EVALUATION OF *Moringa oleifera* AND *Azadirachta indica* EXTRACTS FOR WATER PURIFICATION AND INHIBITION OF SELECTED WATERBORNE BACTERIA

BY

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TITLE PAGE

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCE, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE (B.Sc) IN BIOTECHNOLOGY

SUPERVISOR: DR. I. NWAJIOBI
CO-SUPERVISOR: MRS RUTH UGWU

JUNE, 2026

APPROVAL PAGE

This research, titled "**EVALUATION OF *Moringa oleifera* AND *Azadirachta indica* EXTRACTS FOR WATER PURIFICATION AND INHIBITION OF SELECTED WATERBORNE BACTERIA**", has been assessed and approved by the Departmental Committees in the Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University, Enugu, Nigeria.

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CERTIFICATION

I certify that this research work titled "**EVALUATION OF *Moringa oleifera* AND *Azadirachta indica* EXTRACTS FOR WATER PURIFICATION AND INHIBITION OF SELECTED WATERBORNE BACTERIA**" was carried out by me, Ogbuke Chiamaka Jessica, with Registration Number GOU/U22/BTG/165, under the supervision of Dr. I. Nwajiobi and Mrs. Ruth Ugwu. I confirm that this work is original and has not been submitted elsewhere for any academic award.

_____ _____

Ogbuke Chiamaka Jessica **Date**
GOU/U22/BTG/165

DEDICATION

This project is dedicated to my beloved parents, Ozor Cletus Ogbuke and Lolo Ogochukwu Ogbuke, for their unwavering love, sacrifices, support, and encouragement throughout my academic journey.

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ABSTRACT

This study investigated the effectiveness of *Moringa oleifera* and *Azadirachta indica* (Neem) extracts in the purification of contaminated water and inhibition of waterborne bacteria. The aim was to evaluate their antimicrobial activity and their effects on selected physicochemical and microbiological parameters of water samples. Four contaminated water samples were treated with varying concentrations of aqueous and ethanolic plant extracts. Physicochemical parameters, including pH and turbidity, were analysed before and after treatment. Microbiological analysis was carried out using total heterotrophic bacterial count and Most Probable Number (MPN) techniques for coliform detection. Isolates obtained were further subjected to morphological, microscopic, and biochemical tests for identification. Results showed that the pH of the water samples remained within acceptable limits before and after treatment, indicating minimal impact on chemical properties. However, turbidity was significantly reduced, reflecting improved water clarity. Microbial analysis revealed high bacterial loads in untreated samples, while treated samples showed a drastic reduction in microbial count, with complete absence of coliform bacteria (0/100 mL). Biochemical characterization supported the identification of bacterial isolates, while a residual organism consistent with *Bacillus* species was observed after treatment, likely due to its spore-forming ability and resistance to antimicrobial conditions. Comparative analysis indicated that *Azadirachta indica* exhibited higher antimicrobial activity than *Moringa oleifera*. These findings demonstrate that the plant extracts are effective in reducing microbial contamination and improving water quality, and highlight their potential as low-cost and sustainable alternatives for water purification in resource-limited settings.

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INTRODUCTION

1.1 Background of the Study

Getting clean and safe drinking water has been a major issue in Nigeria. Unavailability of the untreated water has been highlighted as a major human problem. Microbial contamination is one of the factors responsible for water impurities which is more complex because it cannot be seen with our naked eyes, in this, enteric pathogens are responsible for water borne diseases. Almost all the deaths and ill health among kids and adults are caused by germs which enters the body through food and water. Statistics published by the WHO (2013) has approximated that 80% of diseases and sickness in the world today is caused by poor hygiene, insufficient sanitations, and unhealthy conditions. The United Nation (UN) estimates that around 1,500 cubic liters of wastewater are generated annually, which is six times the amount of water in all the streams worldwide (Olasupo *et al.,* 2023).

The detrimental effects of these group of organisms on adult and children have been reported (Karmali *et al*., 2010), (World Health Organization, 2023). Many research studies have been done on the purification of water using chemical methods like chlorine, alum, aluminum sulphate but the major limitations is its high cost . Due to high cost of water treatment and the rapid spread of microbial agent, several investigations are carried out on the use of plant extract to decontaminate water. Plants possess antimicrobial purification properties which is used for the treatment of water, a good amount of effective precipitant and antiseptic have been identified in plant (Recal, *et al* 2023). Medicinal plants such as *Coriandrum sativum* (Llori, 2023) *and Ocimum tenuiflorum (*sharma *et al., 2019)* have been reported to exhibit water purification activities, However no information has been reported on the use of *Moringa oleifera* and *Azadirachta indica* leaves in

water purification. Phytochemicals such as quinones, flavonols, phenols, tannins, coumarins, alkaloids present in plants have been found to be responsible for water purification and antimicrobial activities (Eze *et al.,* 2023).

1.2 Statement of Problem

Access to clean drinking water remains a serious health problems, especially in developing countries where they rely on streams and well water (Delelegn *et al.,* 2018). About 20% of people worldwide lack safe water and 50% lack adequate sanitation. Overtime, there is increase of illness and deaths. The people and societies that are under developed have no access to water purification systems and are forced to use contaminated water which increases risks and exposure to several health issues.

Water treatment and water purification using plant extract is an underrated step used to improve the quality of water. There are so many methods of increasing the quality of water like domestic waste water treatments and municipal waste water treatment but they have their disadvantages like high cost and high use of electricity.

Although multiple researches have stated the antibacterial effects of medicinal plants such as *Moringa oleifera* and *Azadirachta indica*, there is scarce data on their synergistic impact in cleansing polluted water under regional activities. Additionally, differences in plant extract, extraction methods and water source produce the reasons for deeper analysis. Thus, this study sought to assess the capability of this medicinal plants to upgrade water standards and therefore control related illnesses.

1.3 Aim and Objective

Aim

The study was aimed at investigating the possible application of moringa and neem leaf extract in water purification and inhibition of selected waterborne pathogens.

The specific objectives were to:

i. Isolate and identify waterborne bacteria in contaminated water samples.

ii. Obtain and analyze aqueous and ethanolic extracts of neem and Moringa leaf and seed samples as purifying agents and microbial suppressants in water samples.

iii. Determine the before and after effects of utilizing the plant extracts as natural purifying agents in water samples through pH, turbidity and microbial load differences.

CHAPTER TWO

LITERATURE REVIEW

2.1 Challenges in Water Purification

A very important key in the well-being of any community and its individuals is the constant availability of clean and healthy drinking water. The world is actively looking for better ways to purify water and make it safer. Though science and engineering have made progress in securing clean water, a growing global population makes this more challenging. Each year, about 1.5 gigatons of wastewater goes into water supply worldwide, causing water pollution. (Priya *et al*., 2022). Modern things like factories, overpopulation, and cutting down forests worsen these issues. Dirty water can spread diseases like hepatitis A, cholera, and malaria. Antibiotic-resistant bacteria have also become a major worry (Jan *et al*., 2022). Treating water properly could help reduce the spread of these bacteria and cut down on the number of antibiotics in the environment. Concern for the environment also points to the need for clean water. Water quality has a big impact on ecosystems; too much nitrogen or phosphorus can cause eutrophication. Even small amounts of pollution can affect aquatic life. So, studying and improving water purification methods is very important. Current methods applied adsorption, filtration, boiling, osmosis, ionization sterilization and photolysis (Swatia *et al*., 2018). All methods face challenges which demonstrate the need for upcoming technological improvements (Tariq *et al.,* 2023).

Traditionally, the conventional methods of water purification have been developed over the years, primarily through sedimentation and filtration. Sedimentation allows for the settling of impurities but often fails to provide the best outcome. Filtration, with the help of sand, gravel, and cloth, has proven to be very effective against turbidity levels. Heating, such as through the process of boiling, has also been used for purification. However, the process of boiling has some drawbacks, such as

the failure to provide the best outcome for sterilization, as well as the failure to remove chemicals, thereby affecting taste and color. Technologies that are culturally appropriate and compatible with the culture of the people are encouraged, and their development has been remarkable, especially for the people of different regional part of the world. Although it is traditionally used as a bridge to the adoption of modern technologies, these conventional methods provide immediate protection, but their effectiveness has been threatened by microorganisms and the emergence of micro-pollutants. Traditional methods are culturally important for the purification of water, and their effectiveness depends on the level of diversity within the culture of the people. The adaptation of traditional methods to the modern context has proven to be very effective, such as the cloth filter's ability to remove Giardia cysts compared to the conventional ceramic filter. Promoting the conventional methods of purification has proven to be very effective, and the implementation of the dual-path strategy has been very beneficial. This has led to the understanding of the conventional methods, thereby providing the opportunity to develop new technologies to overcome the problem of impurities, such as the ones that persist (Timis *et al*., 2022).

2.2 Water purification techniques

2.2.1. Boiling and chlorination

The use of traditional water purification techniques is still in practice in current day water treatment procedures. The two methods of boiling and chlorination have become the most widely used water purification techniques which people use throughout the world. The process of boiling water eliminates all harmful substances, removing iron, turbidity, bad tasting and bad smelling substances to produce microbiologically safe drinking water. The process produces pathogen-free water which functions as an essential component for maintaining water safety. The method maintains its status as a worldwide preferred choice because of its simple execution process

delivering consistent results. Boiling water not only reduces iron content but also decreases turbidity and enhances its taste. The method of fabric-based purification depends on solar energy which becomes operational when users combine cotton towels with the procedure. Transparent plastic bottles function as solar disinfection devices because they use ultraviolet (UV) rays from sunlight for their disinfection process. The sun's heat establishes a purification system which operates through the use of bottles placed on iron racks. People use transparent bottles to store purified water for safe drinking based on environmental factors which determine their storage needs (Genter *et al*., 2022).

The chlorination process effectively eliminates bacteria together with enteric viruses, which serve as the main pathogens that cause waterborne diseases. The method enables large-scale implementation to treat all drinking water supplies. Chlorination provides a major benefit because it delivers disinfectant protection, which remains active to defend against future contamination. Water contamination that results from poor hygiene during collection can be partially treated through the slow dissolution of chlorine in water. Water systems need a low density of 0.3 mg/L Free Residual Chlorine (FRC) to maintain their disinfection capacity. Water sources become contaminated through direct exposure or they function as breeding grounds for pathogens, which makes it impossible to entirely remove contaminants through manual procedures. The collection stage requires initial disinfection because it can eliminate approximately 60% of all existing contamination. High levels of chlorine in water create dangerous conditions that make water unsafe for drinking. Water sources contain organic material which reacts with chlorine (Cl2) to produce chlorination byproducts (CBPs). The use of large-scale chlorination requires assessment of its potential health risks to human beings and ecological systems and all the various forms of life (Phatthalung *et al*., 2021; Guo *et al*., 2022).

2.2.2 Membrane filtration

Membrane technology has emerged as a vital research field which scientists investigate to create modern water treatment solutions. The technology has received considerable interest because it can purify drinking water by eliminating various contaminants which are present in water sources. Membrane-based treatment systems effectively tackle problems which involve eliminating dangerous bacteria and viruses and suspended particles and sediments, which are contaminants that standard disinfection techniques cannot remove. The technology successfully achieves its goals according to (Chadha *et al*., 2022; Kumar *et al*., 2023) who report its effective results and promising outcomes. Membrane technologies provide a stronger defense system, acting as a barrier against harmful substances that penetrate the defense barriers of the membrane. Membrane filtration technologies simultaneously treat particulate matter and impurities, thereby significantly inhibiting the growth of bacteria through the constraints of increased membrane permeability. Membranes used for the removal of microorganisms are characterized by very restrictive pore sizes. The proposed biotechnologies are developed through two major routes: (i) the advanced manufacturing of the proposed devices, and (ii) the incorporation of flexible operational protocols to enhance the effectiveness of the proposed bioprocesses and biofilters, respectively (Rosenqvist *et al.*, 2023).

2.3 Botanical Water Treatment Methods

They are natural ways of purifying water using medicinal plants or plant based material, without using chemicals or expensive reagents'

There are three major plant base water treatment methods which includes coagulation, adsorption, and disinfection which are discussed below.

2.3.1 Plant based coagulation

As reported by (Karnena *et al.,* 2022), there are plant species which have been used since ancient times because they can coagulate impurities in water, including: *Moringa olifiera, Azadirachta indica, Ocimum sanctum, Cocos nucifera, Ceratonia siliqua, Arachis hypogea, Parkinsonia aculeate, Opuntia ficus-indica, Tamarindus indica, Brassica juncea, Hibiscus esculentus, Luffa cylindrica, Trigonella foenum-graecum, Phaseolus vulgaris, Vigna unguiculata, Cicer arientinum,* amongst several others. Plants can naturally synthesize bioactive compounds like functional proteins, polysaccharides, glycoproteins, poly phenolic compounds, and proteolytic enzymes that perform the coagulation processes of charge neutralization and polymer bridging. Wastewater treatment facilities rely on coagulation as their fundamental operation which serves as a key treatment method. Coagulants that possess positive charges in the coagulation process create floc which binds with colloids by balancing their negative charges. Flocculation increases both the volume and weight of particles that have undergone coagulation (Syam *et al.,* 2020).

