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PHYTODISINFECTION OF WASTEWATER: EFFICACY OF *OCIMUM GRATISSIMUM* LEAF EXTRACT

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MSc ENVIRONMENTAL ENGINEERING AND MANAGEMENT

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DECLARATION AND CERTIFICATION

I, Agyei Awuah Michael (UEMS0501023), hereby declare that this submission is my own work towards the Master of Science (MSc.) Degree in Environmental Engineering and Management, and that, to the best of my knowledge, it contains no material previously published by another person, nor materials which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text

..... …….....

Candidate's Signature Date

This study was conducted under the supervision of Dr. Nana Osei B. Ackerson, in accordance with the guidelines for the supervision of graduate studies.

Ing. Dr. Nana Osei B. Ackerson ..... ……..……...

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Ing. Prof. Francis K. Attiogbe ..... ……..……...

(Head of Department) Signature Date

ABSTRACT

Waterborne microbial pathogens continue to pose a significant public health challenge, especially in resource-limited settings. While plant-based disinfectants offer a promising, sustainable alternative, their direct application in complex water treatment remains underresearched. This study evaluated the antimicrobial efficacy of ethanolic *Ocimum gratissimum* leaf extract against *Escherichia coli*, a primary indicator of microbial contamination. The extract's phytochemical profile was characterized using Fourier Transform Infrared (FTIR) spectroscopy to identify key functional groups. Antimicrobial activity was initially confirmed via a disc diffusion assay, followed by quantitative determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) using broth microdilution. The extract's dose-dependent efficacy was then assessed in both simulated contaminated water and real wastewater samples.

The results showed that *O. gratissimum* extract exhibited significant inhibitory and bactericidal activity against *E. coli*. In sterile contaminated water, a concentration of 50% (v/v) achieved a ≥99.9% microbial reduction, confirming its potential as a potent disinfectant. However, its effectiveness was reduced in real wastewater, where a 50% concentration achieved approximately 71% microbial reduction. This reduced efficacy is attributed to the high-strength wastewater matrix, characterized by high levels of turbidity (800 NTU) and total suspended solids (TSS) (1,400 mg/L). The study's findings confirm the extract's strong dose-dependent antimicrobial potential and its value as a complementary, plant-based disinfectant for decentralized water treatment. This research provides novel insights into the practical application of *O. gratissimum*, suggesting its potential as a sustainable solution in resourceconstrained areas.

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TABLE OF CONTENT

Contents

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1. Background

Water is an essential resource for sustaining life, yet its contamination with microbial pathogens remains a significant health challenge, particularly in developing countries (Treacy, 2019). In many developed regions, the increasing demand for water has led to increased water stress, while in developing communities, the lack of adequate sanitation infrastructure and the prevalence of open defecation make untreated sewage a primary source of contamination (Mustafa & Hassan, 2024; Vega Andrade et al., 2021). The health and socio-economic implications of water contamination are profound. Sewage harbours a variety of pathogenic microorganisms, including bacteria, viruses, and protozoa, which contribute significantly to the global burden of waterborne diseases such as diarrhoea, cholera, and dysentery (Gerba & Pepper, 2019; Shayo et al., 2023).

Effective water treatment is critical for removing contaminants that pose health risks to consumers. Conventional water treatment techniques typically involve a combination of physical, chemical, and biological processes designed to eliminate pathogens and enhance water quality. Physical processes such as filtration and sedimentation serve as preliminary steps, significantly reducing microbial loads but not eliminating them entirely (Kumar et al., 2024). These initial methods are commonly followed by disinfection stages to ensure higher microbial removal efficiency. Among the most widely used disinfection techniques are chlorination, ultraviolet disinfection, and boiling, particularly for small-scale or household applications. Roy et al. (2016) assessed the efficacy of the various disinfection methods for developing countries. They assessed different water sources, including treated drinking water, groundwater, and pond water. While all methods were effective under the right conditions, each presented challenges in terms of energy demand, time requirements, accessibility, and cost.

Among these, chlorination was identified as the most practical option, not without concerns as well. Research has further identified several classes of DBPs, such as haloacetic acids (HAAs), trihalomethanes (THMs), and nitrosamines that result from chlorination and ozonation (Wu, 2020). While chlorine remains the most widely used chemical disinfectant due to its affordability and effectiveness, its long-term implications for human health warrant serious consideration (Kumar et al., 2024).

In recent years, plant-based antimicrobial agents have emerged as a viable, cost-effective, and environmentally sustainable alternative for water treatment, particularly in low-resource settings. These natural materials are not only locally available and safe for use, but they also produce biodegradable by-products, thereby minimizing environmental impact (Lwasa et al., 2024; Tiruneh et al., 2023). In terms of efficacy, many plant-based methods perform comparably to conventional chemical treatments. Numerous studies have confirmed that certain plant species exhibit significant antimicrobial activity (Adeeyo et al., 2020). Vega Andrade et al. (2021) used *Moringa oleifera* as a natural coagulant, significantly reducing turbidity and particulate matter in contaminated water. Similarly, Adeeyo et al. (2020) investigated the antimicrobial properties of ethyl acetate extracts from *Zanthoxylum zanthoxyloides* and *Gongronema latifolium*, reporting positive outcomes against various fungal and bacterial isolates. Pritchard et al. (2009) also reported that aqueous extracts of *Moringa oleifera*, guar gum, and *Jatropha curcas* improved water clarity and purity.

Much of the focus on antimicrobial plants has been given to their medicinal purposes, with little research on their use for water treatment. This study explores the use of *Ocimum gratissimum* as a natural, plant-based solution for treating microbial contamination in water, providing an affordable, accessible method to ensure water safety.

Ocimum gratissimum, commonly known as African basil, is a widely recognized medicinal and aromatic plant in sub-Saharan Africa (Ayeni et al., 2024). Belonging to the genus *Ocimum*,

which comprises herbaceous plants of significant economic and ethnobotanical importance, *O. gratissimum* is widely used for flavouring, perfumery, and traditional medicine (Orafidiya et al., 2004). The plant has a long-standing history in traditional medicine across various cultures. In West Africa, particularly in Nigeria, it is used to manage gastrointestinal disorders, such as stomach ulcers and diarrhoea (Bekoe et al., 2021). In India, it has been used to treat urinary stones and other internal ailments (Agarwal & Varma, 2014). Like many medicinal plants, *Ocimum gratissimum* contains a rich profile of bioactive compounds that contribute to its antimicrobial efficacy. According to Kpètèhoto et al. (2019), ethanol extracts of *O. gratissimum* leaves revealed the presence of three primary phytochemical groups: phenolic compounds, nitrogenous compounds, and steroidal/terpenoid compounds. By investigating the efficacy of *Ocimum gratissimum* in treating microbial contaminants in water, this study addresses a critical gap in water resource management. The findings could provide a low-cost, environmentally friendly, and scalable solution for improving water quality, particularly in underprivileged communities.

1.2. Problem Statement

Water contamination by microbial pathogens remains a critical global health challenge, with pronounced impacts in developing regions where access to safe water is limited. Microbiologically contaminated water can transmit diseases such as diarrhoea, cholera, typhoid, and polio, and is estimated to cause approximately 505,000 diarrhoeal deaths each year (WHO, 2023). In Kassena Nankana Municipality, Ghana, due to inadequate sanitation facilities and poor hygiene practices, 55% of children under 5 were reported to have experienced diarrhoea in the 12 months between 2012 and 2013 (Issahaku et al., 2017). While conventional disinfection methods have demonstrated effectiveness, their implementation in resource-constrained communities is often hindered by factors such as cost, infrastructure constraints, and sustainability challenges.

While plant-based solutions offer a promising alternative, existing research has primarily focused on their medicinal purposes. The few studies that do explore their role in water treatment, such as studies done by (Vega Andrade et al., 2021; Winward et al., 2008) emphasize coagulation and purification while overlooking their specific roles in microbial disinfection.

This study will address this gap by investigating the efficacy of *Ocimum gratissimum* as a natural disinfectant for water, with a focus on its potential for use in rural and resourceconstrained communities.

1.3. Justification

Plant-based solutions have shown considerable potential for inhibiting microbes, primarily due to their rich content of bioactive secondary metabolites, such as terpenoids and other phytochemicals. According to (Ahmad et al., 2022) plant-based disinfectants can achieve levels of efficacy comparable to conventional chemical treatments in water purification. Similarly, Winward et al. (2008) reported that essential oils derived from plants demonstrated significant antimicrobial activity when used for the disinfection of greywater, maintaining inhibition of microbial growth for up to 14 days after achieving disinfection.

Ocimum gratissimum, a widely available medicinal plant, has long been used in traditional medicine for the treatment of various ailments. Its familiarity and cultural relevance will increase its acceptance in rural and traditional communities, particularly when compared to synthetic chemical disinfectants. The local availability of such plant-based resources also presents a cost-effective opportunity to develop accessible water disinfection methods, especially in remote or underserved areas (Shaheed et al., 2009).

Moreover, the use of green technologies for water treatment aligns with Sustainable Development Goals 6 (Clean Water and Sanitation) and 13 (Climate Action), reflecting the global shift toward environmentally sustainable practices. As highlighted by Nzeyimana &

Mary. (2024), green approaches are not only eco-friendly but also economically viable and easier to implement in low-resource settings. Plant-derived antimicrobials are biodegradable, generate minimal sludge, and pose less risk to both human health and the environment, making them an ideal alternative to chemical disinfectants (Lwasa et al., 2024; Tiruneh et al., 2023).

1.4. Research Questions

1. Does *Ocimum gratissimum* leaf extract exhibit inhibitory effects against *Escherichia coli*?
2. What is the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Ocimum gratissimum* leaf extract against *Escherichia coli*?
3. What concentrations of *Ocimum gratissimum* leaf extract are most effective for microbial disinfection in water?

1.5. General Objective

To assess the effectiveness of *Ocimum gratissimum* leaf extract in disinfecting E. coli in water.

1.6. Specific Objectives

1. To confirm the inhibitory effect of *Ocimum gratissimum* extract against *E. coli*.
2. To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Ocimum gratissimum* leaf extracts on *E. coli*.
3. To assess effective concentrations of *Ocimum gratissimum* extracts for effective microbial disinfection.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Water Contamination and Public Health Challenges

Water contamination refers to the introduction of harmful substances into water bodies, rendering them unsafe for human, animal, and environmental health. This phenomenon is driven by a combination of anthropogenic and environmental factors, including industrialisation, urbanisation, inadequate sanitation, regional inequality, agricultural expansion, population growth, and climate change (Kumar et al., 2024; Mustafa & Hassan, 2024; Wu, 2020). Common contaminants include industrial effluents, agricultural runoff containing pesticides and fertilizers, and untreated domestic sewage. In many developed regions, the increasing demand for water has exacerbated water stress, while in developing communities, the lack of adequate sanitation infrastructure and the prevalence of open defecation make untreated sewage a primary source of contamination (Mustafa & Hassan, 2024; Vega Andrade et al., 2021).

The health and socio-economic implications of water contamination are profound. Sewage harbours a variety of pathogenic microorganisms—including bacteria, viruses, and protozoa—which contribute significantly to the global burden of waterborne diseases. These pathogens primarily spread through the faecal-oral route, with ingestion of contaminated water playing a central role in disease transmission. Human exposure can occur through multiple pathways, such as drinking contaminated water, consuming seafood and crops irrigated with it, or engaging in recreational activities like swimming. These exposures have been linked to outbreaks of diseases such as diarrhoea, cholera, and dysentery (Gerba & Pepper, 2019; Shayo et al., 2023).

