International Research Journal of Pharmacy and Medical Sciences
ISSN (Online): 2581-3277

Antibacterial Activity of Lemon Juice Extract Against *Pseudomonas aeruginosa* and *Escherichia coli*

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***Abstract*—** *This study investigated the antibacterial activity of aqueous and ethanol dilutions of lemon juice against Pseudomonas aeruginosa and Escherichia coli, utilizing standard microbiological techniques. The antibacterial efficacy was assessed by measuring the zones of inhibition produced by different concentrations of the dilutions. Results demonstrated a concentration-dependent increase in antibacterial activity. For the ethanolic dilution, Escherichia coli exhibited zones of inhibition of 15.5 mm, 5 mm, 4.5 mm, 4 mm, and 4.5 mm, while Pseudomonas aeruginosa showed zones of 16 mm, 15 mm, 13 mm, 9.5 mm, and 7.5 mm at concentrations of 100mg/ml, 75mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml, respectively. The aqueous dilution also showed antibacterial activity, with Escherichia coli with zones of 13 mm, 10 mm, 6.5 mm, 2 mm, and 1.5 mm, and Pseudomonas aeruginosa zones of 9 mm, 6 mm, 9 mm, 8 mm, and 4 mm at the same concentrations. The minimum inhibitory concentration (MIC) of the ethanolic dilution was found to be 50mg/ml for Escherichia coli and 25mg/ml for Pseudomonas aeruginosa, while the aqueous dilution observed was 100mg/ml concentration to inhibit both bacteria. These findings suggest that lemon juice dilution, particularly ethanolic, have potential as natural antibacterial agents.*

***Keywords*—** *Bacteria; Escherichia coli; Ethanol; lemon extract; natural antibacterial agents; Pseudomonas aeruginosa.*

I. INTRODUCTION

For centuries, plants have traditionally been a cornerstone for natural health products in which their medicinal properties have been extensively utilised throughout history[1]. Globally bacterial infection remains a significant cause of morbidity and mortality which more often results from treatment failure or due to their genetic ability to acquire resistance to drugs [2]. According to World Health Organization, traditional medicines in some instances have provided solutions to issue of drug resistance. Medicinal plants are considered the optimal resource for obtaining a diverse range of medications. Roughly 80% of individuals in developed countries utilize traditional medicine, incorporating compounds sourced from medicinal plants. Hence, there is currently renewed interest in the traditional system of medicine especially in developing countries of the world due to the realisation of the need to use innovative approach of utilising locally accessible medicine and therefore reducing the reliance on expensive imported drugs and its attendant economic, therefore, such plants should be investigated to better understand their properties, safety and efficiency [3,4]. Lemon is medicinal plant from the family Rutaccae. It is used for its alkaloid propeties, which have been reported to having anticancer activities as well as its antibacterial potentiald in crude extracts of different parts ranging from leaves, root, stem, flower, juice, and peels which have been reported to antibacterial against bacterial of clinical importance [5,6]. Lemon juice possesses notable antimicrobial properties attributed to its unique chemical composition, primarily comprising organic acids, phenolic compounds, vitamins, and essential oils. Lemon juice demonstrates broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria due to its acidic pH and bioactive constituents. Lemon juice have been shown to inhibit the growth of *Escherichia coli*, including pathogenic strains such as *Escherichia coli* O157:H7, through its acidic environment and the presence of antimicrobial compounds like citric acid and flavonoids [7]. The citric acid in lemon juice significantly inhibits the growth of *Escherichia coli* by more than 3 log CFU/ml within a few hours. This has also been attributed its acidic pH and presence of other bioactive compounds [8]. The acidic environment disrupts the integrity of the bacterial cell membrane, leading to cell lysis. Flavonoids such as hesperidin and naringin found in lemon juice have been shown to interfere with Escherichia *coli* cell division and DNA replication [9]. The aim of this study is to evaluate the antibacterial activity of lemon juice extract against *Pseudomonas aeruginosa* and *Escherichia coli*.