The coagulants belong to two distinct categories because they either come from artificial manufacturing processes or they exist in nature. The wastewater management systems use flocculants which include lime and aluminum sulphate as their metallic coagulants. The production of lime requires the extraction of limestone or dolomite materials. The two coagulants operate through their ability to eliminate both physical and chemical impurities which exist in water. we always make use of coagulating chemicals which results in the creation of harmful waste materials which remain in water and sludge and these substances harm the environment and human health. Natural coagulants include sources from bacteria, fungi, plants, and animal substances like plant extracts, fruit wastes and other components.

Al-Sahari *et al*., (2020) said that using plant coagulants like plant-based polymers or polysaccharides has become popular because they offer a sustainable solution which reduces the

environmental impact of water treatment systems compared to traditional inorganic coagulants. The process of charge neutralization occurs when coagulants with opposite charges to the colloidal particles complete the process which results in decreased electrostatic repulsion between two colloids that finally create large flocs. The process of polymer bridging occurs when colloidal particles bind to polymers and create large floc structures. Plant coagulants create sufficient bridging bonds which connect particle surfaces of their material. Researchers have discovered bioactive compounds that different plant species use as coagulants throughout their entire plant structure (Karnena *et al.,* 2022).

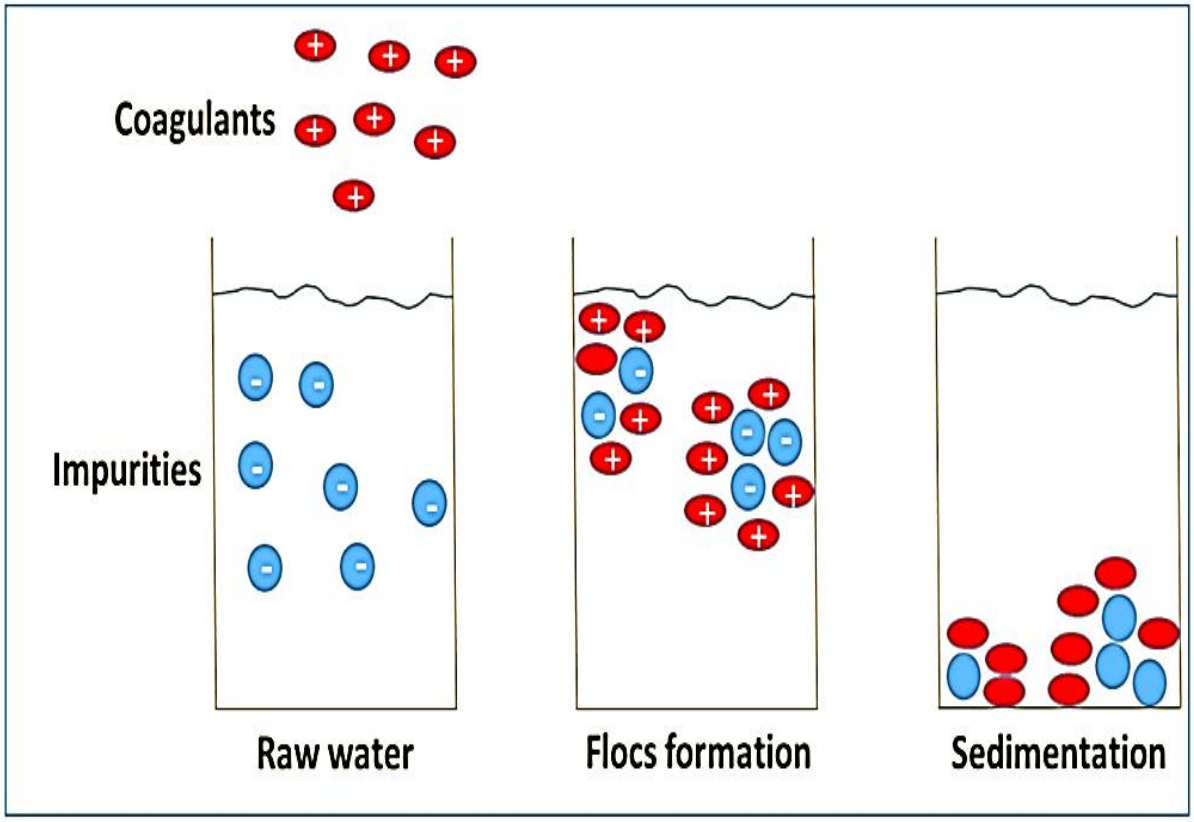

Figure 2.1. The mechanism of coagulation, flocculation and sedimentation to destroy water impurities.

Source: Kumar *et al*., 2024.

2.3.2. Plant base adsorption

Adsorption, which is an economical and easy method of water and waste-water treatment and also a sustainable technology, is employed to remove toxic element from contaminated water such as carbon based compounds including organic dyes and medicines, and also metals such as mercury and cadmium, etc.

Adsorption occurs when substances in the liquid or gas phase (adsorbate) gather on the solid material (adsorbent) because of the adsorbent's extensive surface area. Adsorption may be either physical or chemical in nature. The contaminants must move from the adsorbent boundary to the outer surface and then reach the adsorbent's active pore locations according to (Fig. 2.2). A study which was done by (Sukmana *et al*., 2021) reports that scientists categorize adsorbents into two main groups for their research activities. The first category, conventional adsorbents, encompasses activated carbon materials sourced from wood, coconut shells, coal, and other origins. The second category of non-conventional adsorbents according to (Crini *et al*., 2019) comprises organic absorbents that develop from bio- based materials chitin and polysaccharides together will enable the presence carbon generated from agricultural and industrial solid waste and spill control that use natural materials which include non-organic substances, silica-based materials, and kaolin. The process of converting biomass into biosorbents shows great promise for treating water pollution through its ability to eliminate contaminants from water sources.

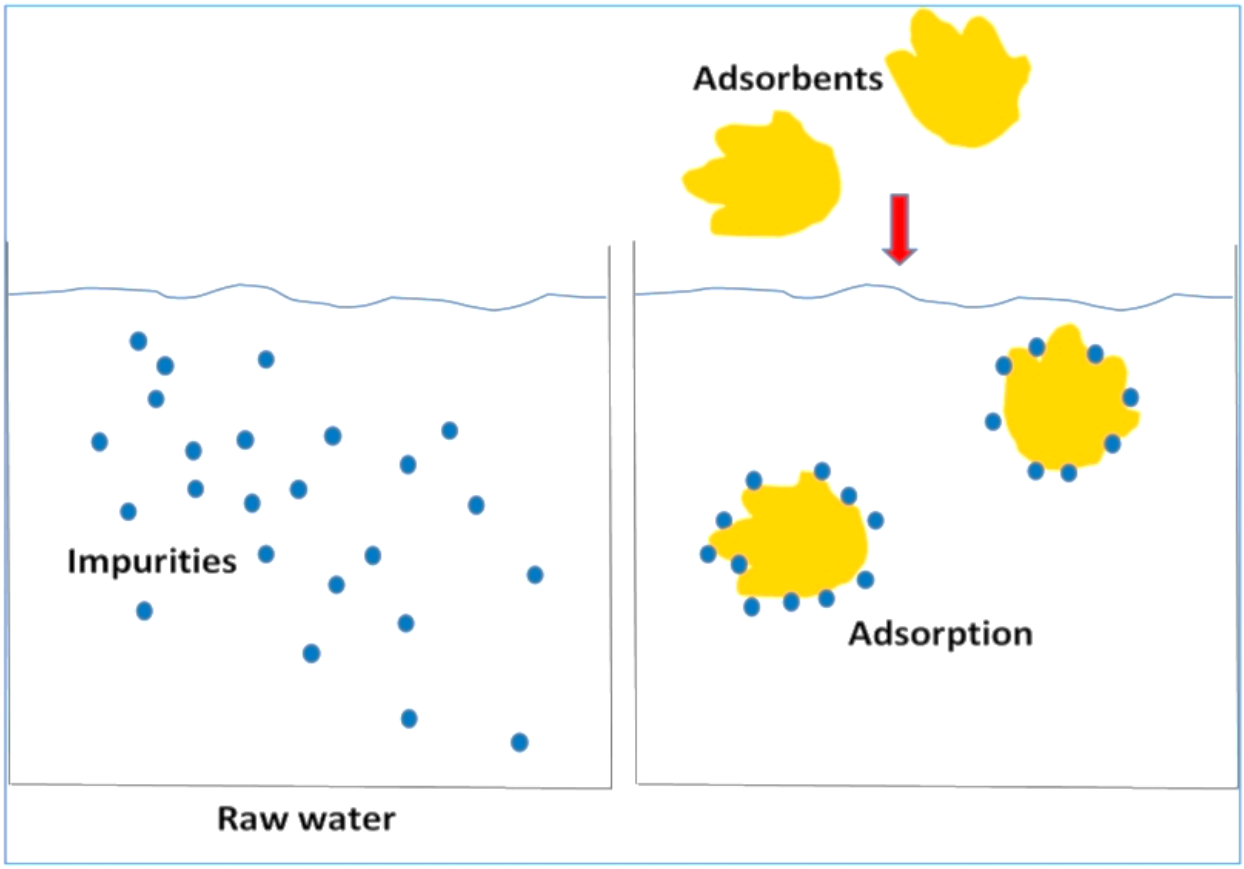

Figure 2.2. Adsorption mechanism for removal of impurities in water.
Source: Kumar *et al*., 2024.

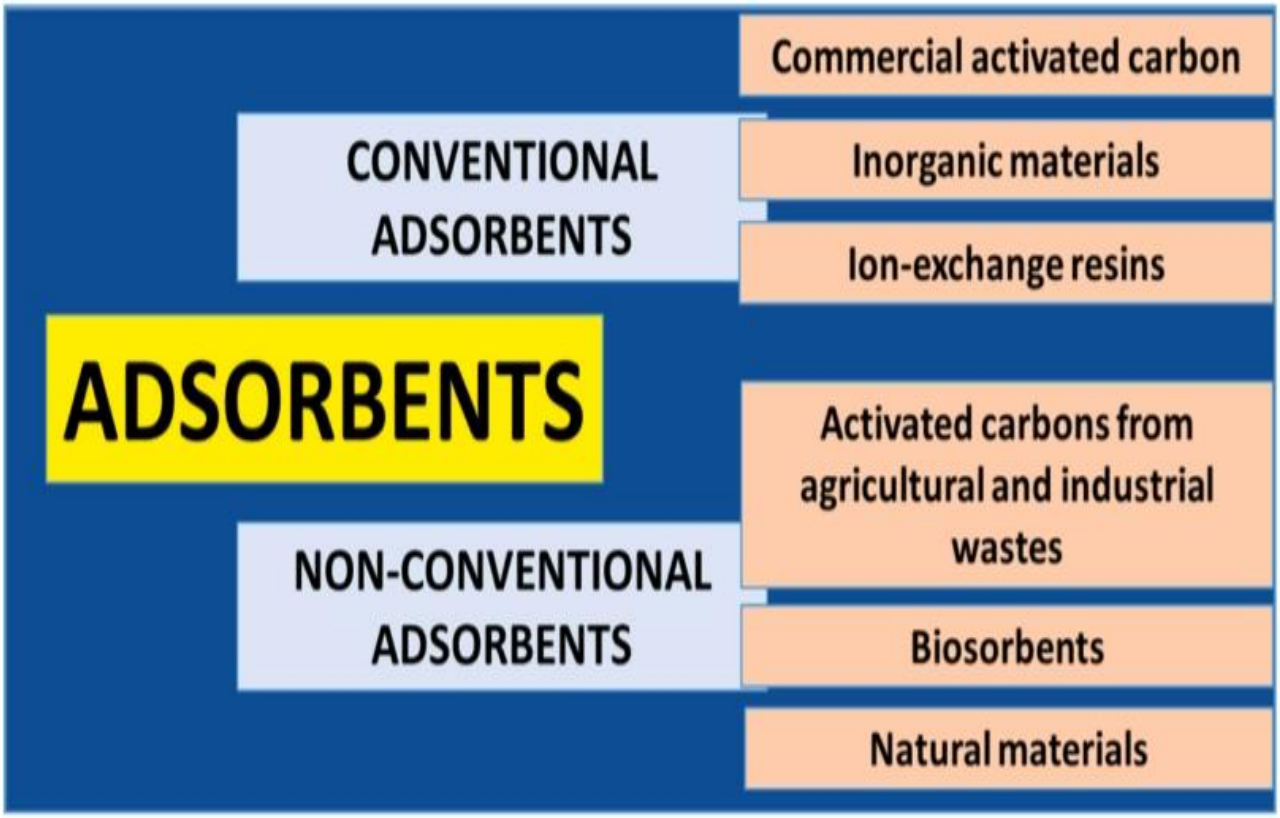

Figure 2.3. Different varieties of adsorbents used in the treatment of water.
Source: Kumar *et al*., 2024.