2.2 Importance of addressing microbial contaminants in water

Microbial pathogens in contaminated water present critical public health risks. Historical records indicate that numerous disease outbreaks, particularly in the early 20th century, were linked to unsafe drinking water. For instance, typhoid fever caused approximately 30 deaths per 10,000 people in the United States during the 1890s until the implementation of water filtration systems and other sanitation interventions, which significantly reduced the mortality rate to 5 per 10,000 (Gerba & Pepper, 2019). In 2012, bacterial waterborne diseases were responsible for an estimated 502,000 deaths due to inadequate access to safe water, and 280,000 deaths due to poor sanitation. Among children under five, 361,000 deaths, equating to 5.5% of global mortality in that age group, could have been prevented through improved water quality and sanitation (Prüss-Ustün et al., 2014)

Microbial contamination can lead to a wide range of illnesses, including diarrhoea, cholera, paediatric infections, and various skin diseases (Dharsono et al., 2022; Mustafa & Hassan, 2024). *Escherichia coli* (E. coli), in particular, has been associated with severe health conditions such as acute renal failure and haemolytic anaemia (Nganje et al., 2020). (Adebolu & Oladimeji, 2005) identified several pathogenic organisms, including *Salmonella typhi*, *S. typhimurium*, *Staphylococcus aureus*, and *E. coli*, as causative agents of diarrheal diseases in humans. Vulnerable populations, especially children and individuals with compromised immune systems, are at heightened risk of infection (Mustafa & Hassan, 2024). Despite advancements in water treatment, microbial contamination continues to be a major challenge in many developing regions, highlighting the urgent need for effective and appropriate interventions (Dharsono et al., 2022; Gerba & Pepper, 2019).

2.3 Role of plant-based materials in the treatment of water

Plant-based antimicrobial agents have emerged as a viable, cost-effective, and environmentally sustainable alternative for water treatment, particularly in low-resource settings. These natural

materials are not only locally available and safe for use, but they also produce biodegradable by-products, thereby minimizing environmental impact (Lwasa et al., 2024; Tiruneh et al., 2023). In terms of efficacy, many plant-based methods perform comparably to conventional chemical treatments. Although chemical coagulants and disinfectants are widely used, their long-term application poses environmental and health risks, especially in settings lacking adequate waste management infrastructure.

Numerous studies have demonstrated the effectiveness of plant-based solutions in enhancing water quality. Vega Andrade et al. (2021) utilized aqueous extract of *Moringa oleifera* seeds as a natural coagulant, significantly reducing turbidity and organic matter in domestic wastewater. Aqueous extracts of *Moringa oleifera* seeds at the optimal dosage of 600 mg/L showed results statistically equivalent to those of alum (200 mg/L), achieving bacterial load, turbidity, and apparent colour removal of 99 %, 92 %, and 66 %, respectively. Similarly, Adeeyo et al. (2020) investigated the antimicrobial properties of ethyl acetate and chloroform extracts from *Zanthoxylum zanthoxyloides* and *Gongronema latifolium*. All plant extracts showed broadspectrum antibiosis against E. *coli*, P. *aeruginosa*, *Klebsiella sp*, S. *pneumoniae*, and B. *cereus*, as well as A. *niger*, A. *flavus*, *Trichoderma sp*, and *Candida* sp.

Pritchard et al. (2009) also reported that aqueous extracts of *Moringa oleifera*, guar gum, and *Jatropha curcas* improved water clarity and purity. All three extracts achieved an efficiency exceeding 90% on shallow well water with a turbidity of 49 NTU. Coliform reduction was about 80% across all extracts. The pH of the water samples increased with dosage but remained within acceptable drinking water levels for all extracts.

Additionally, Winward et al. (2008) demonstrated that essential oils derived from specific plants could effectively disinfect greywater. Over a 30-minute contact time, an organum essential oil concentration of 468 mg/L rendered total coliforms non-detectable in 100mL of

grey water. Collectively, these findings underscore the potential of plant-derived substances as sustainable and scalable solutions for improving water quality in developing countries.

2.4 Microbial Contaminants in Water

Contaminated water typically harbours a wide array of microorganisms, including bacteria, viruses, protozoa, and fungi (Shayo et al., 2023). These microbial contaminants pose significant public health risks, particularly in communities with limited access to effective water treatment systems. Nganje et al. (2020), in their study of water sources in Kumba, Cameroon, they found that all tested water samples were contaminated with pathogenic bacteria such as *Escherichia coli*, *Shigella* spp., *Enterobacter* spp., *Streptococcus* spp., and *Salmonella* spp.—all of which are associated with various waterborne diseases.

Gerba & Pepper. (2019) emphasize that when non-disinfected water is consumed, the primary bacterial threats include genera such as *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Escherichia*, and *Vibrio*. Similarly, Ashbolt. (2015) identifies enteric pathogens as the predominant cause of waterborne illnesses in humans. He reported that, among the classic pathogens are *Vibrio cholerae* (serogroups O1 and O139, which cause cholera), *Salmonella enterica* (subspecies *enterica* serovar Typhi, the causative agent of typhoid fever), and *Shigella* spp. (associated with dysentery). Although conventional water treatment and disinfection methods have substantially reduced the prevalence of these pathogens in developed regions, emerging and persistent threats remain. These include *Shigella sonnei*, shiga toxin- and verotoxin-producing *E. coli*, and pathogenic species of *Campylobacter*, *Salmonella*, *Arcobacter*, *Helicobacter*, and *Yersinia*—many of which are still prevalent in untreated wastewater in developing countries.

Due to the vast diversity and abundance of potential microbial contaminants, it is impractical to test for each individual pathogen. As a result, microbiologists commonly employ indicator

organisms—particularly total coliforms and faecal coliforms—as proxies for faecal contamination. The presence of these indicators in water samples suggests the possible existence of pathogenic microorganisms originating from faecal matter (Forstinus et al., 2016; Gerba & Pepper, 2019). Roy et al. (2016) further confirmed the utility of these indicators in evaluating the efficacy of water disinfection methods. Notably, waterborne pathogens can be excreted in large quantities by infected individuals or animals, even in the absence of clinical symptoms. Moreover, certain pathogens can cause infection at very low doses, heightening the risk of transmission through contaminated water.

2.5 Conventional Methods for Microbial Water Treatment

2.5.1 Overview of conventional treatment methods

Effective water treatment is critical for removing contaminants that pose health risks to consumers. Conventional water treatment techniques typically involve a combination of physical, chemical, and biological processes designed to eliminate pathogens and enhance water quality. Physical processes such as filtration and sedimentation serve as preliminary steps, significantly reducing microbial loads but not eliminating them entirely (Kumar et al., 2024). These initial methods are commonly followed by disinfection stages to ensure higher microbial removal efficiency. Filtration, particularly sand filtration, is often employed prior to chemical disinfection to optimize treatment outcomes. Some researchers, such as Shaheed et al. (2009), have recommended combining filtration with natural plant extracts to enhance microbial reduction. Winward et al. (2008) demonstrated that ultraviolet (UV) light can also be used synergistically with essential oils to improve the disinfection of greywater.

Among the most widely used disinfection techniques are chlorination, ultraviolet disinfection, and boiling—particularly for small-scale or household applications. Roy et al. (2016) assessed the efficacy of various disinfection methods, including chlorination, UV treatment, boiling, and solar radiation. Their findings revealed that the effectiveness of each method is influenced by

factors such as contact time and dosage. For instance, chlorine required a minimum of 30 minutes to achieve effective disinfection, while boiling required one minute for 10 mL of water, and solar radiation needed approximately six hours of exposure. While all methods were effective under the right conditions, each presented challenges in terms of energy demand, time requirements, and cost. Among these, chlorination was identified as the most practical option, albeit with significant caveats.

2.5.2 Limitations of conventional methods in rural and low-income settings.

Despite its effectiveness and cost-efficiency, chlorine must be applied with caution due to the formation of disinfection by-products (DBPs). Roy et al. (2016) cautioned that prolonged or excessive chlorination could lead to harmful DBPs, and Shayo et al. (2023) corroborated this concern by noting that while alternative methods like UV disinfection and boiling are effective, they are often cost-prohibitive. Chlorine, therefore, remains the preferred disinfectant, though it is not without risks. Studies have associated DBPs with adverse health outcomes, including neurodegenerative diseases such as Alzheimer's and various forms of cancer.

Recent research has further identified several classes of DBPs—such as halo acetic acids (HAAs), trihalomethanes (THMs), and nitrosamines—that result from chlorination, chloramination, and ozonation (Wu, 2020). While chlorine remains the most widely used chemical disinfectant due to its affordability and effectiveness, its long-term implications for human health warrant serious consideration (Kumar et al., 2024).

2.6 Plant Based Materials for Water Treatment

2.6.1 Plant-based antimicrobials and their mechanisms of action.

Medicinal plants have long been recognized for their antimicrobial properties, primarily attributed to their rich composition of secondary metabolites. Numerous studies have confirmed that certain plant species exhibit significant antimicrobial activity (Adeeyo et al.,

2020). According to Kumar et al. (2024)., this biological activity arises from the synergistic effects of various secondary metabolites, which include tannins, eugenol, flavonoids, alkaloids, terpenoids, and phenolic compounds.

Recent research has highlighted specific classes of secondary metabolites—such as terpenes, flavones, flavanols, alkaloids, and phenylpropanoids—as promising antimicrobial agents. Their primary mode of action often involves the disruption of microbial cell membranes (Álvarez-Martínez et al., 2021). Flavonoids, for example, are synthesized by plants in response to microbial infections and have been shown to destroy the cell walls of both Gram-negative and Gram-positive bacteria. These compounds also target molecular structures vital for microbial survival (Lwasa et al., 2024). Similarly, tannins exhibit antiviral, antibacterial, and antiparasitic properties, likely due to their ability to inactivate microbial adhesins, enzymes, and cell envelope transport proteins.

2.6.2 Efficacy of medicinal plants in microbial inhibition.

Dharsono et al. (2022) conducted a review on five species of the *Ocimum* genus—*O. americanum*, *O. basilicum*, *O. gratissimum*, *O. campechianum*, and *O. sanctum*—and concluded that these species possess diverse chemical compositions and notable antibacterial activity, with terpenoids being the most prevalent compound. A phytochemical investigation of the methanolic leaf extract of *Ocimum gratissimum* (Lamiaceae) revealed eight known bioactive compounds, including five triterpenes, one flavonoid, and two phytosterols (Nganteng et al., 2022).

Shaheed et al. (2009) reported that seed extracts demonstrated higher antimicrobial efficacy than fruit extracts, attributing this difference to the presence of certain phytochemicals in the seeds that were absent in the fruits. Adebolu & Oladimeji. (2005) further identified eugenol as the key antimicrobial compound in the essential oils of *Ocimum gratissimum*, noting its

effectiveness against *Salmonella typhi*, *S. typhimurium*, *Staphylococcus aureus*, and *Escherichia coli*. Nepolean et al. (2009) examined the antimicrobial activity of both ethanolic and aqueous extracts from the leaves and seeds of *Moringa oleifera*. Their study found these extracts effective against pathogens such as *E. coli* and *S. aureus*. The extracts contained a variety of bioactive phytochemicals, including tannins, saponins, flavonoids, glycosides, terpenoids, and steroids. These findings collectively support the potential of medicinal plants as sustainable and effective alternatives for microbial control, particularly in resource-limited settings.

