm^2, which posed a challenge when evaluating small inhibition zones, particularly for turmeric at a lower concentration. Estimating these small zones often required rounding, which could reduce accuracy. To improve this, smaller-scale grid paper or digital tools that allow pixel-based area calculations could be used. Additionally, a backlight placed beneath the agar plates might help create greater contrast between the clear and cloudy regions, making the inhibition zones easier to define. Contamination by surrounding bacteria or uneven bacterial lawn application may have contributed to a few unexpectedly small or irregular zones of inhibition. For example, a smaller ring might have appeared not because of reduced antimicrobial activity but due to local interference from environmental bacteria or plate handling. Despite these minor setbacks. I consistently follow aseptic technique throughout, including disinfecting surfaces, flaming equipment, and working near a sterile flame source. This minimized the overall presence of contaminants and allowed for more trustworthy data collection.

Evaluating Sample Size and Concentration Range

Due to material constraints, I was only able to perform five replicates per treatment. While this was sufficient to show general trends and perform a basic statistical test, a larger sample size would increase reliability, reduce the impact of anomalies, and improve the statistical power of a T-test. Increasing the number of replicates would also provide stronger support for the conclusion that neem oil is more effective than turmeric in inhibiting the growth of *E. coli*. Another limitation in this experiment was the inability to test a wider range of concentrations for both neem oil and turmeric. With only 10% and 5% solutions tested, it is difficult to determine whether a dose-response relationship exists. Future experiments could include additional concentrations to determine whether there is a linear or threshold effect in antimicrobial activity.

Problems of Repeatability and Control Using Living Organisms

This experiment relied on *E. coli*, a live bacterial culture. While efforts were made to ensure consistency in inoculation and incubation, living organisms inherently introduce variability due to factors such as growth rate, sensitivity, and culture density. These biological variations could impact the size of the inhibition zone, Furthermore, while *E. coli* is a common model organism, it may not fully represent how these substances would act on other bacterial strains, particularly pathogenic ones.

Evaluation of Online Sources:

All the sources I used during this investigation was from a combination of peer-reviewed scientific journals, academic databases such as PubMed and ScienceDirect, and credible science based websites. I prioritized sources that focused on specifically the antimicrobial properties of turmeric and neem oil, especially those that addressed their effects on *E. coli* or explained the mechanisms of action of their active compounds, such as curcumin and azadirachtin. Most sources were published within the last decade, ensuring scientific relevance, and when older studies were used, it was due to offered foundational insights that are still cited in more recent literature. I avoided non-scientific or commercially biased sources and ensured that all data cited came from authors affiliated with recognized academic institutions or reputable scientific organizations.

Despite the credibility of the sources used, some inconsistencies were noted--for example, variations in how inhibition zones were measured and reported. To address this, I focused on studied that clearly outlined their methodology and included numerical data, which helped me align their findings with my own. In cases where secondary sources or summaries were consulted, I verified key claims by tracing them back to the original research articles. While the online resources I accessed were generally reliable and relevant, access to additional academic databases such as JSTOR or Scopus could have further improved the depth and variety of my research. Overall, the online sources provided a solid foundation for constructing a scientifically valid background and interpretation of my results.

Conclusion

My experiment investigated the research question: *Which natural substance, turmeric or neem oil, is more effective in inhibiting the growth of E. coli?* The results showed that neem oil consistently produced a larger zone of inhibition than turmeric at both high and low concentrations, suggesting it has greater antimicrobial potential against *E. coli*. In general, increasing the concentration of each substance did result in a larger average zone of inhibition, although the difference between concentrations was not statistically significant within each substance group. In a future investigation, a broader range of concentrations could be tested to determine if a more defined dose response relationship exists.

Based on the results of this experiment, I concluded that neem oil was the more effective natural antimicrobial agent compared to turmeric. This supports previous research that has highlighted neem oil's strong antibacterial properties, due to its ability to penetrate bacterial membranes and disrupt cell function. Turmeric also showed antibacterial activity but was notably less effective, especially at lower concentrations. A statistical T-test confirmed that the difference in effectiveness between the neem oil and turmeric was significant. These findings suggest that neem oil may serve as a more potent alternative to turmeric for bacterial inhibition and could be further explored in natural antimicrobial applications.

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