2.3.3. Herbal disinfection

Filtration, flocculation, and coagulation help to reduce the quantity of large microorganisms and biomass, but the process does not guarantee the elimination of major pathogens. This is the reason for the need to add the disinfection process to the others to kill the pathogenic microorganisms. Pathogens are the major contributors to diseases associated with contaminated drinking water. Drinking water standards require that water must be free from coliform organisms according to Bureau of Indian Standards guidelines established in (BIS., 2012). Disinfection serves to remove all pathogenic microorganisms which exist in water sources through the process of killing or rendering those organisms inactive. Throughout history, scientists have developed multiple disinfection methods which combine physical and chemical approaches to achieve water purification and the cleansing of other materials. Physical disinfection methods, such as filtration, heating, microwaves, and plasma, are limited by their low capacity, high cost of operation, and the time required for the process. Chlorination stands as the most common disinfection method used throughout the world because it provides an easy and cheap and efficient solution (Azuma *et al.,* 2021). The method serves multiple purposes which include disinfecting water and wastewater and controlling odors and removing iron. The process contains a major disadvantage since disinfectants usually respond with both natural and synthetic compounds which exist in water supply systems. The chemical reactions create carcinogenic by-products which endanger both human health and environmental safety (Mane *et al*., 2021). Thus, there's a strong push towards the development of safer disinfection technologies that circumvent the chlorination challenges. The plant-based products have shown considerable promise as alternatives to conventional disinfection and coagulation chemicals. Plants have the ability to produce secondary metabolites in different parts of the plant as defense mechanisms against predators (Joseph *et al*., 2010). The phytochemicals

have led to the development of antibiotics and new medicines and herbicides and insecticides. Researchers have examined the disinfection properties of plant-based compounds across various domains which include food and alternative medicine and pharmaceuticals and disinfection products. The phenolic compounds of plants have been known to possess free radical scavenging and antioxidant properties. The compounds function as reducing agents which enable hydrogen donation and excited oxygen quenching and metallic chelation (Chew *et al.,* 2009). Scientists have researched Tulsi*,* Neem leaves, *Phyllanthus emblica* and *Acacia nilotica* as effective plant-based disinfectants for cleaning contaminated water. The Italian synthesis of Silvafloc created a plant-based disinfectant which uses Schinopsis balansae bark as its primary ingredient. Silvafloc has been tested on river exterior water and is considered suitable for the purification of drinking water. The product proved more effective for water clarification than aluminum sulfate. (Adeeyo *et al*., (2021).

2.4. Botanical Description and Phytochemistry of *Moringa oleifera*

Botanical description, taxonomy, cultivation and agronomic practices of *Moringa oleifera*

The *M. oleifera* plant grows quickly to become a tree which develops a height of 8 meters when it reaches its full size. The plant displays bipinnate and tripinnate leaf structures which have a length range of 2 to 2.5 centimeters, and its leaves show a dark green color on the top side while the bottom side displays a pale green color. The tree produces clusters of fragrant white flowers on stiff stems. The plant produces long three-sided pods which people commonly call drumstick pods, and these pods measure 90 millimeters in length and 12 millimeters in diameter. The pods hold light brown seeds which (Olson *et al*., 2021) report will start germinating after one week under perfect growth conditions.

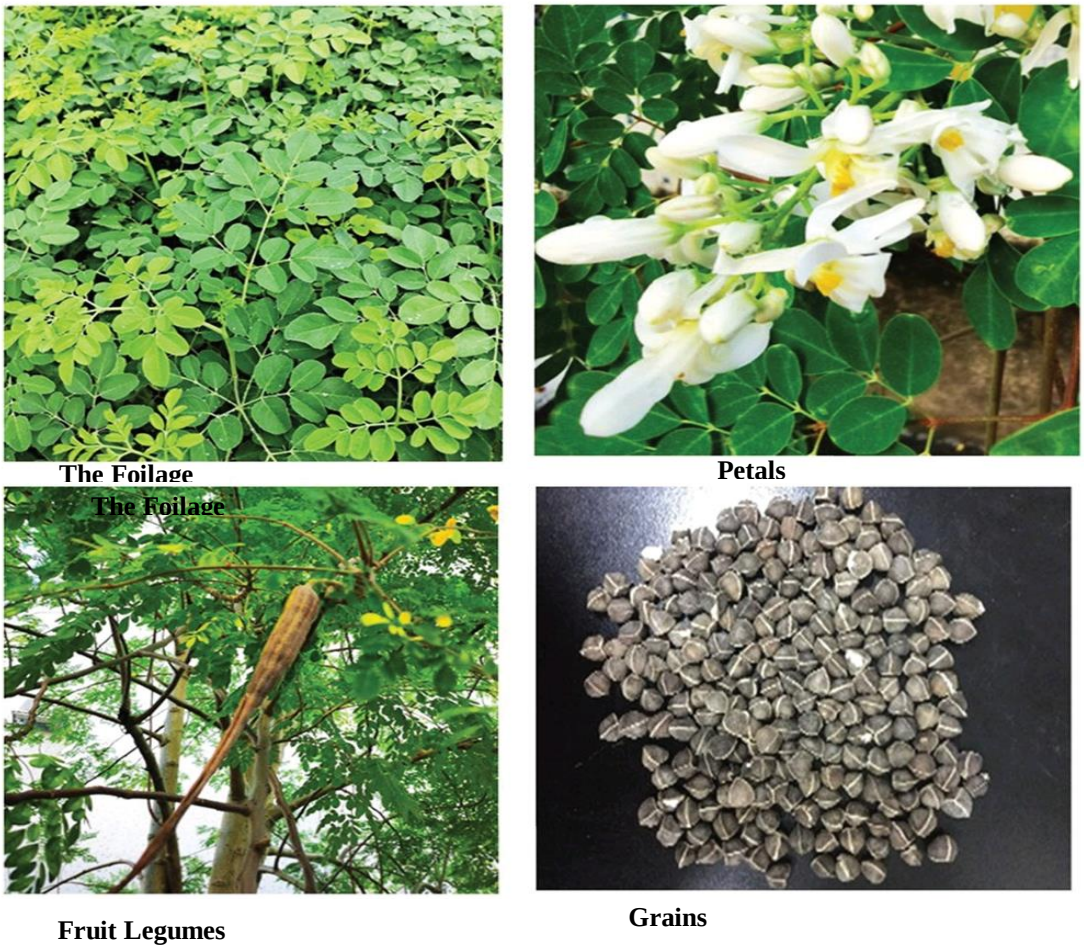

Figure 2.4. The foliage, petals, fruit legumes and grains of *Moringa oleifera*
Source. Liu *et al.,* 2021.

The systematics of *M. oleifera* is represented in the table below;

Table 2.1. Botanical classification of *Moringa oleifera* Lam

Taxonomic level	**Scientific names**
Kingdom	*Plantae*
Subkingdom	*Tracheobionta*
Division	*Spermatophyta*
Superdivison	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Dilleniidae*
Order	*Capparales*
Family	*Moringaceae*
Genus	*Moringa*
Species	*Oleifera*

Source: (Paikra *et al*., 2017; Mallenakuppe *et al*., 2019).

2.4.2 It's growing conditions

Moringa oleifera are versatile species capable of surviving in a range of climates across the tropics and subtropics at altitudes from 0–1000m. It performs well within the temperature ranges of 12–40°C as well as being able to endure short periods of time at intense temperatures as minimal as 1–3°C or as elevated as 48°C (Velázquez-Zavala *et al*., 2016). *M. oleifera* may adapt itself to grow in many different soil types including sandy, loamy, semi-arid, etc.; however, it will typically obtain the best growth characteristics when planted on well-drained soils having a pH close to neutral (near 7.0), although it will tolerate soil pHs ranging from 5.0–9.0. Furthermore, *M. oleifera* can endure low nutrient status/poor soils and therefore provides an opportunity for cultivation where land degradation or climatic conditions affect agricultural production (Leone *et al*., 2015).

2.4.3 It's cultivation

Moringa oleifera propagation methods include using seeds or hardwood cuttings or nursery-raised transplants. Seeds will start their germination process after two weeks when they are planted in soil that has a depth of 2 centimeters. Hardwood cuttings should be collected during the rainy season because tree makers need to take them from mature trees which will establish roots in damp environments. Seeds develop into trees that grow deep roots which enable them to handle drought conditions better than hardwood cuttings which develop shallow roots that enable fast growth (Leone *et al*., 2015) (Swatia *et al*., 2018). Plants grow best when the temperature stays at 30 degrees Celsius in the day and 20 degrees Celsius at nighttime (Abdoun *et al*., 2023). *M. oleifera* enables farmers to harvest their crops three times every year because of its extraordinary growth speed which reaches 3 meters within three months. Tree growers use pruning and pollarding methods to create lateral branches which simplify the harvesting process while enabling new tree

growth. Moderate pruning produces better leaf biomass than both light and heavy pruning methods according to research results of (Abdoun *et al*., 2023).

Crop yields from intensive planting might change greatly depending on the genetics of the planted material, climatic circumstances and the spacing used as far too wide an area will decline the yield potential significantly. Plantation with intensive density (high density of planting) can produce from 40 to 580 tonnes per ha / yr of fresh biomass (Velázquez-Zavala *et al*., 2016). The majority of shoots harvested from intensive plantations will be at a height of between 0.5 and 1.0 metres to encourage new growth; harvesting individual leaves will represent a quicker harvest however may cause the tree to exhibit reduced vigour as time continues. The seed production process requires a field distance of 2.5 to 3.0 meters which will maximize pod development. The pods will reach their complete development three months after the flowering stage and should be collected immediately after they become ripe because this will prevent seed wastage. The number of seeds produced by each tree during each growing season ranges from 15000 to 25000 which depends on the particular tree species and the surrounding environmental conditions (Mahmood *et al*., 2022). *M. oleifera* can thrive outside its native tropical regions because it exhibits successful growth in cooler temperatures which demonstrates its ability to adjust to different weather patterns. The PKM1 and PKM2 cultivars from India now exist in Bulgaria because researchers introduced them into controlled greenhouse and outdoor environments which include plant assessments that start when the plants reach 50 centimeters in height (Olaofe *et al*. 2013) (Leone *et al*., 2015). *Moringa oleifera* has successfully completed growth trials in Portugal and Spain which show that this species grows well in Mediterranean climates when frost protection measures exist during winter months (Trigo *et al*., 2021).

2.4.4 Phytochemistry of *M. oleifera*

Researches has investigated all features of Moringa oleifera and its extracted derivatives because they wanted to study the plant in complete detail. The Moringa genus contains more than 90 different chemical substances which scientists believe provide important health advantages. The compounds of the study include proteins and amino acids according to (Mahmood *et al*. 2020), phenolic acids according to (López-Salazar *et al*., 2019), carotenoids according to (Saini *et al*., 2014), alkaloids was induced by (Sahakitpichan *et al*., 2011) glucosinolates (Rani *et al*., 2018), flavonoids according to (Abdull Razis *et al*. 2014) sterols according to (Anzano *et al*. 2021) and terpenes according to (Matic *et al*. 2018), and tannins and saponins according to (Bhattacharya *et al*. 2014) lipids according to (Faizi *et al*., 2014), glycosides and polysaccharides according to (Olagbemide *et al*., 2014). The chemical analysis of *M. oleifera* revealed multiple secondary metabolites which generate different bioactive compounds. The environmental conditions of temperature and sunlight exposure and soil composition and geographic location determine how bioactive compounds in *M. oleifera* extracts produce their different concentrations (Mbikay, 2022)

2.4.5 Vitamins and minerals

The complete parts of *M. oleifera* plant provides multiple micronutrients, such as Calcium stands out as an essential mineral from Moringa because it supports human growth and development. Fresh *M. oleifera* leaves are estimated to contain between 11300 to 23000 Vitamin A units. Vitamin A plays an essential role in multiple bodily functions which include vision development and prenatal growth as well as immunologic competence and cell division and cell proliferation and the process of apoptosis and the maintenance of epidermal tissue and cognitive abilities (Alam *et al*. 2016).

2.4.6 Flavanoids

M*oringa oleifera* seeds and leaves have so many flavonoids like isorhamnetin, procyanidin, myricetin, rutin, kaempferol, quercetin and catechin. The research conducted by (Abdull Razis.,2014) showed that the plants leave contain high amounts of lutein pigments. The active chemical components that occur naturally in *Moringa oleifera* provide the foundation for its medicinal properties. The researchers (Olagbemide *et al*., 2014) discovered up to 35 chemical compounds found in the plant by using gas tandem mass spectrometry.

2.4.7 Amino acids

The *Moringa oleifera* plant contains multiple essential nutrients which exist as over 90 beneficial chemical substance that include Enzyme and lipids and carbohydrates and dietary fibers (Anwar *et al*., 2017) discovered that proteins function as the main nutrient of the plant because they make up 25 percent of the plants total dry weight. The plant possesses a total of 19 amino acids which include both essential and non-essential types. The seeds contain about 30 percent of their dry weight as their most essential attribute which represents their high lipid content (Abdulkarim *et al*., 2015).

2.4.8 Phenolic acids

Moringa oleifera plant shows presence of various acids which include hydroxycinnamic acid and 4-hydroxy-3, 3-phenylacrylic acid and geneticist corrosive and ferulic acid plus gallate and protocatechuic acid and caffeic acid and epicatechin and p-coumaric acid and ortho-coumaric acid and vanillin. The compounds are classified as phenolic compounds which originate from hydroxycinnamic and hydroxybenzoic acids that occur naturally in plants. The research studies have demonstrated that these substances produce beneficial effects through their anti-mutagenic properties and anti-inflammatory effects and anti-tumor properties and antioxidant properties (Brilhante *et al*., 2018) discovered that *Moringa oleifera* leaves together with fruit and vegetable

nutrients create high nutritional value according to their research. The dry foliage of *M. oleifera* showed the peak level of gallic acid phenolic acid with a weight of 1.034 milligrams per gram of dry leaves. Studies have shown that only tiny quantities of gallic acid exist in previous research. The study results showed that two different acids existed in the sample with chlorogenic acid present at 0.018 to 0.489 mg/g and caffeic acid at 0.409 mg/g (Anzano *et al*., 2021).