2.7 *Ocimum gratissimum*: A Potential Solution

2.7.1 Description, Distribution, and Application of *Ocimum gratissimum*

Ocimum gratissimum, commonly known as African basil, is a widely recognized medicinal and aromatic plant in sub-Saharan Africa (Ayeni et al., 2024). Belonging to the genus *Ocimum*, which comprises herbaceous plants of significant economic and ethnobotanical importance, *O. gratissimum* is extensively utilized in flavoring, perfumery, and traditional medicine (Orafidiya et al., 2004). In Nigeria, the plant is popularly known by several local names, including clove basil, sweet basil, tea bush, scent leaf, and fever plant (Ayeni et al., 2024). Its nickname "scent leaf" arises from its strong, pleasant aroma (Amengialue et al., 2013).

Botanically, *Ocimum gratissimum* is a perennial shrub that thrives in tropical climates and is distributed widely across Africa and Asia. It is especially prevalent in tropical Asian countries, notably India (Agarwal & Varma, 2014; Alexander, 2016).

The plant has a long-standing history in traditional medicine across various cultures. In West Africa, particularly in Nigeria, it is used to manage gastrointestinal disorders, such as stomach ulcers and diarrhea (Bekoe et al., 2021). In India, it has been used to treat urinary stones and other internal ailments (Agarwal & Varma, 2014). Ethnomedical applications also extend to the

treatment of inflammatory conditions, wound infections, epilepsy, fungal infections, and respiratory illnesses such as fever, cold, and catarrh (Okoye et al., 2014; Nweze et al., 2009; O. Ayeni et al., 2022).

Amengialue et al. (2013) confirmed the plant's effectiveness in managing diarrhea, while in southern Nigeria, it is commonly used both as a culinary herb for flavoring food and as a remedy for stomach-related ailments (Okunlola et al., 2019). Furthermore, the essential oils extracted from *O. gratissimum* have demonstrated insecticidal and repellent properties and are also used in oral hygiene formulations (Padilha De Paula et al., 2003). The plant is not only medicinally beneficial but also safe for dietary consumption (Alexander, 2016).

2.7.2 Active Compounds in Ocimum gratissimum

Like many medicinal plants, *Ocimum gratissimum* contains a rich profile of bioactive compounds that contribute to its antimicrobial efficacy. According to Kpètèhoto et al. (2019), ethanol extracts of *O. gratissimum* leaves revealed the presence of three primary phytochemical groups: phenolic compounds, nitrogenous compounds, and steroidal/terpenoid compounds. The phenolic group includes catechin tannins, gallic tannins, flavones, free anthracene derivatives, and combined anthracenic derivatives—particularly reducing agents. Nitrogenous compounds are primarily represented by alkaloids, while the steroidal and terpenoid group is characterized by the presence of saponosides and various steroids. Additionally, cyanogenic glycosides and mucilages—although less well-characterized—were also detected, indicating a broader array of bioactive molecules. Quantitative analysis of the ethanolic extract confirmed the dominance of total phenolics (56.59 mg GAE/100 mg), followed by total flavonoids (13.71 mg QE/100 mg) and total tannins (8.6 mg CE/100 mg), suggesting a strong potential for antioxidant and antimicrobial activity based on their concentrations.

Tella & Oseni. (2019) conducted a comparative phytochemical study involving *O. gratissimum* and another species, identifying a wide range of secondary metabolites, including alkaloids, anthraquinones, cardiac glycosides, flavonoids, phlobatannins, tannins, terpenoids, saponins, and reducing sugars. Their Fourier-transform infrared (FTIR) spectroscopy analysis further confirmed the presence of phenolic phytochemicals, flavonoids, polysaccharides, monoterpenes, sulphur-containing compounds, carboxylic acids, and halogenated phytochemicals across all solvent fractions of *O.gratissimum*.

Akinmoladun et al. (2007) corroborated these findings, reporting the presence of tannins, steroids, terpenoids, flavonoids, and cardiac glycosides in both methanolic and aqueous extracts. The methanolic extract exhibited a total phenolic content of 5.68 ± 0.06 mg/g, although the phenolic concentration in the aqueous extract was not specified. Phenolic compounds, which represent the largest class of phytochemicals in plants, have been widely recognized for their strong antioxidant and antimicrobial activities.

Similarly, Nganteng et al. (2022) conducted a phytochemical investigation of the methanolic leaf extract of O. gratissimum (Lamiaceae) and isolated eight known bioactive compounds: five triterpenes, one flavonoid, and two phytosterols. Dharsono et al. (2022), in a broader review of five *Ocimum* species including *O. gratissimum*, concluded that these plants are chemically rich, with terpenoids being the most dominant compound class and contributing significantly to their antibacterial activity.

2.7.3 Antimicrobial Properties of *Ocimum gratissimum*

The demonstrated presence of diverse bioactive compounds strongly supports the antimicrobial potential of *Ocimum gratissimum*. Several studies have evaluated its efficacy against a range of microbial pathogens. Ayeni et al. (2022). confirmed the antibacterial activity of *O. gratissimum* against fecal coliforms, with findings indicating that its effectiveness increases

proportionally with dosage. de Aquino Lemos et al. (2005) investigated multiple extracts of *O. gratissimum*, including ethanolic and chloroformic fractions, against clinical isolates of *Cryptococcus neoformans*. The study revealed significant in vitro antimicrobial activity: 92% inhibition was observed at a concentration of 62.5 µg/mL with the chloroformic extract, and 100% inhibition was achieved at 250 µg/mL using essential oil and eugenol.

Nweze & Eze. (2009) examined the antimicrobial effects of ethanolic extracts of *O. gratissimum* on clinical isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Escherichia coli*. The extracts demonstrated inhibitory activity against all test organisms at varying concentrations, with the exception of *E. coli*, which showed resistance under the experimental conditions.

Additionally, Nakamura et al. (1999) reported that essential oils (EOs) derived from *O. gratissimum* exhibited significant antimicrobial activity against Gram-negative bacteria, including species from the genera *Proteus*, *Klebsiella*, *Salmonella*, *Escherichia*, and *Shigella*. The minimum inhibitory concentrations (MICs) for these organisms ranged from 3 to 12 µg/mL.

2.8 Factors Affecting Disinfection Efficiency

2.8.1 Contact Time and Concentration of Leaf Extracts

Selecting the most appropriate plant-based coagulants for water treatment involves several key considerations: (i) the plant species and part utilized, (ii) the concentration of chemical or bioactive compounds—which may vary based on soil conditions, growth rate, climate, and agricultural practices, (iii) the extraction method, (iv) the solvent used, and (v) the desired performance outcomes (Lwasa et al., 2024).

Adeeyo et al. (2020) reported that regardless of the extraction solvent used, the dosage of plant extract significantly influenced its antimicrobial effectiveness. In their study, concentrations of

25 mg/L were considered low, while 500 mg/L were deemed high for both chloroformic and ethyl acetate extracts of *Zanthoxylum zanthoxyloides* and *Gongronema latifolium*. Shaheed et al. (2009) suggested that contact time had minimal impact on extract efficacy, with efficiency improving as the dosage increased.

However, contrasting findings were reported by Winward et al. (2008), who observed that both contact time and extract concentration significantly affected the performance of essential oils (EOs) derived from plants in greywater treatment. Specifically, over a 30-minute contact time, *Origanum* EO at concentrations of up to 94 mg/L had a negligible effect on total coliform concentrations, whereas at 468 mg/L, total coliforms were rendered non-detectable in 100 mL of greywater. Moreover, 100% disinfection efficiency was achieved at a concentration of 278 mg/L over a 60-minute contact period.

2.8.2 Solvent Type

The type of solvent used for extraction plays a crucial role in determining the antimicrobial potency of plant extracts. Tiruneh et al. (2023) emphasized that the extraction solvent influences the composition and concentration of bioactive compounds; for instance, they used sodium chloride (NaCl) solutions to dissolve plant proteins but noted that excessive use of the solution could compromise the experiment. Similarly, Ahmad et al. (2022) favoured salt solutions and alcohol-based solvents over water for more efficient extraction.

Akinmoladun et al. (2007) compared aqueous and methanolic extracts of *O. gratissimum*, finding both positive for tannins, steroids, terpenoids, flavonoids, and cardiac glycosides. Notably, anthraquinones were detected only in the aqueous extract, while alkaloids were found solely in the methanolic extract. Neither extract contained saponins. Adebolu & Oladimeji. (2005) compared cold, hot, and steam extracts for their antimicrobial efficacy, concluding that

steam extracts were most effective. This was likely due to the presence of eugenol in the essential oils, which is absent in cold and hot extracts because of the oils' volatility.

Saha et al. (2013) reported that methanol extracts of *Ocimum canum* Sims and *Ocimum tenuiflorum* L. exhibited no antibacterial activity, whereas their essential oils demonstrated significant antibacterial effects. This disparity may be attributed to the higher concentration of phenolic compounds in the essential oils and the low or absent levels in the methanol fractions, underscoring the importance of selecting appropriate extraction methods and solvents tailored to specific plant species.

Tella & Oseni. (2019) highlighted the significance of solvent choice in activating essential bioactive compounds. In their study, the methanolic, n-hexane, and ethyl acetate solvent fractions of *O. gratissimum* contained alkaloids, anthraquinones, phlobatannins, and terpenoids, while butanol and aqueous solvent fractions showed a reduced number of phytochemicals, including cardiac glycosides, flavonoids, and tannins. Saponins and reducing sugars were absent across all solvent fractions of *O. gratissimum*. By contrast, extracts from *Vernonia amygdalina* revealed the presence of alkaloids, flavonoids, terpenoids, and saponins in the methanolic, n-hexane, and ethyl acetate fractions, while butanol and aqueous fractions contained fewer phytochemical constituents, limited to cardiac glycosides, flavonoids, and tannins. Phlobatannins were absent in all *V. amygdalina* solvent fractions. Tella & Oseni. (2019) concluded that the solvent-mediated phytochemical expression in both plants followed a consistent profiling order: "Methanol > Hexane > Ethyl acetate > Butanol > Aqueous distilled water."

2.9 Challenges and Limitations

The full extent of the threat posed by microbial contaminants in water is not yet completely understood. Current detection methods, while advanced, remain insufficient to identify the

entire spectrum of microbial contaminants, indicating the need for innovative approaches capable of addressing both known and emerging pathogens (Forstinus et al., 2016).

Additionally, cross-contamination presents a significant challenge in water disinfection efforts. Addressing this issue requires the development of solutions that prevent secondary contamination, particularly at the point of use (Shayo et al., 2023).

Water quality parameters can also influence the efficacy of plant-based disinfectants. Shaheed et al. (2009) and Winward et al. (2008) reported that the presence of organic matter and particulates in greywater significantly reduced the antimicrobial effectiveness of plant-derived essential oils. Natural organic matter, dissolved solids, and suspended particles can interfere with the interaction between plant-based disinfectants and microbial targets, limiting their practical application in real-world water treatment scenarios.