II. MATERIALS AND METHOD

Study Design and Site

The study was an *in-vitro* quantitative experimental study to determine the antimicrobial activity of ethanolic and aqueous extracts of citrus lemon juice

Plant Collection and Identification

Twenty (20) fresh lemons (*Citrus limon*) were purchased from Ughelli Main Market, Delta State Nigeria. Thereafter transported to the Pharmaceutical Microbiology Laboratory at Site III of the Delta State University, Abraka for analysis. The samples were identified at species level in the Department of Botany.

Preparation of Citrus Lemon Juice

The citrus lemon juice extraction was carried out following an adjusted protocol previously outlined [10,11]. The fresh citrus lemons were washed with distilled water and their surfaces wiped with cotton wool moistened with 70% alcohol. A spatula delicately removed the pericarp before the juice was

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strained into a sterile 100 mL beaker. With the use of an electric blender, about 100 g of the pulp was broken up in distilled water. After sieving, the resulting extract was carefully transferred into a clean, sterile 100 mL beaker. Both the lemon juices from different citrus fruits were tested for their antibacterial activity.

Experimental Microorganisms

Pseudomonas aeruginosa and *Escherichia coli* were obtained from the Pharmaceutical Microbiology Laboratory of the Delta State University, Abarka. The samples were inoculated in a blood agar base using a sterile swab stick and incubated overnight at 37.8ºC to obtain multiple colonies.

Antimicrobial Screening

Standardised test of *Pseudomonas aeruginosa* and *Escherichia coli* isolates suspension (0.5 x 108 McFarland standard) was inoculated uniformly on the entire on the Agar base by steaking. And four wells measuring 8mm in diameter were made using a sterile cork borer on each plate.

Ampiclox served as the positive control, while 50µL of sterile distilled water was used as the negative control. The experiments were conducted in triplicates. The streaked plates were carefully positioned in a laminar flow hood for 30 minutes, allowing the extract to diffuse. Afterwards, the plates were moved to a bacteriological incubator preset at 37. 8°C for 24 hours. The zones of inhibitions resulting from the experiment were measured following the Clinical and Laboratory Standards Institute (CLSI) guidelines.

III. Results

Figure 1 shows a graphical representation of the zones of inhibition (in millimetres) of the test organisms across different concentrations (100mg/ml, 75mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) of the ethanolic dilution of lemon juice. *Pseudomonas aeruginosa* showed a more consistent and higher across concentrations when compared with *Escherichia coli* which recorded a drop in inhibition at a lower concentration.

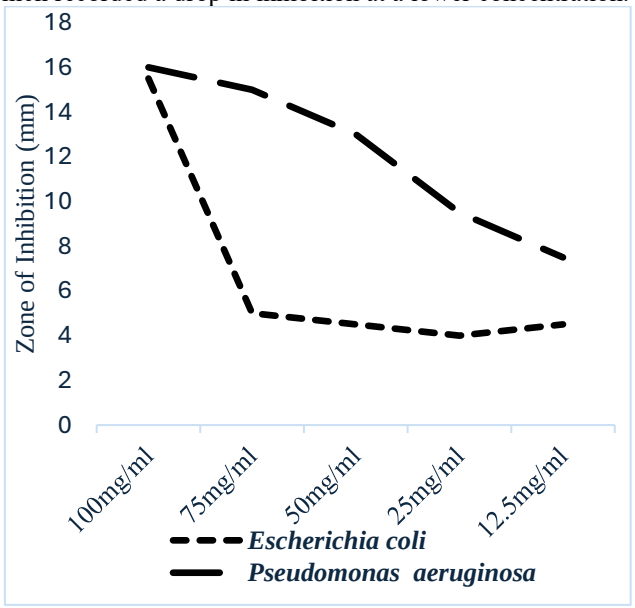

Figure1: Zones of inhibition of lemon juice ethanol dilution at the different concentrations

At 100mg/ml concentration, against *Pseudomonas aeruginosa* and *Escherichia coli* exhibited significant zones of inhibition (16mm – 14mm, respectively). With decreasing concentrations, *Pseudomonas aeruginosa* showed a gradual decline in inhibition which implies that resistance due to decrease in effectiveness. On the other hand, there was a sharp decline between 100mg/ml - 75mg/ml drooping from 15.5mm to 5mm, hitting a minimum at about 25mg/ml.