2.4.9 Alkaloids

Alkaloids function as fundamental nitrogen-based chemical substances which contain nitrogen atoms. Nitrogen atoms in the compounds create amines which scientists group into three classes: primary amines and secondary amines and tertiary amines. The therapeutic abilities of these substances make them extremely appealing to researchers.

2.4.10 Tannins, fatty acids, and sterols

Tannins which are natural polyphenols exist in many plant species that produce fruits and vegetables and seeds. Tannins serve as wine fining agents in the wine industry because they help winemakers stabilize wine color while achieving full flavor development and they prevent the natural growth of spoiled grapes. The Moringa tree shows different tannin concentrations because its dried leaves have the highest tannin content which reaches a value of 20.7 mg/g. The primary fatty acids of the *Moringa oleifera* seed includes octacosanoic acid, palmitic acid, omega-9 fatty acid, and arachidic acid.

2.5 Botanical Description and Phytochemistry of *Azadirachta indica*

2.5.1 Botanical description, taxonomy and biological source of *Azadirachta indica*

Azadirachta indica tree strongly belongs to the Meliaceae clan and grows at a fast pace during all stages of its existence. Neem which originates from the south Asia can now grow in humid and subtropical regions worldwide as a result of its various medicinal and environmental applications.

The neem tree grows between 15 and 20 meters in height and develops a trunk with 2.5 meter circumference while its branches create a dense canopy of dark green foliage. This broad-leaved evergreen tree needs more than two hundred years to complete its full life cycle. The leaves of the plant develop into a pinnate structure which contains oblong leaflets that create a distinctive powerful fragrance. The tree produces yellowish fruit which contains oily seeds and produces tiny white flowers. The tree features rough grayish-brown bark while its seeds and leaves function as the main medicinal elements that people use for treatment purposes (Handa *et al*., 2018). The deep root system of *Azadirachta indica* tree experiences problems from waterlogging yet the tree developed methods to extract soil-based nutrients and water. Neem trees thrive best in arid hot environments that receive between 400 and 1200 millimeters of rainfall while experiencing temperatures that often reach 5°C. High levels of secondary metabolites exist in these fruit kernels according to Hashmat *et al*., (2022a). The plant produces multiple flowering panicles which mainly grow in the leaf axils. The sepal plant has ovoid petals which measure about one centimeter in length and present a white oblanceolate fragrance. The plant produces golden drupes which measure 12 to 20 millimeters in length and have an elliptical shape. The green fruits usually changes it color to yellow when. It gets ripe and produces a strong odor which resembles garlic. The flowering process starts in March and April when new foliage emerges. The period from April to August marks the time when fruits ripen according to the specific location as recorded by Hashmat *et al*., (2022b).

Figure 2.5. Leaves of the neem tree
Source: Khare *et al*., 2025.

Figure 2.6. Bark of the neem tree
Source: Khare *et al*., 2025.

2.5.2 It's taxonomy

As of recent times, neem trees are known to successfully grow in more than 72 countries all around the world which consists of North America Central America South America all of Asia and all of Africa and all of Australia (Jhariya *et al*., 2023). The taxonomic categorization of neem follows this system in table 2.2.

Table 2.2. Taxonomical classification of neem leaves

Taxonomical Rank	**Categorization**
Kingdom	Plantae
Order	Rutales
Suborder	Rutinae
Family	Meliaceae
Subfamily	Melioideae
Tribe	Melieae
Genus	*Azadirachta*
Species	*Indica*

Source: (Uzaman, 2020; Ayeshabi *et al*., 2023).

2.5.3 Biological source and medicinal part of the Indian lilac (*Azadirachta indica)*

The neem tree exists as a member of the Meliaceae family. People use preparations that contain neem tree seeds and bark and leaves and fruits for various medical and other uses.

1. Seeds

The neem tree produces a seed oil which contains azadirachtin and exhibits potent insecticide and antimicrobial effects. The neem seed oil is regarded as a topical treatment for skin infections and healing wounds (Khan *et al*., 2015).

2. Leaves

The leaves used in treatment addresses skin conditions which include acne and eczema and fungal infections. The substance functions as an insecticide while it also serves to treat fevers and gastrointestinal disorders (Subapriya *et al.,*2023).

3. Bark

The substance from here shows both antidiabetic and antiinflammatory effects. The substance is commonly used in decoction form to treat malaria fever and gastrointestinal problems (Rana *et al*., (2017).

4. Neem oil

This extract comes from seeds which manufacturers use to make medicinal and cosmetic products. The product is effective for treating bacterial and fungal infections which also include dry scalp conditions and lice and dandruff (Singh *et al*., 2018).

2.5.4 The phytochemistry of *azadirachta indica*

Neem trees contain their most abundant compound azadirachtin which researchers use as a biopesticide (Chaudhary *et al*., 2017). The traditional Indian health services has used neem tree parts for more than 1000 years because they supposedly treat 14 different medical conditions

according to (Alzohairy *et al*., 2016). *A. indica* provides all its components which include gum, stem, bark, leaves, seeds, fruits, flowers, roots and other parts for use as home remedies to treat human health problems. Neem twigs serve as dental hygiene chewing sticks which millions of people worldwide use according to (Gupta *et al*., 2017). Today scientists who study modern medicine and infectious diseases research the properties of the neem tree because this tree shows promise as an antimicrobial solution while also establishing its presence in oncology, dentistry, dermatology and endocrinology practices. The neem plant's phytochemical compounds can be recovered through extraction methods that utilize different solvents to isolate the plant's active ingredients, yet researchers in laboratories depend on water, ethanol, methanol, chloroform and ether extracts to examine the biological active compounds of neem. Research studies most commonly use methanol and ethanol extracts to conduct their antimicrobial assessments. The neem oil extracts which researchers tested demonstrate their complete biological function because they contain various secondary plant metabolites that include isoprenoids and non-isoprenoids. There are 300 distinct compounds found in the Neem tree yet researchers found that azadirachtin, gedunin and nimbolide serve as the main phytochemicals which deliver its essential biological functions and medicinal properties (Saleem *et al*., 2018).

Table 2.3 Phytochemical compounds of neem plants

Phytochemicals	Plant parts	Type of Compound	Reported Antimicrobial Activity	Mechanism of Action
Nimbolide	Leaves, Flowers	Limonoid	Antibacterial, Antifungal, Antiparasitic	triggers ROS stress , disrupt microbial nucleic acid synthesis
Azadirachtin	Seed, Leaves	Limonoid (Triterpenoid)	Bactericidal, Antifungal, Antiviral	Destroys bacterial bioprocesses and cell replication.
Nimbin	Bark, Plant oil	Triterpenoid	Antibacterial, fungicidal	Disrupts cell membrane stability.
Gedunin	Seeds	Limonoid	Antimalarial, Antibacterial	Protein denaturation, mitochondrial disruption.
Nimbidin	Spores, plant oil	Triterpenoid	Antibacterial, Antifungal	Blocks germ growth, wall assembly, reduces inflammation.

Source: Khare *et al*., 2025.

2.6 Mechanism of Water Clarification by *Moringa oleifera*

The research team demonstrated that *Moringa oleifera* leaves provide effective water purification solutions (Pandey *et al*., 2020a). The year-round availability of leaves enables scientists to use them as a solution for seed powder's restricted availability and its short shelf life. The study demonstrated that Moringa oleifera leaf extracts successfully eliminate biological and chemical and physical contaminants from ground water through their use in various organic solvents which established Moringa oleifera as an effective water purification agent. The research team demonstrated that plant leaves which scientists combined for water purification testing showed better results than their individual testing (Pandey *et al*., 2020b). The study demonstrates that combined plant extract from Moringa and Neem outperforms the individual plant extracts according to its research findings. Their study involved testing Moringa leaves with seed extract and found that their combined application produced better coagulating results than the separate components (Alam *et al*., 2020). Moringa-treated water studies demonstrate that Moringa treatment improves turbidity removal results (Freitas *et al*., 2016). Researchers believe that Moringa and alum water treatment reduces aluminum levels because Moringa binds with aluminum ions which results in safer drinking water. Moringa water treatment with alum demonstrates two benefits because it maintains alum coagulation power while producing larger floc particles that workers can remove easily (Boulaadjoul *et al*., 2018).

The *Moringa oleifera* serves as an effective primary coagulant for water treatment which performs at the same level as alum. Researchers have studied *M. oleifera* since many years ago as an affordable natural water purification solution for developing regions throughout the world. The tree provides useful parts that make it suitable for various purposes, including, food, bee nectar, spices, traditional medicine, fertilizer, natural coagulants, and fodder. The edible plant is widely

consumed because it offers people both super nutritious and healing benefits (El-Hack *et al*., 2018). The powdered seeds of *M. oleifera* function as a human-safe substance which contains coagulating abilities used for decreasing water turbidity together with pH and remaining suspended particles and hardness (Hartman., 2015; Virk *et al*., 2019). So many researchers have done several analysis to identify main hazardous reduction methods which use *M. oleifera* for adherence and charge balancing and particle disruption, which happens when M. oleifera cationic proteins interact with water colloids (Virk *et al*., 2019). The polypeptides acts as a framework which supports eight electropositive amino acids together with 15 glutamine residues. The substance contains both antioxidant and antimicrobial capabilities which enable it to destroy bacteria. The active phytochemicals in *M. oleifera* enable it to function as an effective water purification solution for drinking water and factory effluent (Virk *et al*. 2019). The process starts with charge neutralization which begins when positively charged proteins bind to suspended particles for charge neutralization which leads to decreased repulsive forces that enable particles to combine. The process begins with protein molecules that attach to multiple particles which creates bridges that connect the particles to produce bigger clumps that can settle. Floc formation requires extra interactions between particles that include hydrogen bonding and Vanderwal forces for floc creation. Natural proteins demonstrate polyelectrolyte behavior because they possess biodegradable non-toxic properties that match synthetic polyelectrolyte requirements. *Moringa oleifera* biochemical pathways show potential as sustainable chemical coagulant alternatives. (Al-Jadabi *et al*., 2023). The reliable solution requires only small changes to create a cost-effective drinking water filter system which will decrease waterborne disease rates in the community (Kurniawan *et al*., 2020).

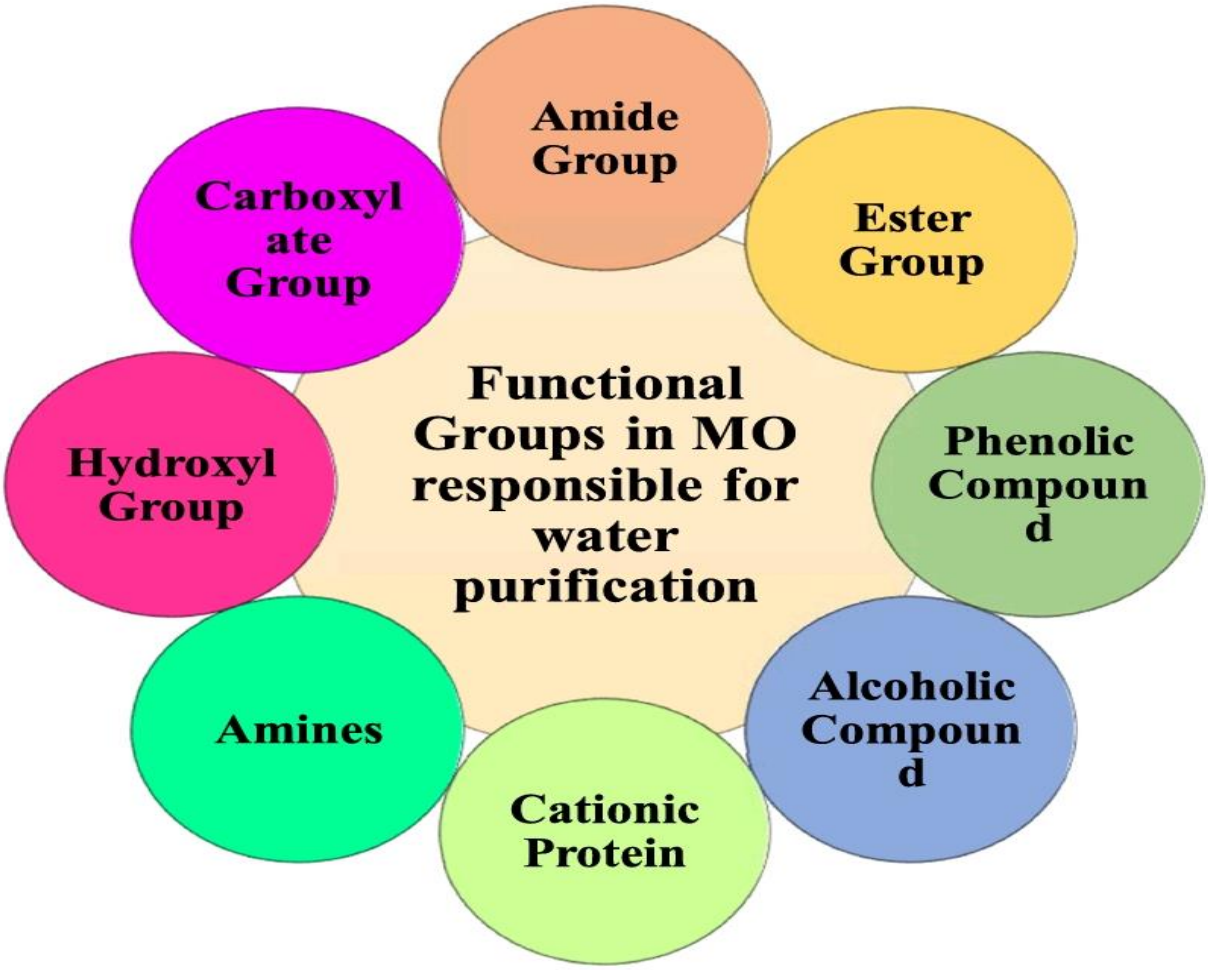

Figure 2.7. The *Moringa oleifera* active functional groups for it's coagulation activity
Source: Kakkar *et al*., 2025.