Most research on plant-based disinfection methods has been conducted at the laboratory scale, highlighting the need for further exploration into their commercial viability (Ahmad et al., 2022). Kumar et al. (2024) concurred, emphasizing that issues such as slow treatment rates, inadequate standardization, and challenges in extraction and purification processes must be resolved to facilitate industrial-scale implementation. Additional concerns include the potential for unpleasant taste and undesirable health effects from consuming concentrated plant extracts, as well as the need to secure a sustainable supply of high-quality raw plant material (Shaheed et al., 2009).

For plant-based disinfectants to be feasible in developing countries, it is essential to develop safe, user-friendly systems for their application. Addressing these challenges will require interdisciplinary collaboration and innovation to ensure both effectiveness and scalability of plant-derived disinfection solutions (Pritchard et al., 2009).

2.10 Knowledge Gaps and Research Opportunities

Although several conventional methods for treating microbial contaminants in water are in use, many developing regions continue to experience significant impacts from waterborne diseases. This persistent challenge underscores the need for innovative approaches, either by enhancing existing systems or by introducing new, context-specific solutions (Forstinus et al., 2016). The persistent prevalence of waterborne diseases, despite decades of research and the implementation of various potential solutions, highlights the need for re-evaluating and strengthening current water treatment strategies in these regions.

A holistic approach to addressing water contamination is essential. Multiple studies emphasize that effective water disinfection is inextricably linked to improved sanitation and hygiene practices (Mustafa & Hassan, 2024; Shayo et al., 2023). Without integrating these factors, efforts to combat waterborne diseases in developing countries are likely to fall short.

Regarding plant-based water treatment solutions, there are notable knowledge gaps that merit further investigation. According to Tiruneh et al. (2023), Other parts of known plants must be investigated to fully realize the plant's potential. For example, in the use of the banana plant for coagulation, much attention has been given to the banana peel, whereas evidence indicates that the stem is the most effective. Moreover, the efficiency of plant-based water treatment depends heavily on the optimization of extraction processes. Ahmad et al. (2022) point out that different plant species require unique extraction protocols, necessitating further research into process optimization to enhance the yield and effectiveness of bioactive compounds.

Safety concerns, particularly the potential toxicity of concentrated plant-based solutions, must also be addressed to ensure the safe application of these treatments in water purification (Pritchard et al., 2009). Additionally, combining conventional methods such as filtration with plant-based treatments could enhance overall effectiveness and provide more comprehensive solutions (Shaheed et al., 2009).

2.11 Conclusion

Water contamination by microbial pathogens remains a critical global health challenge, with pronounced impacts in developing regions where access to safe drinking water is limited. While conventional disinfection methods have demonstrated effectiveness, their implementation in resource-constrained communities is often hindered by factors such as cost, infrastructure, and sustainability.

Plant-based solutions, particularly those leveraging the antimicrobial properties of secondary metabolites, offer a promising alternative. *Ocimum gratissimum*, a widely available medicinal plant known for its diverse bioactive compounds—including phenolics, flavonoids, terpenoids, and essential oils—has demonstrated significant potential in microbial inhibition. Despite its documented medicinal applications for treating infections and other ailments, limited research has explored its specific role in water disinfection.

The effective application of *O. gratissimum* for water treatment requires careful consideration of extraction methods, solvent type, dosage, and contact time to maximize its antimicrobial efficacy. However, challenges such as the potential for off-flavors and the need for precise dosage determination must be addressed. Additionally, combining *O. gratissimum* extracts with conventional treatment methods, such as sand filtration, has been recommended to enhance overall disinfection efficiency, especially in turbid water conditions.

In summary, *Ocimum gratissimum* presents a viable, locally sourced, and environmentally sustainable alternative for water disinfection in developing regions. Continued research is essential to optimize its application, assess safety concerns, and facilitate its integration into broader water treatment strategies.

CHAPTER 3

MATERIALS AND METHODS

3.1 Preparation of Plant Extract

Fresh leaves of *Ocimum gratissimum* were collected from a cultivated plot within a residential area in Fiapre, Ghana (7°22′00″N, 2°21′00″W). Leaves without visible contaminants were sealed in sterile bags for transport to the UENR chemical laboratory after identification and authentication by the Department of Parks and Gardens, Sunyani. At the laboratory, the leaves were thoroughly washed with distilled water, air-dried at room temperature for 5-7 days, and

ground into a powder using a commercial-grade electric blender (Binatone, BGL-403). Analytical-grade ethanol (99%) was procured from Bemburto Enterprises, Accra, Ghana, for the preparation of a 70% ethanol solution with sterile distilled water. The blender's components were sterilised before use by wiping with 70% ethanol solution, followed by a rinse with distilled water. The powdered leaves were packed into airtight containers to prevent moisture absorption and degradation of bioactive compounds before extraction. 75 g of the powdered *Ocimum gratissimum* leaves was submerged in 1000 mL of 70% ethanol and left to stand at room temperature (25 ± 2°C) for 72 hours with occasional stirring. The resulting mixture was filtered through Whatman filter paper (diameter of 125 mm, pore size of 11 µm) to remove plant debris. The resulting filtrate was concentrated using a rotary evaporator (Stuart rotary evaporator, CAT No. RE400/MS) to remove the ethanol, and the crude extract was stored in sterile glass bottles in a refrigerator (4°C) for up to 96 h before the disinfection study.

3.2 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis was performed to identify the functional groups present in the extract before treatment. Peak assignments were made by comparing observed absorption bands with standard FTIR correlation tables (Socrates, 2001; Pavia et al., 2014; Coates, 2000). FTIR analysis of the ethanolic extract of *Ocimum gratissimum* was performed using a Bruker FTIR spectrometer equipped with an ATR accessory. Spectra were recorded in the mid-infrared region (4000–400 cm^{-1}) at a resolution of 4 cm^{-1}, with an average of 32 scans per spectrum. The extract was placed directly on the diamond ATR crystal without further preparation. The resulting spectra were processed using OPUS software for baseline correction and peak identification. The characteristic absorption bands were compared to standard references to assign functional groups associated with bioactive compounds.

3.3 Microbial Culture and Identification

Clinical isolates of *Escherichia coli* were obtained from biological specimens (blood and urine samples) and first cultured in nutrient broth at 37 °C for 24 hours. A loopful of the broth culture was streaked onto MacConkey Agar No. 3 (REF: CM0115B, OXOID) to ensure purity and aid in the selective isolation of lactose-fermenting colonies. The MacConkey agar medium was prepared according to the manufacturer's instructions: the required amount of dehydrated powder was suspended in distilled water, dissolved by heating with gentle agitation, and sterilized by autoclaving at 121 °C for 15 minutes. The sterile medium was then poured into Petri dishes under aseptic conditions and allowed to solidify before inoculation. After incubation, colonies showing characteristic pink to red coloration, indicative of lactose fermentation, were selected. These colonies were further subjected to Gram staining and observed microscopically, confirming their identity as Gram-negative rods consistent with *Escherichia coli*.

3.4 Preparation of Disk and Dilutions

Sterile 6 mm filter paper disks were used to test the extract's efficacy. A two-fold serial dilution of the extract was prepared, ranging from an initial concentration of 100% down to 3.125%. The specific concentrations tested were 100%, 50%, 25%, 12.5%, 6.25%, and 3.125% (v/v). Each sterile disk was then impregnated with 20 µL of each diluted extract concentration and allowed to air-dry under aseptic conditions in a biological safety cabinet before being used in the assay. This step is crucial to ensure that the solvent evaporates, leaving only the active compound on the disk.

3.5 Preparation of Simulated Contaminated Water

To simulate contaminated water, 1 mL of prepared *E. coli* suspension (7.38×10^5 CFU/mL) was aseptically inoculated into 100 mL of sterile distilled water for each treatment group. The

samples were immediately mixed by gentle but continuous hand-swirling for 30 seconds to ensure uniform distribution of the bacteria. Following this, 5 mL of various concentrations (100% v/v, 50%v/v, 25%v/v, 12.5%v/v, 6.25%v/v, 3.125% v/v) of the extract was introduced into each sample. The samples were then mixed again by hand-swirling for 1 minute to facilitate complete contact between the extract and the bacterial cells. Two control setups were included:

1. Negative Control – Simulated contaminated water with no extract.
2. Solvent Control – Simulated contaminated water with diluted ethanol only (5ml ethanol and 100ml sterile distilled water).

   These controls serve as baselines to evaluate the specific antimicrobial effect of the plant extract.

3.6 Wastewater Collection

Contaminated water was obtained in Ejisu, a rural community located in the Ashanti Region, Ghana (6°43'0" N and 1°28'0" W). Wastewater was collected from a communal drainage outlet serving a high-density, mixed-use residential and commercial zone. This source was chosen to obtain a representative sample of real-world wastewater, reflecting the complex composition of domestic greywater and commercial effluents. The samples were expected to contain high levels of organic matter and suspended solids, as well as a wide variety of microbial pathogens, typical of such environments due to a combination of human activities and inadequate sanitation. These characteristics were crucial for assessing the performance of the plant-based extract under challenging, field-like conditions. A negative control (wastewater only) was included for comparison. After incubation, the samples were analysed by culture on MacConkey agar to enumerate viable E. *coli* cells

3.7 Antimicrobial Activity Assay

The antimicrobial activity of the crude ethanolic extract of *Ocimum gratissimum* was assessed using the agar disk diffusion method. *Escherichia coli* isolates were cultured and standardized to 0.5 McFarland turbidity, then uniformly swabbed onto sterile Mueller–Hinton agar plates to form a bacterial lawn. Sterile disks were aseptically placed on the inoculated agar surface, ensuring adequate spacing to avoid overlapping zones. Solvent-impregnated disks served as negative controls, while a commercial AXIOM multi-antibiotic disc for Gram-negative isolates (containing 5µg ciprofloxacin, 10µg gentamicin, 30µg tetracycline, 30µg chloramphenicol, 10µg ampicillin, and 30µg ceftriaxone) was included as the positive control on separate plates. The inoculated plates were incubated at 35 ± 2 °C for 18–24 hours. Following incubation, the diameters of the zones of inhibition, including the 6 mm disk, were measured in millimetres and recorded for analysis.

3.8 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The broth dilution method was used to determine the MIC of the extract. Dilutions ranging from 3.125% to 100% of 5ml of the extract were prepared in nutrient broth and inoculated with 100 µL of E. coli suspension. After incubation at 37°C for 18 hours, bacterial growth was assessed by measuring optical density (OD) at 600 nm. The MIC was defined as the lowest extract concentration that resulted in no visible bacterial growth, which was quantitatively determined by an OD_{600} of less than 0.1.

Aliquots from the tubes showing no visible growth in the MIC test were plated on nutrient agar and incubated for 24 hours. The MBC was determined as the lowest extract concentration at which no bacterial colonies were observed. For this study, the Minimum Bactericidal

Concentration (MBC) was chosen as the optimal concentration, as the aim was to achieve complete elimination of *E. coli* rather than just inhibition.

3.9 pH Monitoring

Throughout the experiment, the pH of all solutions was monitored using a calibrated digital pH meter (Hanna HI9813-6*)*. Prior to use, the pH meter was calibrated at three points using commercial buffer solutions of pH 4.0, 7.0, and 10.0 according to the manufacturer's instructions. The electrode was rinsed with distilled water and blotted dry with a lint-free tissue between each measurement. The values were maintained within an acceptable range of 6.5–8.5 to mimic natural water conditions and to eliminate potential pH-related biases in microbial inhibition.