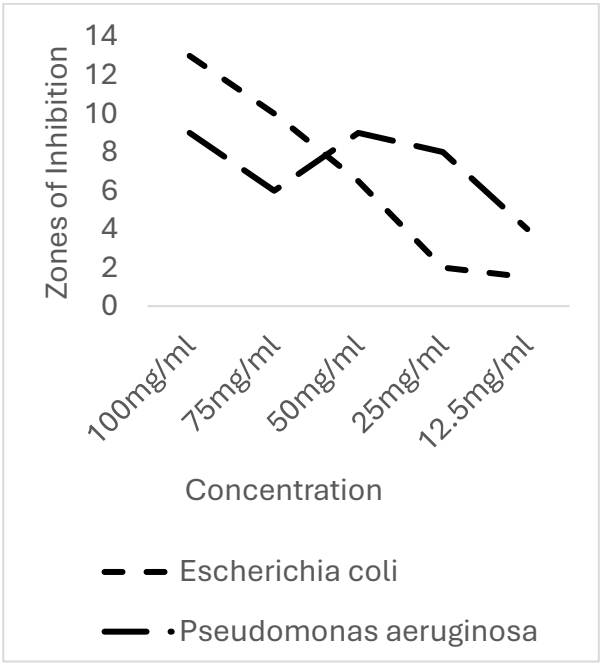

Figure 2: Zones of inhibition lemon juice aqueous dilution at different concentrations.

IV. Discussion

The results obtained from this study demonstrate that both ethanolic and aqueous dilution of lemon juice possess antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli.* The zones of inhibition recorded in this study provide insight into the efficacy of these extracts at various concentrations and reveal a concentration-dependent relationship where higher concentrations exhibited greater antibacterial effects.

From this study, it was observed that the ethanolic dilution is more effective against *Pseudomonas aeruginosa* at higher concentrations compared to *Escherichia coli.* The higher zone of inhibition across all concentration indicates the greater sensitivity of *Pseudomonas aeruginosa* to the lemon juice extract. These non- liners response imply that a certain concentration is required to effectively inhibit the growth of *Escherichia coli* below which the organism is more likely to survive or adapt.

The minimum inhibitory concentrations (MICs) of the ethanolic extract were 50mg/ml for *Escherichia coli* and 25mg/ml for *Pseudomonas aeruginosa,* further substantiating the potent antibacterial activity of the ethanolic dilution. The aqueous dilution also exhibited antibacterial activity, although it was less potent compared to the ethanolic extract. The MIC for both *Escherichia coli* and *Pseudomonas aeruginosa* was

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100mg/ml, indicating that a much higher concentration of the aqueous dilution is required to inhibit bacterial growth.

The findings from this study align with existing literature that highlights the antibacterial properties of lemon juice, and its extracts exhibited considerable antibacterial activity against a range of gram-negative bacteria, including *Escherichia coli* and *Pseudomonas aeruginosa* [12]. The present study indicated that the ethanolic dilution was more potent compared to the aqueous dilution. This have been attributed to the enhanced solubility and extraction of active phytochemicals in ethanol [13]. Lemon juice extracts showed significant zones of inhibition against *Escherichia coli* at higher concentrations, further emphasising that the presence of citric acid, flavonoids, and ascorbic acid in lemon juice contributes to its antimicrobial efficacy. Additionally, the study noted that ethanol serves as an excellent solvent for extracting these compounds, which is consistent with the results of the current findings. Ethanolic extract of citrus fruits, including lemon, had substantial antibacterial activity against both gram-positive and gram-negative bacteria [14]. The MIC values (100mg/ml and 50mg/ml concentration) reported were in a similar range to those found in this study, indicating the effectiveness of lemon extracts in inhibiting bacterial growth at higher concentrations. The variation in the efficacy of the aqueous extract compared to the ethanolic extract can be attributed to the differential solubility of active compounds in water and ethanol. Ethanol, being less polar than water, can dissolve a wider range of phytochemicals, including non-polar compounds that might contribute significantly to the antibacterial activity [13,15,16].

In conclusion, our study demonstrates that lemon juice dilutions, particularly the ethanol dilution, possess substantial antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa*. These findings indicated the potential of natural extracts as alternatives or complements to synthetic antibiotics. Future research should focus on elucidating the mechanisms of action, optimizing extraction techniques, and assessing safety for broader applications in food preservation and pharmaceutical formulations.

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