2.7 Antimicrobial Attributes of Neem Extract

The antimicrobial features of neem are significant in mitigating various infections caused by bacteria, fungi and viruses. Research shows that neem extracts can stop multiple pathogens. The properties of neem make it a suitable option for creating new antimicrobial solutions which have become essential because of increasing issues with antibiotic resistance. The antimicrobial properties of neem stem from its numerous phytochemical compounds which damage microbial cell membranes and block their vital biological functions. The broad-spectrum antimicrobial activity of neem establishes it as an essential resource which both traditional medicine and modern medical practices use to treat infections which do not respond to typical antibiotic treatments (Mohideen *et al*. 2022).

2.7.1 It's Active antimicrobial compounds of Neem

Neem, which is commonly called *A. indica*, contains multiple phytochemicals that demonstrate strong antimicrobial effects. Such as:

1. Azadirachtin

This is one of the most important neem compound exists because it demonstrates insecticidal properties together with possible antimalarial activity. The substance serves as an essential element of neem's medicinal power because it hinders vital functions in disease-causing organisms. (Srivastava *et al*., 2020).

2. Nimbin

Nimbin acts as another essential bioactive compound which displays both anti-inflammatory and antimicrobial properties thus supporting neem's traditional function as an infection treatment (Saleem *et al*., 2018).

3. Terpenoids

The similar compounds like nimbidiol and gedunin demonstrate antibacterial and antifungal properties. The medicinal effects of neem depend on these components (Saleem *et al*., 2018).

4. Flavanoids

The high concentration of flavonoids present in the neem extracts demonstrates their ability to function as antioxidants and antimicrobial agents, which increases the medicinal properties of these extracts (Khanal, 2021).

5. Saponins

These compounds display surfactant characteristics which have the ability to break down microbial membranes through their surface-active properties, resulting in the destruction of cells (Ujah *et al*., 2021).

2.7.2 Neem extracts activity against selected water pathogens

The antibacterial activity of the Neem Plant is rather at a broad spectrum rate and has been recorded to have activity against a wide array of microbes including *Escherichia coli, Vibrio* spp. *Staphylococcus* spp. *Mycobacterium tuberculosis*, *Bacillus* spp. *Pseudomans* spp. *Salmonella* spp. *Enterococcus* spp. *Shigella* spp. and so much more as indicated in the findings of other researchers below.

Neem tree exhibits strong antimicrobial properties that effectively kill *Mycobacterium tuberculosis* and all three bacterial strains. Researches were done and states the use of agar diffusion method to conduct laboratory tests which examined the antibacterial capabilities of neem plant extracts in acetone and ethanol and methanol from its bark and leaves. The researchers conducted their experiments to create extracts which they used to analyze the presence of different phytochemical compounds. The study found that bark and leaf extracts both contained five distinct compounds

which included alkaloids and reducing sugars and flavonoids and tannins and polyphenols and saponins (Maleki *et al*., 2018).

2.7.3 Effect of Plant Extracts on Water Quality Parameters

Plant extracts used in water purification like Moringa seeds and powder influence analytical parameters of water which can be measured, and they include the following;

1. Turbidity: The measurement uses a calibrated turbidimeter implementation of the Hach Q system which generates results in Nephelometric Turbidity Units. The calculation of turbidity removal efficiency will determine the percentage reduction from initial turbidity measurements.

2. pH: The water acidity or alkalinity changes after coagulant application through precise pH measurements obtained from a pH meter (e.g., Hanna Instruments pH) serves as a measurement tool for pH. The comparison between MO buffering capacity and alum pH depression effect requires this method as a critical measurement. The primary benefit of comparing M. oleifera to alum as a water treatment solution operates through its power to maintain steady pH levels in water that undergoes treatment. The acidic coagulant alum establishes a chemical process through which water pH decreases because it produces hydrogen ions during its hydrolysis reactions. The decrease in pH requires treatment facilities to use alkaline substances such as lime or soda ash for pH restoration which results in higher treatment expenses and more chemical usage and additional difficulties for operations (Crittenden *et al.* 2012). Making use of alum for raw water treatment at a starting pH level of 7.5 leads to a pH reduction which reaches 6.0-6.5 with proper dosage (Sivasubramanian *et al*., 2018). Moringa oleifera studies have shown that it keeps water pH levels stable because the pH value remains close to the original measurement through different dosing levels (Desta *et al.,* 2021). The use of MO on raw water with a starting pH of 7.5 shows that the

water maintains pH between 7.4 and 7.6 which proves its natural buffering ability (Charles *et al*. 2022).

3. Microbial load analysis: The assessment needs more complete testing because the antimicrobial properties of MO requires testing both fecal indicators and *Escherichia coli (E. coli)* through standard membrane separation or most probable number(MPN). The test evaluates how well coagulants can decrease the presence of harmful pathogens (Gupta, 2025). *Moringa oleifera* exhibits more than its main function as a coagulating agent because it contains natural antimicrobial properties which help decrease bacterial contamination in water treatment systems (Anwar *et al*., 2007). *M. oleifera* spores have active components of protein and peptide origins, which exhibit bactericidal and bacteriostatic effects counter to the expansion of water pathogens like *Escherichia coli, and Salmonella typhi*. Studies have documented major decreases in E. coli and total coliforms which typically fall between 80% and 99% based on the MO dosage and water characteristics (Bhuptawat *et al.,* 2020; Desta *et al.,* 2021). The antimicrobial effect creates an extra purification method which proves especially useful in areas that lack access to modern disinfection systems. The MO treatment effectively decreases microbial content yet fails to deliver complete disinfection which achieves the rigorous standards needed for drinking water because it does not eliminate viral pathogens. Water treatment facilities must implement post-treatment disinfection measures which include chlorination and UV irradiation to achieve complete water protection especially for potable water use (World Health Organization, 2017). The MO coagulant introduces residual organic matter which raises the risk of microbial regrowth if it remains untreated because bacteria can use it as a nutrient source (Desta *et al.*, 2021).

Turbidity removal efficiency

The two substances *Moringa oleifera* and alum demonstrate excellent capacity to decrease water turbidity which serves as an essential measure of water quality. The original cloudiness of raw water together with the proper dose and the various physical chemicals properties of the water will determine their effectiveness. The conventional chemical coagulant alum achieves efficient turbidity removal which frequently results in over % reduction for different water sources when used in properly controlled conditions (Costa *et al*., 2024). The research conducted on highly turbid river water demonstrated that alum could reduce turbidity to below NTU levels through the application of optimal dosages which ranged between - mg/L (Sivasubramanian *et al*., 2018). Moringa oleifera demonstrates exceptional capacity to decrease turbidity which reaches between 75% and 97% efficiency according to (Silva *et al.,* 2024). The cationic proteins of MO function as the main active components which enable the system to neutralize negative charges found in suspended particles. Research on synthetic turbid water demonstrated that MO could remove 50% of NTU turbidity when treated with dosages ranging from - mg/L (Desta *et al.,* 2021). The environmental impact analysis shows that MO turbidity removal on low to medium turbidity water achieves similar results to alum or even better results according to the study (Sharma *et al.,* 2017).

2.8 Comparative Studies on Natural Coagulants and Antimicrobials

Natural coagulants provide multiple benefits because they protect the environment while offering affordable and environmentally friendly solutions which developing countries can easily implement. The system operates at all pH levels which exist in raw water while maintaining safety for human health and showing no effects from antibiotics on different bacterial and fungal species. Natural coagulants in wastewater treatment provide a budget-friendly solution which effectively decreases wastewater alkalinity when compared to chemical coagulants.

Table 2.4 Comparison of *moringa oleifera effectiveness in* water treatment using antimicrobial coagulants (Alum and ferric)

Criteria	*Moringa oleifera*	Aluminum (Alum)	Ferric Trichloride
Origin	From Natural Plant	Industrially Synthesized chemical compound	Industrially Synthesized chemical compound
Mode of Action	Seeds cause coagulation through natural cationic proteins (polyelectrolytes)	Aids in charge neutralization through hydrolysis to form Al^3 ions	Enables particle destabilization through Fe^3 ion hydrolysis
Sensitivity to pH	Works well across a wide pH range	Depends on optimum pH of around 6-7	Optimal at a slightly acidic pH of 5-6
Pathogen Removal	Exhibits moderate antimicrobial activity	Most effective when combined with disinfectant	Most effective when combined with disinfectant
Typical Dosage	50–250 mg/L	10–50 mg/L	5–30 mg/L
Environmental Impact	Eco-friendly, biodegradable and renewable	Unsustainable with sludge disposal causing environmental problems	Same situation with even heightened environmental sustainability concerns
Sludge Characteristics	Releases eco-friendly 0sludge with minimal toxicity	Generates high volume sludge with aluminum residues	Results in dense sludge containing iron residue
Efficiency in Turbidity Removal	Moderate to high at 60-90% based on specific conditions	Very high efficiency at above 95%	Very high efficiency at above 95%
Cost Implications	Low - cost fees especially where Moringa is locally available at all times	Moderately priced and accessible	Quite more expensive than alum
Shelf Life and Storage	Short shelf life and needs fresh and well- preserved preparation	Long lasting effect and stable under standard conditions	Long lasting effect and stable under standard conditions
Best Use Case	Good for decentralized, rural or resource-limited places	Suited for municipal or urban water treatment areas	Typically used in industrial and advanced treatment system areas
Health and Safety	Non - toxic and Eco-friendly	May leave aluminum residues, posing health risks and concerns	The remaining iron may impart water taste and color

Source: Kakkar *et al.,* 2025.

Moringa oleifera as an organic coagulant

Research investigations have shown that Moringa oleifera works effectively to purify multiple types of water systems which include turbid surface waters and domestic wastewater and industrial effluent streams. The solution proves effective when it removes turbid materials and suspended particles because it achieves considerable decreases in organic materials and heavy metal content and pathogens (Rahid *et al*., 2007). The Indian water treatment market has identified Moringa oleifera as an effective approach for water treatment systems that operate outside centralized systems and serve rural and peri-urban communities (Kumar *et al.,* 2016). Recent research shows that MO operates as an environmentally friendly sewage water treatment system which achieves over 95 percent turbidity removal efficiency while treating chemical oxygen demand (COD) and biological oxygen demand (BOD) parameters (Silva *et al*., 2024). some research demonstrates findings Moringa oleifera has strong antimicrobial effects because E. coli and total coliforms decreased by 99% and 96% according to the study results (Bhuptawat *et al.,* 2020).

Alum as an antimicrobial chemical coagulant

Alum has served as the main coagulant for traditional water treatment systems during the past 100 years because all public drinking water facilities and industrial wastewater treatment plants around the world use it, which includes its widespread application throughout India (American Water Works Association, 2011). The ability of Alum to create heavy flocs that settle quickly makes it ideal for applications that require both high treatment rates and stable discharge quality. The material finds multiple uses across different industrial sectors including paper production and textile dyeing and pharmaceutical manufacturing which need to treat wastewater and eliminate contaminants (Crittenden *et al*., 2012).

2.9. Research Knowledge Gaps in Existing Studies

Researchers have continuously studied the coagulation and flocculation process since scientists first introduced coagulants to the world more than 100 years ago. The research developed coagulation mechanisms and theories together with advanced studies to achieve water quality standards. The field of biocoagulants and bioflocculants shows multiple knowledge gaps which scientists have documented through research (Fig. 2.8) (Saleem *et al*., 2019). Biocoagulants and bioflocculants stand out from other coagulants used in water treatment because they combine economic advantages with complete biodegradability and effective performance and easy access.