3.10 Characterisation of Contaminated Water Sample

The contaminated water samples were characterized to establish baseline conditions, ensuring their suitability for a real-world validation study. The following physicochemical parameters were measured: Turbidity, Total Suspended Solids (TSS), Biochemical Oxygen Demand after five days (BOD_5), Chemical Oxygen Demand (COD), and pH.

Turbidity was measured using a calibrated digital turbidimeter (Hach 2100Q). The results are reported in Nephelometric Turbidity Units (NTU). This method adheres to the EPA Method 180.1.

Total Suspended Solids (TSS) were determined using the Standard Gravimetric Method (APHA 2540D). A 500 mL sample was filtered through a pre-weighed Whatman GF/C glass fibre filter of pore size 1.2 µm. The filter was then dried in an oven at 103°C to a constant weight. The mass increase was used to calculate the TSS concentration, which is expressed in mg/L. This method is effective for measuring particulate matter in water.

The TSS concentration was calculated using the following formula. The units are typically expressed in milligrams per litre (mg/L).

$$Tss(mg/L) = \frac{W(final) - W(initial) \times 1000}{V(sample)} \quad (1)$$

- $W(final)$: Final constant weight of the filter and dried solids (in mg).
- $W(initial)$: Initial weight of the clean, dried filter (in mg).
- $V(sample)$: Volume of the water sample filtered (in L).

Biochemical Oxygen Demand (BOD_5) was determined using the Standard Dilution Method (APHA 5210B). The initial dissolved oxygen (DO) was measured with a DO meter (YSI ProSolo). The diluted sample was then incubated in the dark at 20°C for five days. The final DO was measured after the incubation period, and the BOD_5 was calculated from the DO depletion and the dilution factor. This process simulates the natural oxygen consumption by microorganisms in the water. The equation for BOD_5 is:

$$\text{BOD}(5)\ \left(\frac{mg}{L}\right) = \frac{DO(initial) - DO(final)}{P} \quad (2)$$

Where:

- $DO(initial)$: Initial DO of the diluted sample (mg/L).
- $DO(final)$: Final DO of the diluted sample after 5 days (mg/L).
- P: The decimal volumetric fraction of the sample used.

Chemical Oxygen Demand (COD) was determined using the Closed Reflux Colorimetric Method (APHA 5220D). Samples were digested with a potassium dichromate solution in a COD reactor (Hach DRB200) at 150°C for two hours. Following digestion, the absorbance of the reacted solution was measured at a wavelength of 600 nm using a spectrophotometer (Hach DR3900). The COD concentration was then determined using a previously established standard calibration curve. The water samples were measured using a digital pH meter (Hanna

HI98136). The meter was calibrated daily using standard buffer solutions of pH 4.0, 7.0, and 10.0 to ensure accuracy before each measurement.

Following characterization, 5 mL of the effective plant extract concentrations (determined from a preliminary simulated water study) were applied to 100 mL of the real wastewater samples. This procedure aimed to evaluate the extract's efficacy within a more complex environmental matrix.

3.11 Data analysis

All experimental procedures were performed in triplicate to ensure the reproducibility and reliability of the data. The raw data were initially compiled and organized in Microsoft Excel 2022, where they underwent preliminary quality checks for consistency and accuracy. Statistical analysis was conducted using IBM SPSS Statistics version 28 and Python 3 within a Jupyter Notebook environment. For each treatment concentration, descriptive statistics, including the mean and standard deviation (mean ± SD) of the bacterial counts, were calculated and are presented in the Results section for clarity.

A dose-response model (specifically a sigmoidal or logistic curve) was fitted to the data using Python 3 to determine the inhibitory concentration 50% (IC_{50}) and effective concentration 50% (EC_{50}). These values represent the extract concentration required to inhibit or eliminate 50% of the bacterial population. The correlation between the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was also analysed and presented. Prior to inferential statistical analysis, the data were assessed for adherence to the assumptions of parametric tests. Normality of the data was assessed using the Shapiro-Wilk test, and the Homogeneity of variances was checked using Levene's test. To assess the statistical significance of the differences in bacterial counts among the various treatment groups, a oneway Analysis of Variance (ANOVA) was performed. The treatment concentration was designated as the independent variable, while the bacterial colony-forming unit counts

(CFU/mL) served as the dependent variable. From the ANOVA output, the partial eta squared (η_p^2) effect size was calculated to determine the biological significance of the results. A significance level of $p<0.05$ was used for all tests. Following a significant ANOVA result, Tukey's Honest Significant Difference (HSD) post-hoc test was applied to identify the specific pairs of treatment groups that exhibited statistically significant differences. The complete inferential statistics, including ANOVA tables, post-hoc comparison results, and the raw replicate data, are included in the Appendix.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 FTIR Analysis of *Ocimum gratissimum* Extract

The FTIR spectrum of the *O. gratissimum* extract **(**Figure 4-1**)** revealed characteristic absorption peaks corresponding to a range of functional groups. These peaks indicated the probable presence of phytochemicals such as flavonoids, tannins, terpenoids, glycosides, and essential oil components including thymol and eugenol derivatives. The absorption bands

related mainly to hydroxyl, carbonyl, aromatic, and ether functional groups, confirming that the extract contained bioactive compounds (Table 4-1) known for antimicrobial properties.

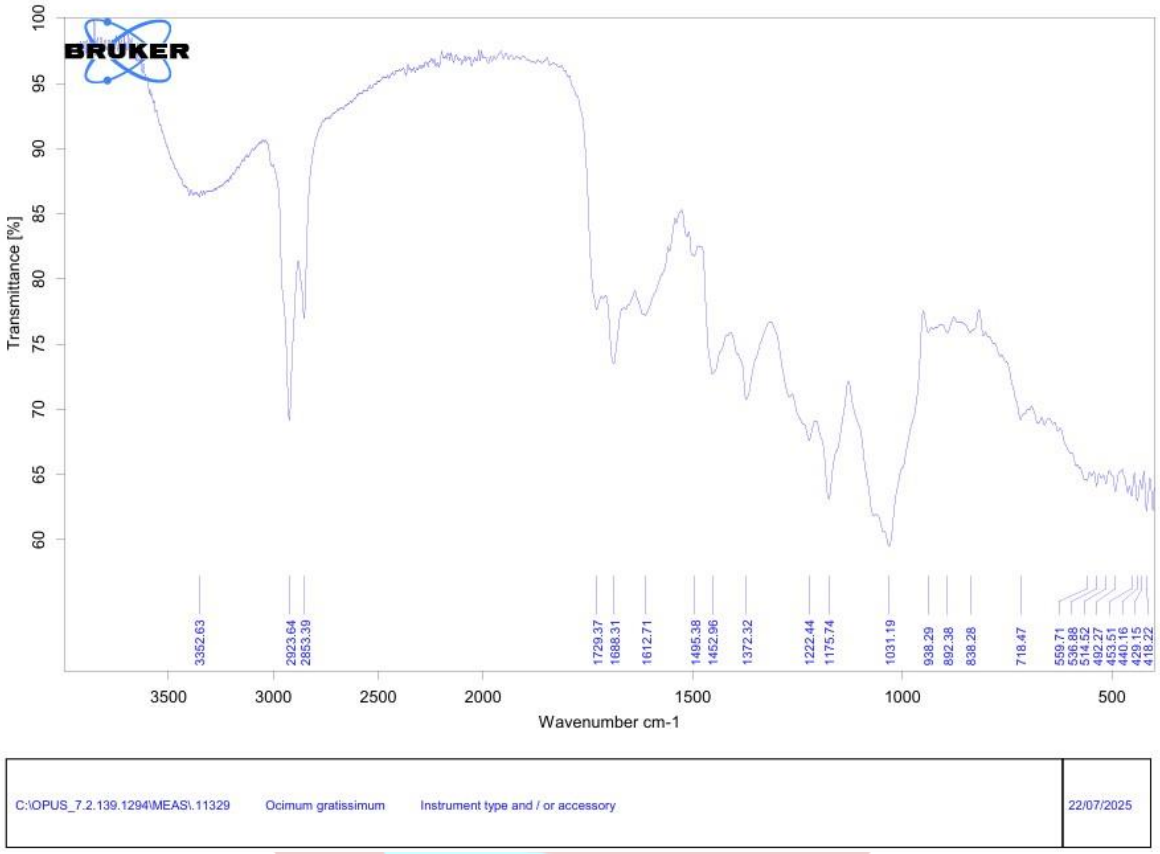

Figure 4-1:FTIR Spectrum of Ocimum gratissimum showing major functional groups.

Table 4-1:FTIR Spectral Assignments and Putative Phytochemicals of Ocimum gratissimum Extract

Wavenumber (cm^{-1})	**Functional Group**	**Possible phytochemicals**
3352	Broad O–H stretching (phenols, alcohols)	Flavonoids, tannins, glycosides
2923 and 2853	C–H stretching (alkanes)	Terpenes, hydrocarbons, fatty acids
1729	C=O stretching (aldehydes, ketones, carboxylic acids.)	Eugenol derivative, aldehydes, fatty acid esters
1688	C=C stretching (aromatic ring)	Aromatic compounds, especially those with conjugated carbonyls

1612 and 1495	Aromatic skeletal vibrations	Thymol, Eugenol, other phenolic compounds
1452 and 1372	C–H bending (methyl groups)	Terpenoids, lipids
1222 and 1175	C–O stretching (alcohols, esters, or ethers)	Essential oil components, secondary alcohols
1031	C–O–C stretching (ether linkages)	Glycosides and ethers
938, 892, 838, and 718	Out-of-plane C–H bending (aromatic substitution)	substituted benzene rings

Data are presented as mean ± standard deviation (SD). Wavenumber is given in cm^{-1}. Functional groups and possible phytochemicals were assigned by comparing the observed absorption bands with established FTIR correlation tables.

The broad O–H stretching band at 3352 cm^{-1} suggests abundant phenolic compounds which indicates the presence of flavonoids, tannins, glycosides, consistent with reports by Tella & Oseni., (2019) and Nepolean et al., (2009), who identified flavonoids and tannins as key bioactive in extracts of *O. gratissimum* and *Moringa Olefeira* as they were effective against E. *coli*, S. *aureus* and similar pathogens. Phenolics are well known to exert antimicrobial effects through protein precipitation and disruption of microbial enzymes (Mandal and Domb, 2024). A distinct C=O stretching vibration at 1729 cm^{-1} confirms the presence of carbonyl groups, indicating compounds such as aldehydes, carboxylic acids, esters, or ketones. It is important to note that while some derivatives of eugenol, such as acetyl eugenol, may contain a carbonyl group, the core Eugenol molecule itself does not. The presence of the carbonyl peak at 1729 cm^{-1} therefore suggests other compounds, potentially aldehydes or esters, which are also often found in essential oils and may contribute to the extract's antimicrobial activity.

The C=C and aromatic skeletal vibrations observed between 1688–1495 cm^{-1} further support the presence of aromatic phenols such as thymol and eugenol. According to Kumar et al.,

(2024) these compounds have been demonstrated to act synergistically with other plant metabolites in inhibiting the growth of Gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*.