According to (Saleem *et al*., 2019), the plant lant-based coagulants remain the most popular coagulant choice because they exist in more quantity and are much easier to obtain than animal and microbial coagulants Organizations should conduct research to identify more microorganisms that scientists want to study because this procedure will help them discover better candidate organisms for their research work. Water treatment facilities need to finish extraction and purification procedures before they can use biocoagulants that come from animal sources, microorganism sources and plant sources. The biocoagulants and bioflocculants originate from different sources which include animal, microorganism and plant sources require different preparation techniques, extraction and purification methods that affect the quality and yield of its active coagulating elements. The current extraction techniques of biocoagulants which use salt and acid should combine with water extraction methods to achieve better extraction results. The methods used for pretreatment and extraction during coagulation process directly impact the effectiveness of coagulation because different methods produce different elemental compositions of active biocoagulation and bioflocculation materials (Baptista *et al*., 2017). The extraction process of polysaccharides which includes chitosan, alginate and pectin requires washing to eliminate undesired materials before they undergo

extraction through solvent heating or cooling in aqueous solution, followed by precipitation and drying processes. The extraction method requires improvement and modification according to the specific domain of knowledge. The active coagulant compounds present in the coagulant determine how effectively the coagulation and flocculation process operates. The research should focus on developing new methods which include operational units and material development and optimization methods and coagulation condition development to enhance the quality and yield of biocoagulant and bioflocculant components which will result in faster success of the coagulation-flocculation procedure. The standard biocoagulant application problem occurs due to the high organic material residue which causes more bacteria development and results in extra water treatment needs. The organic matter that is found in the water will create operational difficulties for the filtration system after the completion of the coagulation-flocculation treatment. According to (Ang *et al*., 2015) membrane biofouling occurs during membrane filtration and reverse osmosis when organic matter levels reach high values. The biocoagulant extraction process will lead to the availability of non-coagulating impurities (carbohydrate and lipids) because they restrict contact between coagulants and pollutants which results in poor pollutant removal performance that requires extra purification (Ding *et al*., 2018).

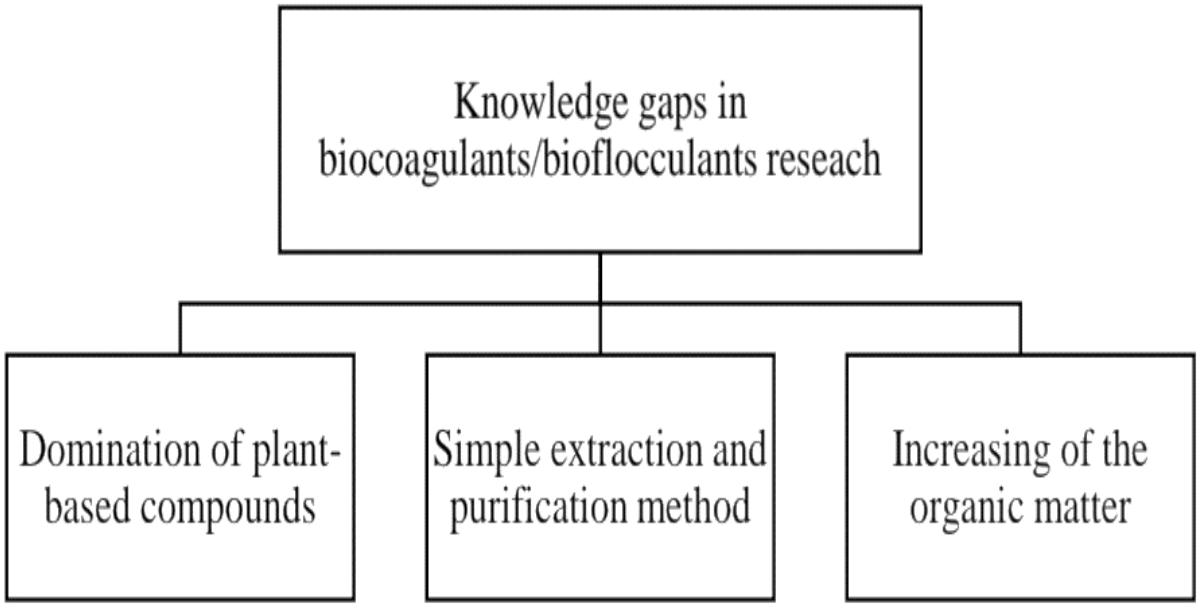

Figure 2.8. The Gap in research knowledge on plant based water treatment methods
Source: Kurniawan *et al*., 2022

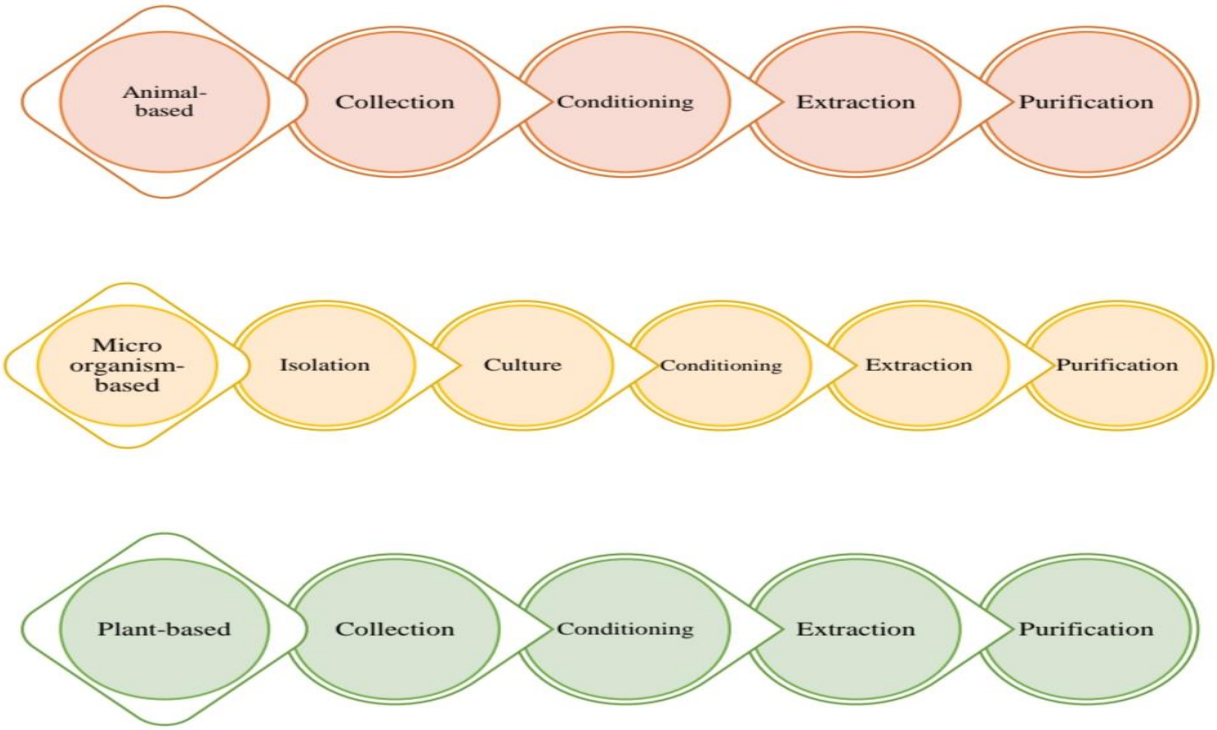

Figure 2.9. The preparation procedure for biocoagulants and bioflocculants
Source: Kurniawan *et al*., 2022.

CHAPTER THREE

MATERIALS AND METHOD

3.1. Experimental Design

The study was conducted to evaluate the water purification and microbial pathogens inhibition efficacy of *Moringa oleifera* (Moringa) seeds and leaves and *Azadirachta indica* (Neem) leaves. A total of 16 treatments, divided into Three treatments each and One negative control from the collected water sample (Thinkerscorner well, Emene river, Abakpa River and Trans-Ekulu well were utilised here.

Table 3.1; Experimental Design of Water Sample treatment

Code	Water Source	Treatment
W1	Thinkers Corner Well	Control
W2	Thinkers Corner Well	Moringa
W3	Thinkers Corner Well	Neem
W4	Thinkers Corner Well	Moringa + Neem
W5	Emene River	Control
W6	Emene River	Moringa
W7	Emene River	Neem
W8	Emene River	Moringa + Neem
W9	Abakpa River	Control
W10	Abakpa River	Moringa
W11	Abakpa River	Neem
W12	Abakpa River	Moringa + Neem
W13	Trans-Ekulu Well	Control
W14	Trans-Ekulu Well	Moringa
W15	Trans-Ekulu Well	Neem
W16	Trans-Ekulu Well	Moringa + Neem

Table 3.2. Water samples treatment regimen using neem leaves and moringa seeds and leaves respectively

Water samples	Negative control only	Moringa seeds and leaves only	Neem leaves only	Neem leaves + Moringa seeds and leaves
T1 W1	+	—	—	—
T1 W2	—	+	—	—
T1 W3	—	—	+	—
T1 W4	—	—	—	+
T2 W1	+	—	—	—
T2 W2	—	+	—	—
T2 W3	—	—	+	—
T2 W4	—	—	—	+
T3 W1	+	—	—	—
T3 W2	—	+	—	—
T3 W3	—	—	+	—
T3 W4	—	—	—	+
T4 W1	+	—	—	—
T4 W2	—	+	—	—
T4 W3	—	—	+	—
T4 W4	—	—	—	+

Key; (—): wrong column per each sample type; (+): correct column per sample.

Key;

T1 = Treatment 1, Well water 1 from Thinkerscorner

T2 = Treatment 2, Emene River

T3= Treatment 3, Abakpa River

T4 = Treatment 4, Transekulu well

W1 = Untreated water

W2 = Water Sample + Moringa Seeds and Leaves only

W3 = Water Sample + Neem Leaves only

W4 = Water Sample + Neem Leaves + Moringa Seeds Leaves

Water Samples keys;

Emene river	Abakpa river	Thinkers Corner well	Trans-Ekulu well
A	B	C	D

3.3. Sample Sourcing and Preparation

The *Moringa oleifera* (Moringa) seeds and leaves were plucked from Moringa tree at the Front Gate Entrance of Godfrey Okoye University, Thinkers Corner Campus. About 100 grams in total (50 grams each) were harvested and taken to the microbiology laboratory in the school inside sterile Ziploc bags. The *Azadirachta indica* leaves (100g) on the other hand were freshly picked from the Neem tree at Godfrey Okoye University Clinic, Thinkers Corner Campus, and taken to the laboratory. These seeds and leaves were respectively dipped quickly in bleach for 2 seconds (counted using a stopwatch) and rinsed off thoroughly with sterile distilled water four times to completely sterilize the surface, region shade-dried in the laboratory for 4 days at room temperature. Shade drying was used in order to protect the sensitive active phytocompounds and possible coagulants within the respective plant materials. Thereafter, the moringa seeds were further dried in a hot air oven until crisp at 50 °C to a constant weight and then crushed using a grinder to powder form. The rough textured powder gotten was then passed through a 2 mm sieve to obtain the fine powder. The fine powder from the sieve was then stored in sterile plastic containers until further analysis (Sandhya *et al.,*2018)

3.4 Preparation of Extracts

Aqueous and Ethanolic extraction was carried out for the preparation of extracts, for the aqueous extraction 100g of powdered plants sample was soaked in 1000ml of distilled water and was shakened thoroughly and allowed to settle for 3 days then it was kept in the water bath for a week**.**

For the ethanolic extraction 100g of powdered plants sample was soaked in 1000ml of 100% ethanol and was shakened thoroughly and allowed to settle for 3 days then it was kept in the water bath for 3 days.

3.5. Phytochemical Screening of Neem Leaves and Moringa Plant Parts

Phytochemical examinations of flavonoid, phenols, alkaloid, tannins, saponins, glycosides, steroids, triterpenoids and proteins/amino acids present in the Moringa plant was done according to the methods described by Khalid *et al.,* (2018), Sudha *et al.,* (2021), Sharma *et al.,* (2020) and Olaoye *et al.,* (2021).

3.5.1 Test for saponins

Two grammes of powdered sample was boiled in 20 mL of distilled water. Ten millilitres of filtrate was diluted with 5 mL of distilled water were shakened vigorously. The appearance of frothing indicated the presence of saponins (Sharma *et al.,* 2020).

3.5.2 Test for alkaloid

In 1% v/v HCl, the plant extract is mixed, warmed and filtered. To 2 mL of extract, 1 mL of Meyer's reagent was added. The presence of pale yellow precipitate indicated the presence of alkaloids (Khalid *et al.,* 2018).

3.5.3 Test for tannin and phenol

About 20 ml of distilled water and gelatin reagent (for tannins detection) was poured into a test tube containing the powdered sample of leaves, and was boiled and cooled then filtered. The addition of 3 drops of 0.1% v/v Ferric chloride to the filtered sample color changed to blue, which indicates the presences of phenols, while whitish precipitates indicates the presence of tannins (Khalid *et al*., 2018).

3.5.4 Test for flavonoids

About 4 mL of extract solution, 1.5 mL of 50% methanol solution and small magnesium chunk were warmed. About 6 drops of concentrated HCl was added, brown color was observed for flavonoids (Sharma *et al.,* 2020).

3.5.5 Presence of glycosides

Ground Moringa leaves and seeds and dilute HCl were mixed and legal's reagent was used to dissolve sodium nitropruside in pyridine and NaOH. Glycosides were confirmed due to the appearance of blood-red color (Sudha *et al.,* 2020; Olaoye *et al.,* 2021).

3.5.6 Test for steroids

Steroids were detected by Salkowski's test. In this the Neem leaves and Moringa leaf and seed were respectively dissolved in chloroform, filtered and then, mixed with concentrated H_2SO_4. Brown color appeared to indicate the presence of steroids (Sudha et al., 2020; Olaoye et al., 2021).

3.5.7 Detection of protein and amino acids

Protein was confirmed by following Xanthoproteic test. Neem leaves and Moringa leaves and seeds were treated with few drops of concentrated HNO_3, which resulted in the formation of yellow colored precipitates. Amino acids were indicated by Ninhydrin test. Extract was mixed with 0.25% Ninhydrin reagent and boiled for few minutes; blue colored precipitates appeared (Sudha *et al.,* 2020; Olaoye *et al.,* 2021).

3.5.8 Detection of triterpenoids

Triterpenoids were detected by Salkowski's test in which Moringa leaf and seed were dissolved in chloroform. Two/three drops of H_2SO_4 was added after filtration and mixed thoroughly. Golden color appeared to indicate the presence of triterpenoids (Sudha *et al*., 2020)

3.6. Experimental Process of Water Purification Through Coagulation

The different solutions were then stirred manually using sterile glass rod for about 15 minutes before being allowed to settle down for the particles to sediment for one hour, and the supernatant was filtered and further analysed for pH, turbidity and microbial load changes against to the negative control sample (Rubini *et al*., 2019).

3.6.1 pH Determination

pH is a measure of the concentration of hydrogen Ion H^+ in a solution which is measured mathematically on a scale of 0 to 14; defining 0 to 6 as being acidic, 7 being neutral and 8 to 14 being alkaline or basic. For this experiment, it was determined using a pH probe metre. The pH probe was first calibrated by inserting into distilled water before transferring into the 250 mL beakers respectively. The pH values were recorded for the untreated negative control and the introduced coagulant concentrations.

3.6.2 Turbidity measurement

The turbidity is a measure of the degree of cloudiness of water as a result of suspended particles in the water. This was determined using a UV-Vis spectrophotometer at 675nm. The spectrophotometer was calibrated using distilled water before the untreated and treated water samples were loaded into the curvette after thoroughly shaking to obtain homogeneous mixture. The length of the curvette was determined in cm as the path of light for the turbidimetric calculation. The samples in the curvette were properly cleaned around it before it was loaded into the sample holder of the spectrophotometer, and it's absorbance was noted.

3.7. Enumeration and Isolation of Total Heterotrophic Bacteria Count

Serial dilutions were carried out for the four water treatment samples and negative control before adding the Moringa seeds and leaves and neem leaves, and also after adding to the water samples up to 10^{-5} and dilution 10^{-2} and 10^{-4} were selected for the microbial count. The standard plate count method was used to test the samples which required culturing 0.1 ml of all water samples on nutrient agar plates to measure heterotrophic bacteria through the pour-plate technique. The plates were incubated at 37°C for 24 hours for the total bacterial count. The colonies were counted after 24 hours of incubation and the following formula was used to determine the count in colony-forming units per ml.

CFU/ml= number of colonies × dilution factor ÷ volume of inoculum.

To determine how much of the present bacteria was inhibited as a result of the treatments, log reduction values for the bacteria count were calculated as;

$$LR = Log_{10}(A) — Log_{10}(B).$$

Where: A is the number of organisms before treatment, and B is the amount after treatment

For percentage bacteria reduction, the following mathematical formula was employed:

$$P = (1\text{-}10^{-L}) \times 100.$$

Where P= Percentage microbial reduction, L= Log reduction (Ogofure *et al.,* 2023).

3.7.1. Determination of most probable number (total coliform count)

To reveal the presence of coliform in the water sample, the ISO, (2012) method was employed. The following items were prepared for this;

Ten millilitres test tubes containing MacConkey broth were prepared and sterilised at 121 °C in an autoclave according to manufacturer's instructions, and allowed to cool with Durham tubes inversely inserted inside. A volume of 0.1 mL of dilutions 10^{-2} and 10^{-4} of all water samples were inoculated into the broth and incubated at 37°C for 24 to 48 hours. Positive results were observed as a colour change of the broth from pinkish to yellow indicating presence of acid and the formation of bubbles at the base of the inverted Durham tubes, indicating presence of CO_2 gas (ISO, 2012). The positive result tubes were then plated on MacConkey agar and Eosin Methylene Blue (EMB) agar respectively by aliquoting 0.1 mL of the dilution broth tubes unto prepared petri dishes containing the MacConkey and EMB agar respectively and incubated at 37 °C for 24 to 48 hours. The resultant formation of pinkish colonies on the MacConkey agar indicates coliforms of the *Enterobacter* spp. while the formation of dark greenish coloured bacteria with a metallic like sheen on Eosin Methylene Blue agar indicates the presence of *Escherichia coli.* These were expressed in colony forming units after physical counting of colonies.

3.8. Determination of the Morphological and Biochemical Characteristics of the Bacteria Isolates

The morphological and biochemical characters of the obtained isolates were determined using the following tests

3.8.1 Gram stain reaction

All isolates were gram-stained to determine their morphological characteristics. Bacterial isolates were thinly smeared into the surface of a glass slide with a few drops of sterile water. The sample underwent heat fixation using a Bunsen burner, followed by the application of crystal violet, which was allowed to sit for one minute. After this period, the slide was rinsed with running tap water. Subsequently, Gram iodine was added as a mordant and left for an additional minute before another rinse. Decolorizer was then applied for 1 second, followed by a final rinse with tap water. Safranin, serving as the counterstain, was introduced to the slide in the last step. Once the slide had air-dried, it was examined under a microscope using oil immersion at 100x magnification. The staining results allow for differentiation of bacteria as Gram-positive bacteria appear purple due to their thick cell wall, while Gram-negative bacteria appear pink, reflecting differences in cell wall structure (Chauhan *et al.,* 2018).

3.8.2 Catalase test

On a sterile glass slide surface, loopful of bacteria isolates was placed using sterile wire loop and flooded with a drop of 3% hydrogen peroxide. The solution was mixed well using the wireloop. A positive catalse result is indicated by bubble formation, whereas a negative result shows absence of bubble formation (Mannan *et al.,* 2017).

3.8.3 Oxidase test

A sterile glass slide was used as the isolates were placed on its surface and reacted with the cytochrome oxidase reagent. The formation of blue-purple coloration indicates positive oxidase

whereas formation of pink coloration indicates oxidase negative result. The bacteria being positive or negative is based on their change in color (Dimri *et al.,* 2020).

3.8.4 Indole test

In a test tube containing 5ml of peptone water, the test organism was inoculated and placed in an incubator at 37°C for 24 hours to enable the organism adsorb the indole. After 24 hours, approximately 0.5ml of Kovac's reagent was introduced into the tube. A red colour appearance at the topmost layer indicated positive while a yellow colouration depicted negative for the test (Edem *et al.,* 2022).

3.8.5 Citrate utilization test

About 10ml of Citrate medium was added into several test tubes and was placed in an autoclave at a temperature of 121°C. It was left for 15 minutes so that the medium can be properly sterilized. The organism used for test was transferred to the sterilized media by inoculation method and was incubated for a period of 48 hours at 37°C. The presence of a deep-blue colour showed that the reaction was positive (Edem *et al.,* 2022).

3.9. Molecular Identification of Waterborne Isolates

200ul DNA Pre-Wash Buffer was added to the Zymo-Spin™ ICR column in a new collection tube and centrifuged at 10,000 x g for 1 minute. 500ul of g-DNA Wash Buffer was added to the Zymo-Spin™ ICR Column and centrifuged at 10,000 x g for 1 minute. Zymo-Spin™ ICR Column was transferred to a clean 1.5 ml microcentrifuge tube and 100 pl (35ul minimum) DNA Elution Buffer was added directly to the column matrix and centrifuged at 10,000 x g for 30 seconds to elute the DNA.

Two isolates of persistent bacteria in the already treated water samples using the Neem and Moringa seeds and leaves extracts respectively were selected for this phase. The study used a three-step process which included DNA extraction and PCR amplification and phylogenetic analysis to study the bacterial DNA. The boiling DNA extraction method which (Chakravorty

et al., 2007) developed was applied to extract DNA from a 2 mL nutrient broth culture of the bacterium. The pellets were mixed with sterile distilled water, then vortexed and heated before it was centrifuged t-o obtain pure DNA from the supernatant for further analysis. Ten microliters of this DNA were utilized for gene amplification through PCR. The PCR reaction mixture required various elements which included GoTaq® buffer and MgCl2 and dNTPs mix and specific primers and Taq DNA polymerase and the DNA template which collectively required a final volume of 42µL.

3.10 PCR amplification

The PCR process used a GeneAmp 9700 thermal cycler to perform an initial denaturation step and then executed 30 cycles which included denaturation at 94 to 95°C for 3-5 minutes and completed with a final extension step. The amplified PCR products were stored at 4°C until when needed for future experiments. The 1% agarose gel electrophoresis method confirmed the integrity of the amplified DNA fragment. The gel was prepared with TAE buffer and ethidium bromide and solidified before being submerged in a gel tank. Samples which contained loading dye were added into the wells and performed electrophoresis at 120V for 45 minutes. Ultraviolet light was used to visualize the gel and the size of the PCR products were compared with a molecular weight ladder. Ethanol served as the purification agent to remove PCR reagents from the amplified DNA fragments. The PCR product was combined with ethanol and sodium acetate and then subjected to vortexing before being stored at a temperature of 20 degrees Celsius. The mixture went through centrifugation which resulted in the supernatant being thrown away while the pellet underwent washing with ethanol and then went through another round of centrifugation before it was dried and finally suspended in sterile distilled water. The purification process was confirmed through agarose gel electrophoresis testing and Nano-Drop spectrophotometer analysis. The Applied Biosystems™ Genetic

Analyzer together with the BigDye™ terminator kit was used to sequence the amplified DNA according to the manufacturer's specifications.

3.11 Data Analysis of Result

The experiment was conducted in triplicates of independent investigations. The results were analysed using descriptive statistics to determine the significance difference between mean sets from the data generated on Excel Spreadsheet Package 2013.

CHAPTER FOUR

RESULTS

4.1. Effect of Neem and Moringa Extract on pH of Water Samples before and after Treatment

Upon collection of the water samples for analysis, two physical parameters were analyzed and used as a focal point for determining the purifying efficacy of the selected plant extracts for this study (Moringa leaves and seeds and neem leaves respectively). The parameters which were the pH and turbidity were tested before treatment in this case, and the result of the pH as indicated in Table 4.1 shows a fairly neutral and acceptable range between 6.76 to 7.76 according to WHO standards. The turbidity (Table 4.1) on the other hand, which is measured in Nephelometric Turbidity Unit (NTU) recorded values within the ranges of 16.0 to 20.9 NTU across the four water samples, which were above the acceptable turbidity limit for safe water according to the WHO.

4.2. Effect of Coagulants (neem and moringa extracts) on pH of Water Samples after Treatment

The aqueous and ethanolic Neem and Moringa extracts caused some drastic changes to the standing pH of the water samples upon application as a treatment regimen. The results as seen in Table 4.2 shows varying pH ranges, that of the neem at both concentrations being between 4.84 to 7.35 while the Moringa being between 4.70 to 7.10, thus indicating the extracts were either unable to neutralize the acidity of the water or caused acidification of the raw water samples. From this result, the neem extracts may have a slightly higher neutralizing effect on acidity in comparison to the Moringa extracts, although both have very close ranges in terms of their effect against pH of the water samples respectively.

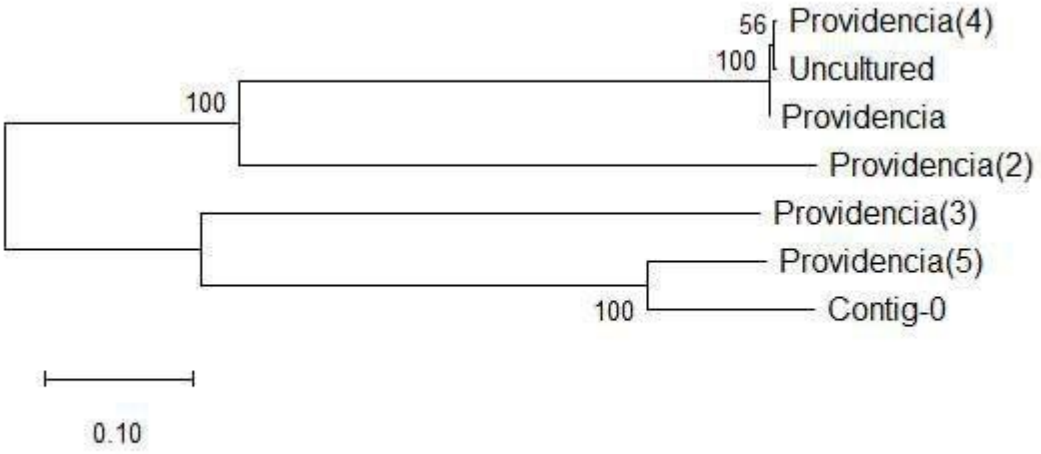

Figure 4.1: Molecular identification and result

Table 4.1 Phytochemical Test of the Plant Extracts

	Plant Extracts	
Phytochemicals	**Moringa leaves**	**Neem leaves**
Alkaloids	+	+
Glycosides	+	+
Saponins	-	+
Tannins	-	+
Flavonoids	+	+
Resins	+	+
Proteins	+	+
Steroids	+	+
Terpenoids	+	+

Key: (+): Presence of phytochemical compound; (-): Absence phytocmical compound.