Similarly, the aliphatic C–H stretches (2923-2853 cm^{-1}) and bending vibrations (1452-1372 cm^{-1}) are indicative of terpenes and terpenoids, secondary metabolites widely reported to exhibit antimicrobial activity by disrupting microbial membranes (Akinmoladun et al., 2007; Nganteng et al., 2022). The C–O and C–O–C stretches (1222–1031 cm^{-1}) confirm the presence of alcohols, esters, and glycosides, which have also been linked to antimicrobial activity in medicinal plants (Nepolean et al., 2009). Furthermore, the peaks between 938 cm^{-1} and 718 cm^{-1} correspond to out-of-plane C–H bending vibrations, which are indicative of substituted benzene rings in the aromatic compounds present. These signals, particularly when analysed in combination with the other bands, provide a detailed fingerprint of the various complex phytochemicals in the extract.

Overall, the FTIR spectrum of the *O. gratissimum* extract provides a comprehensive molecular fingerprint that confirms the presence of several key phytochemical classes. The co-occurrence of specific functional groups allows for the identification of these complex compounds. For instance, the combination of O–H stretching (3352 cm^{-1}) and aromatic C=C vibrations (1688–1495 cm^{-1}) strongly indicates the presence of phenolic compounds such as flavonoids and tannins. Similarly, the presence of aliphatic C–H stretches (2923–2853 cm^{-1}) along with C–O stretches (1222–1031 cm^{-1}) is characteristic of terpenoids.

This integrated analysis, which moves beyond identifying single functional groups, provides crucial evidence for the types of compounds present in the extract. These findings align with previous studies, such as Kpètèhoto et al. (2019) that describe the plant as being rich in antimicrobial constituents. The presence of these specific phytochemicals directly corroborates the observed antimicrobial effects of the extract.

4.2. Antimicrobial assay

The antimicrobial assay showed that the Ocimum gratissimum extract exhibited a concentration-dependent inhibitory effect on *Escherichia coli*. The inhibition effect was highest (16.33 ± 1.15 mm) at the highest tested concentration (100% v/v) , indicating a strong antimicrobial activity but decreased gradually with dilution to a concentration of 6.25% v/v (Table 4-2). The absence of activity in the solvent control confirms that the antimicrobial effect was attributable to the phytochemicals in the extract rather than to the solvent.

A one-way ANOVA was conducted to determine whether there were statistically significant differences in the mean zones of inhibition across the five concentrations. p-value (Sig. = 0.000), $p<0.001$ showed the difference was significant. The post-hoc analysis confirmed that the higher concentrations (100% v/v and 50% v/v) produced significantly larger zones than the lower concentrations (6.25% v/v, 12.50% v/v, and 25.00% v/v), with all p-values < 0.05. The differences in zone size between the lowest concentrations (6.25% v/v vs. 12.50% v/v and 12.50% v/v vs. 25.00% v/v) were not statistically significant, meaning their effects on the zones were not distinct enough to be considered different.

A comparative analysis with standard antibiotics used as positive controls further contextualises the extract's efficacy. The *O.gratissimum* extract at 100% concentration displayed a significant inhibitory effect (16.33 mm) that was superior to that of several other conventional antibiotics, including tetracycline (4 mm), co-trimoxazole (8 mm), ceftriaxone (0 mm), ampicillin (0 mm), and gentamicin (0 mm). The complete lack of an inhibitory zone for ceftriaxone, ampicillin, and gentamicin suggests the *E. coli* strain used in this study possesses resistance mechanisms to these established antibiotics. This observation is not unique; similar studies on other antimicrobial plants like *Origanum majorana* and *Cinnamomum zeylanicum* have also failed to inactivate *E. coli* strains (Joshi et al., 2009), indicating that this bacterial species is

particularly resilient to a range of other antimicrobials. The superior efficacy of the *O. gratissimum* extract (16.33 mm), relative to several of these antibiotics, is therefore a significant finding. This result is notable because it demonstrates that the extract's compounds are effective against a resistant *E. coli* strain that was not susceptible to certain conventional antibiotics. These results are consistent with previous reports on the antimicrobial potential of *O. gratissimum* (Tella & Oseni, 2019) .

Table 4-2:Zone of Inhibition (mm) of Ocimum gratissimum Leaf Extract and Standard Antibiotics Against Escherichia coli

Sample (n = 3)	**Concentration**	**Mean ± SD (mm)**
Leaf Extract[a]	100%	16.33 ± 1.15
	50%	13.67 ± 0.58
	25%	10.00 ± 1.00
	12.5%	8.00 ± 1.00
	6.25%	7.3 ± 1.0
Control solvent	N/A	0

Positive controls		
Chloramphenicol	5 µg	21
Ciprofloxacin	30 µg	20
Co-trimoxazole	30 µg	8
Tetracycline	30 µg	4
Ceftriaxone	30 µg	0
Ampicillin	10 µg	0
Gentamicin	10 µg	0

[a] *Concentrations in v/v*

Data are presented as the mean diameter of the zone of inhibition in millimeters (mm) ± the standard deviation (SD). N/A indicates "not applicable." All values are the average of triplicate experiments.

The extract's inhibitory zone of 16.33 mm at 100% v/v is notably higher than the activity reported for methanolic and aqueous extracts in other studies (Adebolu & Oladimeji, 2005; Saha et al., 2013) highlighting the efficiency of ethanol to dissolve a wide range of bioactive compounds. These findings align with the study of Adeeyo et al. (2018) and Nganteng et al. (2022), who demonstrated that ethanol is a more effective solvent for extracting bioactive compounds compared to methanol, ethyl acetate, butane, hexane, chloroform and distilled water. As documented by Kpètèhoto et al. (2019), ethanol's polarity allows for the efficient solubilization of a wide range of antimicrobial phytochemicals, including flavonoids, tannins, and terpenoids, which are often less soluble in other solvents. The higher efficacy observed in this study can therefore be attributed to the enhanced extraction of these key bioactive compounds.

4.3 MIC and MBC Determination

At a concentration of 25% v/v, growth was suppressed in the broth medium, and this was recorded as the minimum inhibitory concentration (MIC). At this level, visible growth was inhibited compared to the growth (OD_{600} = 0.80) recorded in both the untreated growth control and the solvent control (Table 4-3). This suggests that the extract contains bioactive compounds capable of reducing microbial proliferation at relatively moderate concentrations.

To further quantify this effect (figure 4-2), dose-response modelling was used to determine the concentration required for 50% inhibition. The Inhibitory Concentration 50% (IC_{50}) was determined to be approximately 4.70% v/v, which is consistent with the observed dosedependent inhibition.

Table 4-3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Ocimum gratissimum Extract Against E. coli

Concentration (%v/v)	Growth (OD_{600} after 18 h)	MIC	MBC
100	0.05	-	-
50	0.05	-	YES
25	0.06	YES	NO
12.5	0.10	NO	NO
6.25	0.20	NO	NO
3.175	0.50	NO	NO
Growth control	0.80		
Solvent control	0.80		

OD_{600}: Optical Density (OD) was measured at a wavelength 600 nm to determine bacterial growth. MIC (Minimum Inhibitory Concentration) (OD less than 0.1). MBC (Minimum Bactericidal Concentration). 'YES' indicates the condition was met, and 'NO' indicates it was not. A dash (-) indicates the condition was not applicable.

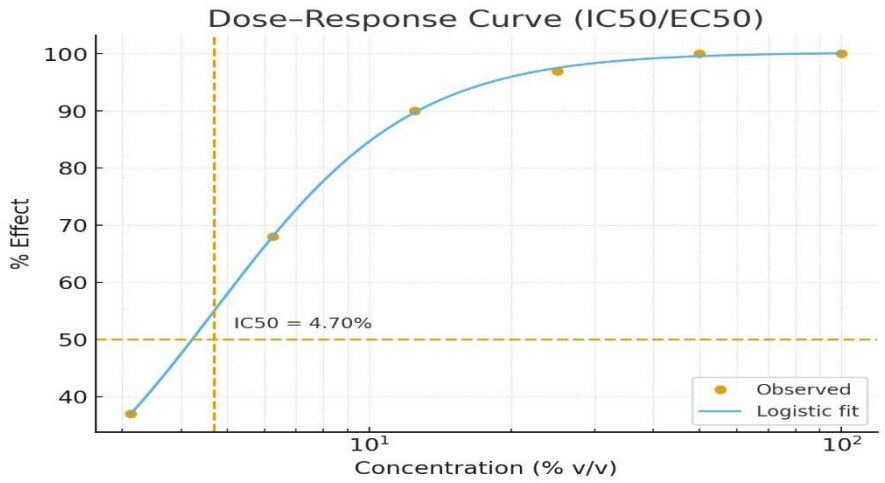

Figure 4-2:Dose-Response Curves IC_{50} of O. gratissimum Extract against E. coli.

Concentrations of 25% v/v and higher (50% and 100% v/v) were subsequently plated to determine the minimum bactericidal concentration (MBC). Unlike the MIC, the MBC was recorded at 50% v/v because no bacterial growth was observed at this concentration, whereas some growth was still present on the plates at 25% v/v. This distinction indicates that while the extract demonstrated bacteriostatic activity at 25% v/v, complete bactericidal action required a higher concentration.

Table 4-4:Pearson Correlation Analysis of Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC)

		MBC	MIC
MBC	Pearson Correlation	1	-.756
	Sig. (2-tailed)		.454
	N	3	3
MIC	Pearson Correlation	-.756	1
	Sig. (2-tailed)	.454	
	N	3	3

The relationship between the extract's inhibitory and bactericidal effects was assessed using a Pearson correlation as measured by optical density (OD_{600}), as shown in Table 4-4. The results show a Pearson correlation coefficient of -0.756. This indicates a strong negative correlation between MBC and MIC, suggesting that as the concentration required to inhibit microbial growth (MIC) increases, the concentration required to kill microbes (MBC) decreases.

However, the correlation is not statistically significant as the p-value (Sig. 2-tailed) is 0.454, which is greater than the conventional significance level of 0.05. This lack of significance is likely due to the very small sample size (N=3), which makes it difficult to draw reliable statistical conclusions.

The low optical density (OD_{600} = 0.05) at both 100% and 50% concentrations supports the extract's bactericidal potential. The increasing OD values at 12.5%, 6.25%, and 3.125% v/v indicate reduced extract efficacy at lower concentrations, suggesting a dose-dependent effect. Such dose-response patterns are well documented in phytochemical studies, where secondary metabolites such as phenolics, flavonoids, and terpenoids contribute to antimicrobial activity in a concentration-dependent manner(Tiruneh et al., 2023; Vidhya et al., 2020)

The MIC and MBC values obtained in this study are comparatively higher than studies reported by Adebolu & Oladimeji. (2005), MBC value was low as 0.1% when using essential oils of *O. gratissimum* against *E. coli, S.aureus, S.typhi and S.typhimurium*. Saha et al., (2013) documented an MIC of 62.5 µg/ml for methanolic extracts of *O. gratissimum* against *E. coli* which is lower compared to similar studies in weight per volume concentrations even though direct comparisons could not be made for this study. These variations can be attributed to differences in extraction methods, test organisms, and inoculum size. Essential oils of *O. gratissimum* have been consistently reported as more effective compared to other solvent extracts, due to their higher concentration of volatile phytochemicals such as eugenol, thymol, and other terpenoids (Saha et al., 2013).

The comparatively higher concentrations observed in this study may be explained by the use of crude extracts and their direct dilutions. This approach does not provide an exact quantification of the active compounds dissolved at each concentration, thereby reducing potency relative to purified extracts or essential oils. Furthermore, the bacterial inoculum used in this study was relatively high compared to the levels employed in some previous works (Joshi et al., 2009; Saha et al., 2013). A larger initial bacterial load can demand higher concentrations of extract to achieve bacteriostatic or bactericidal effects, which may partly account for the observed differences.

Despite these variations, the results of this study remain consistent with reports that ethanolic extracts of *O. gratissimum* are effective against *E. coli*. Overall, these findings confirm that *O. gratissimum* possesses antimicrobial properties, with a 25% v/v extract concentration sufficient to inhibit growth (MIC) and a 50% v/v concentration required to achieve bactericidal activity (MBC).

4.4 *O. gratissimum* Extract in Simulated Contaminated Water

The extract demonstrated a clear dose-dependent bactericidal activity, as illustrated in (Figure 4-3). The highest concentration tested, ≥50% v/v, was highly effective, achieving a ≥3.0 log reduction, or ≥99.9% reduction, of the initial *E. coli* population (7.38×10^5 CFU/mL). This level of microbial inactivation meets the World Health Organization's (WHO) guidelines for effective water disinfection (WHO, 2011), confirming that the Minimum Bactericidal Concentration (MBC) was achieved at this dosage.

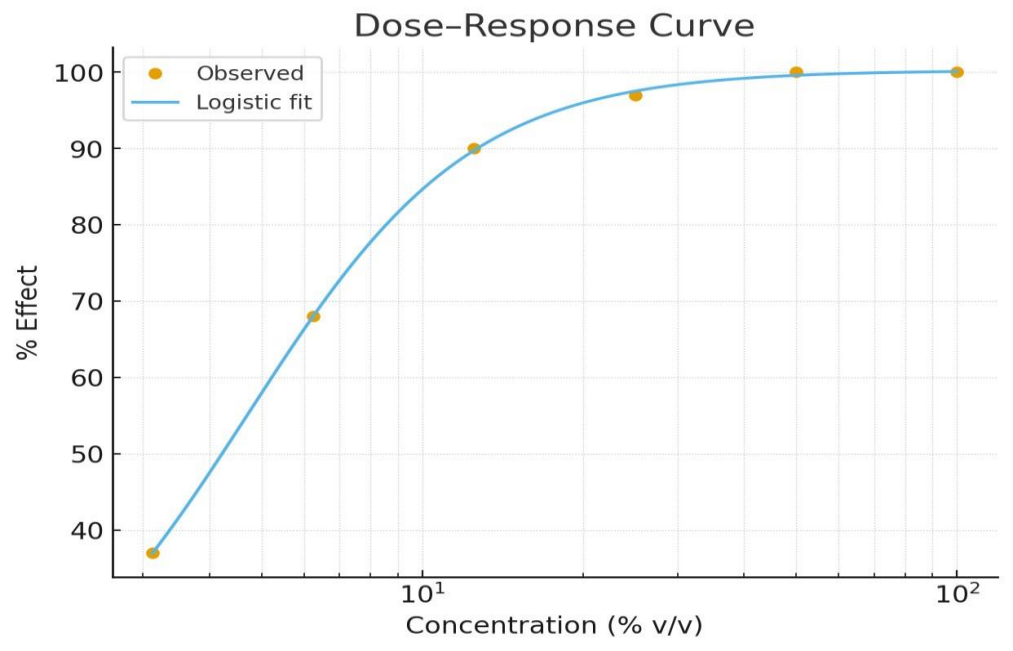

Figure 4-3: Dose-Response Relationship of O.gratissimum Extract in Simulated Contaminated Water

Lower concentrations produced progressively less inhibition. A 25% v/v concentration resulted in a substantial 97% reduction (1.5 log), while 12.5% v/v yielded a 90% reduction (1.0 log). At the lowest concentrations (6.25% and 3.125% v/v), the extract showed only modest inhibitory effects, with reductions of 68% and 37% respectively. These findings are consistent with previous research on plant-based extracts, which often exhibit concentration-dependent activity against enteric bacteria (Adebolu & Oladimeji, 2005; Saha et al., 2013).

Following the Shapiro–Wilk test and Levene's test which confirmed no deviation from normality for all groups ($p>0.05$) and equality of the variance as non-significant, respectively, a one-way ANOVA showed a statistically significant ($F=164.399$, $p<0.001$) effect of the extract's concentration on bacterial count. This confirms that the treatment concentrations caused a statistically significant reduction in bacterial growth. The post hoc test reveals which specific concentration pairs differ significantly. The 50% and 100% concentrations were not statistically different from each other ($p=1.000$), indicating that the bactericidal effect plateaus

at 50% (v/v). The 25% (v/v) concentration is not statistically different from the 12.5% (v/v) concentration ($p=0.132$), suggesting similar inhibitory effects at the two dosages. All other comparisons between the higher concentrations (12.5%, 25%, 50%, 100% v/v) and the lower concentrations (3.125% and 6.25% v/v) were statistically significant ($p<0.05$).

The antimicrobial action of the *O. gratissimum* extract was not mediated by changes in pH. The pH values remained remarkably stable throughout the 18-hour incubation period, as shown in Table 4-5. The initial and final pH values of the extract-treated samples (e.g., 50% v/v extract changed from 6.85 to 6.80) closely paralleled those of the growth and solvent controls (6.90 to 6.88 and 6.89 to 6.87, respectively). This stability aligns with a study made by Matthews et al. (2009) who reported that pH was not a contributing factor to coliform inactivation in a study in which Neem oil achieved similar results across different water sources with highly varying pHs.

Table 4-5: Initial and Final pH Values of Treatment and Control Samples

Sample	Initial pH	Final pH
100% extract	6.83	6.80
50% extract	6.85	6.80
25% extract	6.83	6.79
12.5% extract	6.85	6.84
6.25%	6.88	6.87
Growth Control	6.90	6.88
Solvent Control	6.89	6.87

Initial and final pH values of the extract-treated samples and controls recorded after 18 hours of incubation.

This demonstrates the significant dose-dependent antimicrobial activity of *Ocimum gratissimum* extract against *E. coli* in contaminated water. The results clearly show that the extract's efficacy is directly proportional to its concentration, with the highest reductions

observed at the top dosages. Specifically, the 100% and 50% (v/v) concentration proved to be the most effective, achieving a substantial ≥99.9% reduction in *E. coli* population. These findings confirm the extract's potential as a powerful natural bactericidal agent for water purification, highlighting the critical role of concentration in its application.

4.5 Validating with Real Waste Water

The antimicrobial efficacy of *Ocimum gratissimum* extract was validated using real-world wastewater, confirming its potential beyond controlled laboratory conditions. The baseline wastewater parameters from Table 4-6, highlight a high turbidity of 800 NTU and TSS of 1,400 mg/L, providing tangible evidence of this complex matrix. The wastewater sample had a high microbial load of 6.72×10^5 CFU/ml, serving as the untreated control (Table 4-7). Consistent with the findings from sterile water, the extract demonstrated a concentration-dependent reduction in the total microbial count. The 50% v/v concentration was the most effective, reducing the total count to 1.93×10^5 CFU/ml, while the 100% achieved a similar reduction, representing approximately 71% and 70% reductions, respectively. The 25% v/v concentration also showed measurable activity, reducing the total count to 4.32×10^5 CFU/ml, a reduction of approximately 34%.

Table 4-6: Physicochemical Parameters of the Raw Wastewater

Parameter	**Value**	**Unit**
Turbidity	800	NTU
Total Suspended Solids (TSS)	1400	mg/L
Biochemical Oxygen Demand (BOD_5)	500	mg/L
Chemical Oxygen Demand (COD)	1200	mg/L
pH	8.1	-

The values presented in this table characterize the baseline conditions of the contaminated wastewater sample before treatment.

Table 4-7: Total Microbial Count in Real Wastewater Samples Treated with O.gratissimum Extract

Concentrations	**Total count/ml**
100%	$1.98 \times 10^5 \pm 1.2 \times 10^3$
50%	$1.93 \times 10^5 \pm 3.2 \times 10^3$
25%	$4.32 \times 10^5 \pm 5.5 \times 10^3$
Control water	$6.72 \times 10^5 \pm 8.8 \times 10^3$

Data are presented as the total microbial count in Colony-Forming Units per millilitre (CFU/ml), with values as the mean ± standard deviation.

Statistical analysis was performed to determine whether different concentrations of O. *gratissimum* extract had a significant effect on microbial load in wastewater samples.

Normality of the data was assessed using the Shapiro–Wilk test. Results showed no significant deviation from normality for all treatment groups ($p > 0.05$), indicating that the assumption of normality was satisfied. The Levene's test for equality of variances was non-significant, $F(2,6) = 1.568$, $p = 0.283$ $F(2, 6) = 1.568$, $p = 0.283$ 2,6 1.568 p=0.283, confirming that the homogeneity of variances assumption was met.

A one-way ANOVA confirmed a highly significant effect of the treatments ($F(3,8) = 2205.2$, $p < .001$), indicating that at least one of the concentration groups had a statistically significant impact on the microbial population. To identify which specific groups differed, a Tukey HSD post hoc test was conducted. The analysis revealed a clear dose-dependent effect, with the following statistically significant differences:

- The microbial count in all three treatment groups (25%v/v, 50%v/v, and 100%v/v) concentrations) was significantly lower than that of the untreated control group ($p<.001$).
- The 25%v/v concentration group also had a microbial count that was significantly higher than both the 50%v/v and 100%v/v concentration groups ($p<.001$).

Interestingly, the Tukey HSD test and the homogeneous subsets table showed that the 50% v/v and 100% v/v concentration groups were not statistically different ($p=.883$). This suggests that although a significant reduction in microbial load occurred, the extract's antimicrobial effect began to plateau at 50% v/v. The homogeneous subsets table further reinforced these findings, grouping the 50% and 100% v/v concentrations into a single statistical subset, separate from the 25% v/v concentration and the untreated control.

Despite these significant reductions, neither concentration achieved complete disinfection. This reduced efficacy compared to the lab-based experiment is likely due to the complex matrix of the real wastewater. These high levels of suspended solids and the corresponding organic matter (indicated by a high BOD of 500 mg/L and COD of 1,200 mg/L) are consistent with previous research that has reported a decline in the effectiveness of plant-based disinfectants and essential oils in the presence of natural organic matter (NOM), suspended solids, and dissolved ions. Real wastewater contains high levels of NOM, suspended solids, and dissolved ions. These substances can interfere with the antimicrobial action of the extract's bioactive compounds, either by binding to them or by shielding the microorganisms (Shaheed et al., 2009; Winward et al., 2008). Additionally, Matthews et al. (2009) suggested that naturally occurring microbial populations in real wastewater may be more resilient due to prior adaptation to environmental stressors, making them more resistant than laboratory-cultured isolates.

While the scope of this study did not include an investigation into whether *E. coli* resistance increases with the level of water contamination, it is noteworthy that other studies have achieved 100% disinfection using fluids squeezed from *O. gratissimum* leaves against naturally occurring *E. coli* (192 CFU/ml) in well water (Ayeni et al., 2022). In another study using a different plant, Vega Andrade et al. (2021) suggested that the reduced effectiveness was likely due to the low dosage applied, noting that Moringa oleifera used in their study was intended to function both as a coagulant and a disinfectant, given the wastewater quality. This study's findings with *O. gratissimum* contradict that conclusion. Our results indicate that even at higher concentrations and effective dosages, disinfection efficiency was limited. Our results reinforce the critical role that initial water quality can play in the overall effectiveness of the disinfectant. For future applications, it is recommended that pretreatment methods such as filtration and sedimentation be considered to substantially reduce these interfering parameters. Alternatively, a synergistic approach could be explored, combining the *O. gratissimum* extract with other plant-based materials known for their purification properties. This would be a crucial step toward achieving total disinfection of *E. coli* in more challenging, real-world conditions.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study successfully demonstrated the potent, dose-dependent antimicrobial activity of *Ocimum gratissimum* leaf extract against *Escherichia coli*. The extract's efficacy is attributed to its diverse phytochemical profile, including phenolics, flavonoids, and essential oils such as eugenol and thymol. In a controlled laboratory setting using sterile, *E. coli*-inoculated water, the extract proved highly effective, with a minimum inhibitory concentration (MIC) of 25% v/v and a minimum bactericidal concentration (MBC) of 50% v/v, achieving≥99.9% microbial reduction and meeting the established WHO disinfection guidelines.

However, the extract's performance was significantly influenced by the aqueous conditions. When applied to real wastewater, its efficacy was notably reduced, achieving only 71% microbial reduction at 50% (v/v). This discrepancy highlights the critical role of external factors in a complex water matrix, where high microbial loads, organic matter, and suspended solids can interfere with the bioactive compounds in the extract.

Overall, the findings confirm that *O. gratissimum* possesses significant antimicrobial activity, making it a valuable candidate for water disinfection. While its efficacy may be limited in complex systems, it holds promise as a sustainable, low-cost, and locally accessible complementary disinfectant, particularly for preliminary microbial reduction in decentralised water treatment.

5.2 Recommendations

Based on the findings of this study, several key recommendations are proposed to enhance the practical application and understanding of *Ocimum gratissimum* extract as a natural water phytodisinfectant.

For effective large-scale application in complex matrices like wastewater, pre-treatment is essential. Future work should focus on integrating *O.gratissimum* extract with primary physical treatment methods, such as sedimentation, sand filtration, or coagulation. This approach would reduce the concentration of organic matter and suspended solids, thereby minimizing interference with the extract's bioactive compounds and significantly improving its antimicrobial efficacy under field conditions.

Research should be directed toward optimising the extraction techniques to maximise the yield and concentration of active phytochemicals. By refining methods, it may be possible to reduce the volume of plant material required, making the process more cost-effective and sustainable.

Additionally, investigating the long-term stability and shelf life of the extract is crucial for practical storage and distribution.

Given the extract's reduced efficacy in real wastewater, future studies should explore synergistic formulations. Investigating the combined effects of *O. gratissimum* with other plant-based disinfectants or natural coagulants could enhance disinfection efficiency, particularly in turbid, microbially dense waters. This would provide a more robust, comprehensive solution for water treatment, addressing both microbial and physical contamination.

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APPENDIX

This appendix contains the detailed data and statistical output from the analysis of *O. gratissimum*'s efficacy in both simulated and real wastewater.

Table A.1: Disinfection Efficacy of O. gratissimum in Simulated Contaminated Water

Conc(%v/v)	**Approx. Log reduction**	**Remaining (CFU/ml)**	**% Reduction**
100%	≥3.0	≤ 7.38×10^1	≥99.9%
50%	≥3.0	≤ 7.38×10^1	≥99.9%
25%	1.5	2.33×10^3	97%
12.5%	1.0	7.38×10^3	90%
6.25%	0.5	2.33×10^4	68%
3.125%	0.2	4.65×10^4	37%

This table summarizes the results of the experiment, showing the effectiveness of various concentrations of the *O. gratissimum* leaf extract in reducing microbial contamination in a simulated wastewater sample. The data illustrates the relationship between the concentration of the extract and the resulting microbial reduction, as measured by log reduction, remaining colony-forming units (CFU/ml), and percentage reduction.

Table A. 2:Normality tests for CFU/ml data at different concentrations in simulated contaminated water

Tests of Normality

	Concentration	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
cfu/ml	3.08%	.314	3	.	.893	3	.363
	6.12%	.345	3	.	.839	3	.213
	12.50%	.212	3	.	.990	3	.810
	25.00%	.228	3	.	.982	3	.742
	50.00%	.192	3	.	.997	3	.893
	100.00%	.353	3	.	.822	3	.169

a. Lilliefors Significance Correction

Results of normality analysis for CFU/ml data using the Kolmogorov-Smirnov and Shapiro-Wilk tests. A significance value ($p>0.05$) indicates that the data is normally distributed. The 'df' (degrees of freedom) for each test is 3. Note that the Lilliefors Significance Correction was applied for the Kolmogorov-Smirnov test.

Table A.3:Results of Levene's Test for Homogeneity of Variances on CFU/ml data in simulated contaminated water.

Test of Homogeneity of Variances cfu/ml

Levene Statistic	df1	df2	Sig.
10.273	5	12	.001

The table displays the results from Levene's Test, which was used to assess the assumption of **homogeneity of variances** for the CFU/ml data. With a Levene Statistic of 10.273 and a significance value of p=0.001, the null hypothesis (that the group variances are equal) is rejected. This indicates a statistically significant difference in the variances among the groups.

ANOVA

Table A.4:One-way ANOVA for Microbial Reduction in Simulated Wastewater cfu/ml

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4289300262.500	5	857860052.500	164.399	.000
Within Groups	62618054.000	12	5218171.167		
Total	4351918316.500	17			

Multiple Comparisons

Table A.5:Tukey's HSD Post-Hoc Test for Multiple Comparisons for simulated wastewater

Dependent Variable: cfu/ml

Tukey HSD

(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3.08%	6.12%	20633.333*	1865.149	.000	14368.45	26898.22
	12.50%	35098.333*	1865.149	.000	28833.45	41363.22
	25.00%	40253.333*	1865.149	.000	33988.45	46518.22
	50.00%	42472.000*	1865.149	.000	36207.11	48736.89
	100.00%	42470.000*	1865.149	.000	36205.11	48734.89
6.12%	3.08%	-20633.333*	1865.149	.000	-26898.22	-14368.45
	12.50%	14465.000*	1865.149	.000	8200.11	20729.89
	25.00%	19620.000*	1865.149	.000	13355.11	25884.89
	50.00%	21838.667*	1865.149	.000	15573.78	28103.55
	100.00%	21836.667*	1865.149	.000	15571.78	28101.55
12.50%	3.08%	-35098.333*	1865.149	.000	-41363.22	-28833.45
	6.12%	-14465.000*	1865.149	.000	-20729.89	-8200.11
	25.00%	5155.000	1865.149	.132	-1109.89	11419.89

	50.00%	7373.667*	1865.149	.018	1108.78	13638.55
	100.00%	7371.667*	1865.149	.018	1106.78	13636.55
	3.08%	-40253.333*	1865.149	.000	-46518.22	-33988.45
	6.12%	-19620.000* -	1865.149	.000	-25884.89	-13355.11
	12.50%	5155.000	1865.149	.132	-11419.89	1109.89
25.00%						
	50.00%	2218.667	1865.149	.834	-4046.22	8483.55
	100.00%	2216.667	1865.149	.834	-4048.22	8481.55
	3.08%	-42472.000*	1865.149	.000	-48736.89	-36207.11
	6.12%	-21838.667*	1865.149	.000	-28103.55	-15573.78
	12.50%	-7373.667*	1865.149	.018	-13638.55	-1108.78
50.00%						
	25.00%	-2218.667	1865.149	.834	-8483.55	4046.22
	100.00%	-2.000	1865.149	1.000	-6266.89	6262.89
	3.08%	-42470.000*	1865.149	.000	-48734.89	-36205.11
	6.12%	-21836.667*	1865.149	.000	-28101.55	-15571.78
100.00%	12.50%	-7371.667*	1865.149	.018	-13636.55	-1106.78
	25.00%	-2216.667	1865.149	.834	-8481.55	4048.22
	50.00%	2.000	1865.149	1.000	-6262.89	6266.89

*. The mean difference is significant at the 0.05 level.

Table A.6:Homogeneous Subsets Based on Tukey HSD Test

cfu/ml Tukey HSD

Concentration	N	Subset for alpha = 0.05			
		1	2	3	4
50.00%	3	28.00			
100.00%	3	30.00			
25.00%	3	2246.67	2246.67		
12.50%	3		7401.67		
6.12%	3			21866.67	
3.08%	3				42500.00
Sig.		.834	.132	1.000	1.000

Means for groups in homogeneous subsets are displayed. a.
Uses Harmonic Mean Sample Size = 3.000.

Table A.7:Normality tests for CFU/ml data at different concentrations in real waste water

Tests of Normality[a]

	Concentration	Kolmogorov-Smirnov[b]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
CFU/ml	0.25	.238	3	.	.976	3	.702
	0.5	.328	3	.	.871	3	.298
	1	.189	3	.	.998	3	.908
	control	.299	3	.	.915	3	.433

a. There are no valid cases for CFU/ml when Concentration = .000. Statistics cannot be computed for this level.

b. Lilliefors Significance Correction

Table A.8:One-way ANOVA for Microbial Reduction in Wastewater

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	466399000000.000	3	155466333333.333	2205.196	.000
Within Groups	564000000.000	8	70500000.000		
Total	466963000000.000	11			

This table presents the results of a one-way analysis of variance (ANOVA) test. The ANOVA was performed to determine if there were statistically significant differences in microbial reduction among the different concentrations of *O. gratissimum* extract used in the

experiment. The "Sig." (significance) value indicates the probability that the observed differences are due to random chance

Table A. 9:Tukey's HSD Post-Hoc Test for Multiple Comparisons

(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	0.25	237666.667*	6855.655	.000	215712.45	259620.89
	0.5	478000.000*	6855.655	.000	456045.78	499954.22
	1	473000.000*	6855.655	.000	451045.78	494954.22
0.25	control	-237666.667*	6855.655	.000	-259620.89	-215712.45
	0.5	240333.333*	6855.655	.000	218379.11	262287.55
	1	235333.333*		.000	213379.11	257287.55
0.5	control	-478000.000*	6855.655	.000	-499954.22	-456045.78
	0.25	-240333.333*	6855.655	.000	-262287.55	-218379.11
	1	-5000.000	6855.655	.883	-26954.22	16954.22
1	control	-473000.000*	6855.655	.000	-494954.22	-451045.78

0.25	-235333.333*	6855.655	.000	-257287.55	-213379.11
0.5	5000.000	6855.655	.883	-16954.22	26954.22

*. The mean difference is significant at the 0.05 level.

This table shows the results of Tukey's Honestly Significant Difference (HSD) post-hoc test. This test was conducted after the one-way ANOVA (Table A.2) confirmed a significant difference between groups. The purpose of this test is to identify exactly which specific pairs of extract concentrations had a statistically significant difference in their microbial reduction efficacy. A significant value ("Sig.") less than 0.05 indicates a significant difference between the two concentrations being compared

Table A.10:Homogeneous Subsets Based on Tukey HSD Test

Concentration	N	Subset for alpha = 0.05		
		1	2	3
0.5	3	192666.67		
1	3	197666.67		
0.25	3		433000.00	
Control	3			670666.67
Sig.		.883	1.000	1.000

Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.000.

This table presents the results of the Tukey Honestly Significant Difference (HSD) test, organizing the treatment groups into "homogeneous subsets." Groups that are in the same subset are not statistically different from one another in their microbial reduction efficacy. This provides a more detailed view of the pairwise comparisons from Table A.3, clearly showing which concentrations of the *O. gratissimum* extract had a similar effect on the microbial population.