Table 4.2. Physical characteristics of raw water samples before treatment regimen (ph and turbidity)

Sample ID	pH before of Raw Water	WHO/NSDWQ 2017 Standard Values	Turbidity of Raw Water (NTU)	WHO/NSDWQ 2017 Standard Values
A	7.76		17.8	
B	7.53		17.9	
C	7.09	6.5-8.5	16.0	5.0
D	6.76		20.9	

Key: (A): Emene River Water; (B): Abakpa River Water; (C): Thinkers Corner Well Water; (D): Trans-Ekulu Well Water; (WHO): World Health Organisation; (NSDWQ): Nigerian Standard for Drinking Water Quality.

4.3. Effect of Coagulants (Neem and Moringa Extracts) on Turbidity of Water Samples after Treatment

Comparing the effect of the neem leaf extracts and Moringa seed extract in clearing the turbidity of the raw water as natural coagulants shows distinct results as recorded in Table 4.3. In regards to Turbidity reduction, the Moringa seed extract at lower concentration of 1.5g/50mL was able to perform better than the neem leaf extract at same lower concentration by producing consistently lower Turbidity levels. The turbidity levels after treatment with the moringa seed extracts significantly reduced between 5.1 NTU and 18.1 NTU at lower concentrations, whereas the neem leaf extracts was between 5.3 NTU to 19.5 NTU at same concentration. At even higher concentration of 4.5g/50mL, the Moringa seed extract had lower turbidity values between 5.5 to 8.8 NTU, whereas the neem leaf extract had NTU values between 8.3 to 10.5 NTU. This could entail that the Moringa seed extract was a better agent, to clump up and remove suspended particles from raw water, thus purifying it efficiently.

4.4. Morphological and Gram Stain Features of Microbial Isolates from Raw Water Samples and Occurrence of Microbes across Water Samples

From the four different raw water samples, there were prevalent concentrations of coliform bacteria in all water samples and a mix of lactic acid producers and fermenters in two different water samples from the mix. The morphological characteristics of the isolates from the four water samples are observed in Figure 4.1 and also detailed in Table 4.4, with the likely identities of the isolates stated as well.

4.5. Microbial Load Count of Water Samples before Treatment

There was an observable high concentration of bacteria present in the raw water samples during the screening exercise. The initial microbial load count as expressed in CFU/mL for all four water samples are indicated in Table 4.5, with Sample A having the most bacteria present as indicated by the individual number of colonies counted across the three selected dilution

factors; too numerous to count (TNTC), 281 and 129 CFU/ml respectively, and Sample D having the least at 190 and 139 CFU/ml as recorded via the same procedure. The results as shown in Table 4.6 indicate the average bacteria load concentration of the samples as follows; Sample A: 1.40×10^9 CFU/mL, Sample B: 1.30×10^8 CFU/mL, Sample C: 1.24×10^8 CFU/mL and Sample A: 1.65×10^9 CFU/mL. Sample D had the highest average concentration of present bacteria at 1.65×10^9 CFU/mL, which is followed closely by Sample A at 1.40×10^9 CFU/mL, then Sample B and Sample C respectively.

4.6. Microbial Load Count of Water Samples after Treatment

After using the plant extracts as treatment regimen at different concentrations in the raw water, the initial average microbial load concentration decreased to negligible levels at perfect logarithmic percentage reductions. As seen in Table 4.6, Sample A had a microbial count decrease from 1.40×10^9 CFU/mL to a least count of 3.2×10^3 CFU/mL, indicating a percentage reduction of 99.99%, while Sample B recorded a microbial count decrease from 1.30×10^9 CFU/mL to 3.27×10^3 CFU/mL, also indicating a 99.99% reduction. Samples C and D also recorded a 99.99% reduction as the microbial load decreased from 1.24×10^3 CFU/mL and 1.65×10^3 CFU/mL respectively to less than 1CFU/mL. The plant extracts were able to significantly reduce the microbial contaminants initially present in the water to even zero colony counts per each sample treated and tested. The coliform count also gave very low to zero values as well, especially for the water samples from the two well sources, as is expected of underground water sources to have absence of coliform bacteria in them.

Table 4.3. Effect of the plant extracts on pH of water samples after treatment

Concentration (g/50mL)	Sample ID	Moringa Leaf Extract	Neem Leaf Extract
1.5 Aqueous	A	5.12 ± 0.19	4.84 ± 0.19
	B	4.95 ± 0.72	5.97 ± 0.72
	C	4.7 ± 1.87	7.35 ± 1.87
	D	5.1 ± 1.01	6.53 ± 1.01
1.5 Ethanol	A	5.5 ± 0.25	5.85 ± 0.25
	B	6.2 ± 0.21	5.9 ± 0.21
	C	5.8 ± 0.21	6.1 ± 0.21
	D	6.0 ± 0.25	6.25 ± 0.25
4.5 Aqueous	A	4.9 ± 0.67	5.85 ± 0.67
	B	6.88 ± 0.27	6.5 ± 0.27
	C	7.1 ± 0.14	6.9 ± 0.14
	D	6.85 ± 0.23	6.53 ± 0.23
4.5 Ethanol	A	5.85 ± 0.25	6.2 ± 0.25
	B	5.05 ± 0.88	6.3 ± 0.88
	C	5.5 ± 0.63	6.39 ± 0.63
	D	5.25 ± 0.29	5.66 ± 0.29

Key: (A): Emene River Water; (B): Abakpa River Water; (C): Thinkers Corner Well Water; (D): Trans-Ekulu Well Water; (±): Standard Deviation across Mean Rows via single factor ANOVA.

Table 4.4. Effect of the plant extracts on turbidity of water samples after treatment

Concentration (g/50mL)	Sample ID	Moringa Seed Extract (NTU)	Neem Leaf Extract (NTU)
1.5 Aqueous	A	17.6 ± 6.08	9.0 ± 6.08
	B	6.6 ± 0.07	6.5 ± 0.07
	C	5.4 ± 0.07	5.5 ± 0.07
	D	5.1 ± 0.14	5.3 ± 0.14
1.5 Ethanol	A	18.1 ± 6.08	9.5 ± 6.08
	B	7.4 ± 0.28	7.8 ± 0.28
	C	5.3 ± 0.49	6.0 ± 0.49
	D	5.9 ± 0.0	5.9 ± 0.0
4.5 Aqueous	A	6.1 ± 3.11	10.5 ± 3.11
	B	7.3 ± 0.85	8.5 ± 0.85
	C	7.9 ± 1.20	9.6 ± 1.20
	D	5.5 ± 1.84	8.1 ± 1.84
4.5 Ethanol	A	8.8 ± 0.42	8.2 ± 0.42
	B	6.2 ± 1.49	8.3 ± 1.49
	C	8.1 ± 0.57	8.9 ± 0.57
	D	8.2 ± 0.85	9.4 ± 0.85

Key: (A): Emene River Water; (B): Abakpa River Water; (C): Thinkers Corner Well Water; (D): Trans-Ekulu Well Water; (±): Standard Deviation across Mean Rows via single factor ANOVA.

4.7 Most probable number (MPN) of coliform bacteria in water sample

The MPN results showed that all untreated water samples contained coliform bacteria, indicating faecal contamination. However, after treatment with plant extracts, no coliform bacteria were detected (0/100 mL) in all samples, demonstrating the effectiveness of the treatment.

Table 4.5 Most probable number (MPN) of coliform bacteria in water sample

Sample	Before Treatment (MPN/100 mL)	After Treatment (MPN/100 mL)
A	160	0
B	110	
C	90	0
D	75	0

Table 4.6. The morphology and gram stain characteristics of microbial isolates from water

Water Samples Bacteria Isolates	Morphological Description	Gram Stain Feature	Colour	Size	Elevation	Shape	Likely Organism
A	Black round shape colonies with a sheen	Gram-negative rod like shape	Pale yellow	3-4	Raised	Irregular	*Escherichia coli.*
	Mucoid like colonies with pale yellow centres	Gram-positive cocci shape	yellowish	3-6	raised	Circular	*Staphylococcus aureus.*
	Small pale colonies with shiny texture	Gram-positive rod shaped colonies	Whitish	2-4	Flat	Irregular	*Bacillus* sp.
	Large translucent colonies with pale color	Gram-negative rod shaped colonies	Creamy	4-8	Raised	Circular	*Klebsiella* sp.
B	Mucoid like colonies with pale yellow centres	Gram-positive cocci shape	Creamy	3-7	Raised	Circular	*Staphylococcus aureus*
	Fairly large translucent pale colored colonies	Gram-negative rod shaped colonies	Pale	4-8	flat	Irregular	*Enterobacter* sp
C	Large translucent pale colored colonies	Gram-negative rod shaped colonies	Pale	5-9	Flat	Irregular	*Enterobacter* sp.
	Small size translucent colonies with pale color	Gram-negative rod shaped colonies	Creamy	2-5	Raised	Circular	*Klebsiella* sp.
D	Fairly large translucent pale colored colonies	Gram-negative rod shaped colonies	Pale	4-8	Flat	Irregular	*Enterobacter* sp.
	Small size translucent pale colored colonies	Gram-negative rod shaped colonies	Creamy	2-5	Raised	Circular	*Klebsiella* sp.

Key: (A): Emene River Water; (B): Abakpa River Water; (C): Thinkers Corner Well Water; (D): Trans-Ekulu Well Water.

Figure 4.1: The Morphological features of the bacteria from Emene River

Figure 4.2: The Morphological features of the bacteria from Abakpa River

Figure 4.3: The Morphological features of the bacteria from a Thinkers Corner Well

Figure 4.4: The Morphological features of the bacteria from Trans Ekulu well

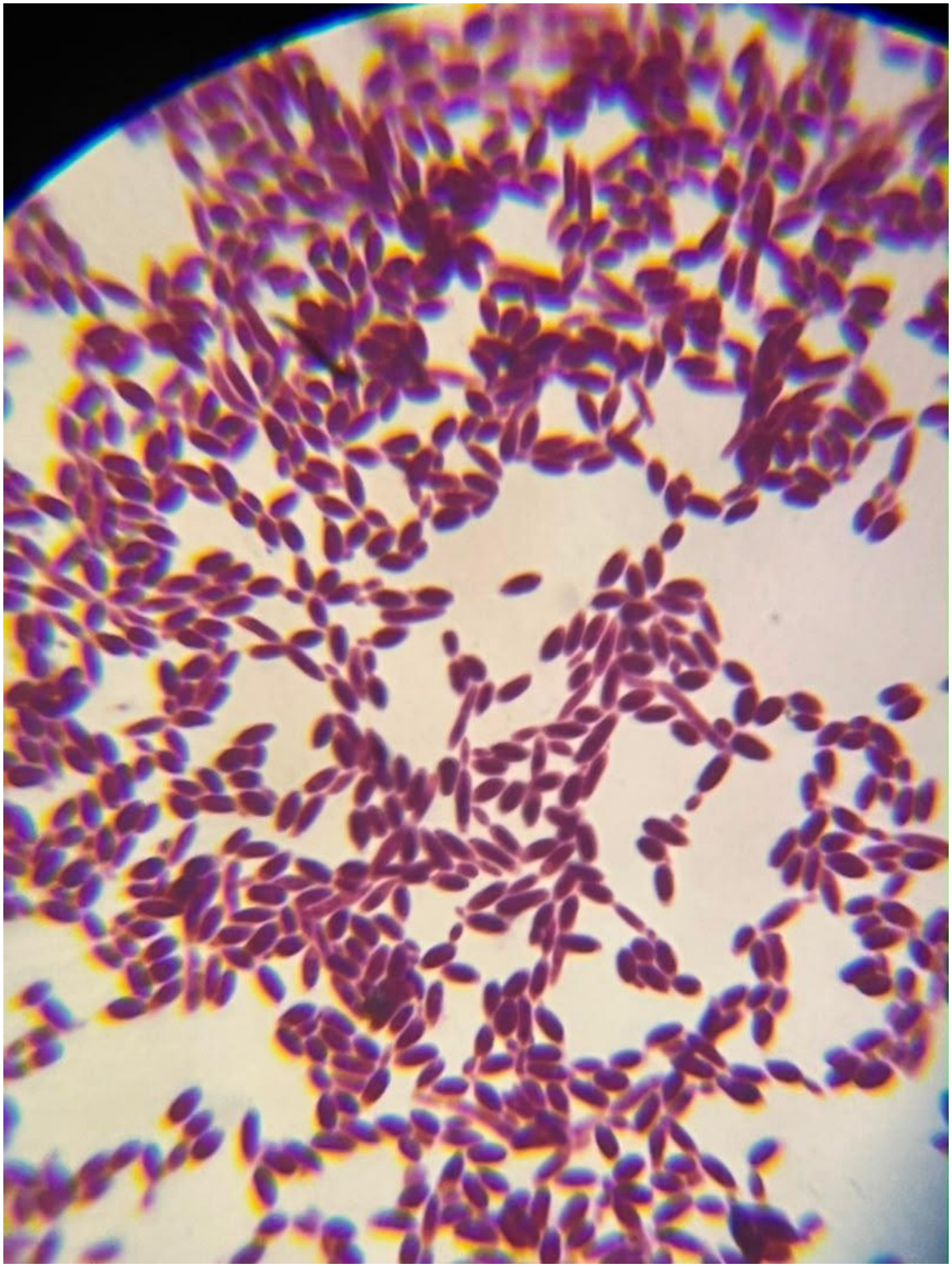

Figure 4.5: Microscopic visualization of recurring bacteria isolates from gram negative rods, likely *Escherichia coli*

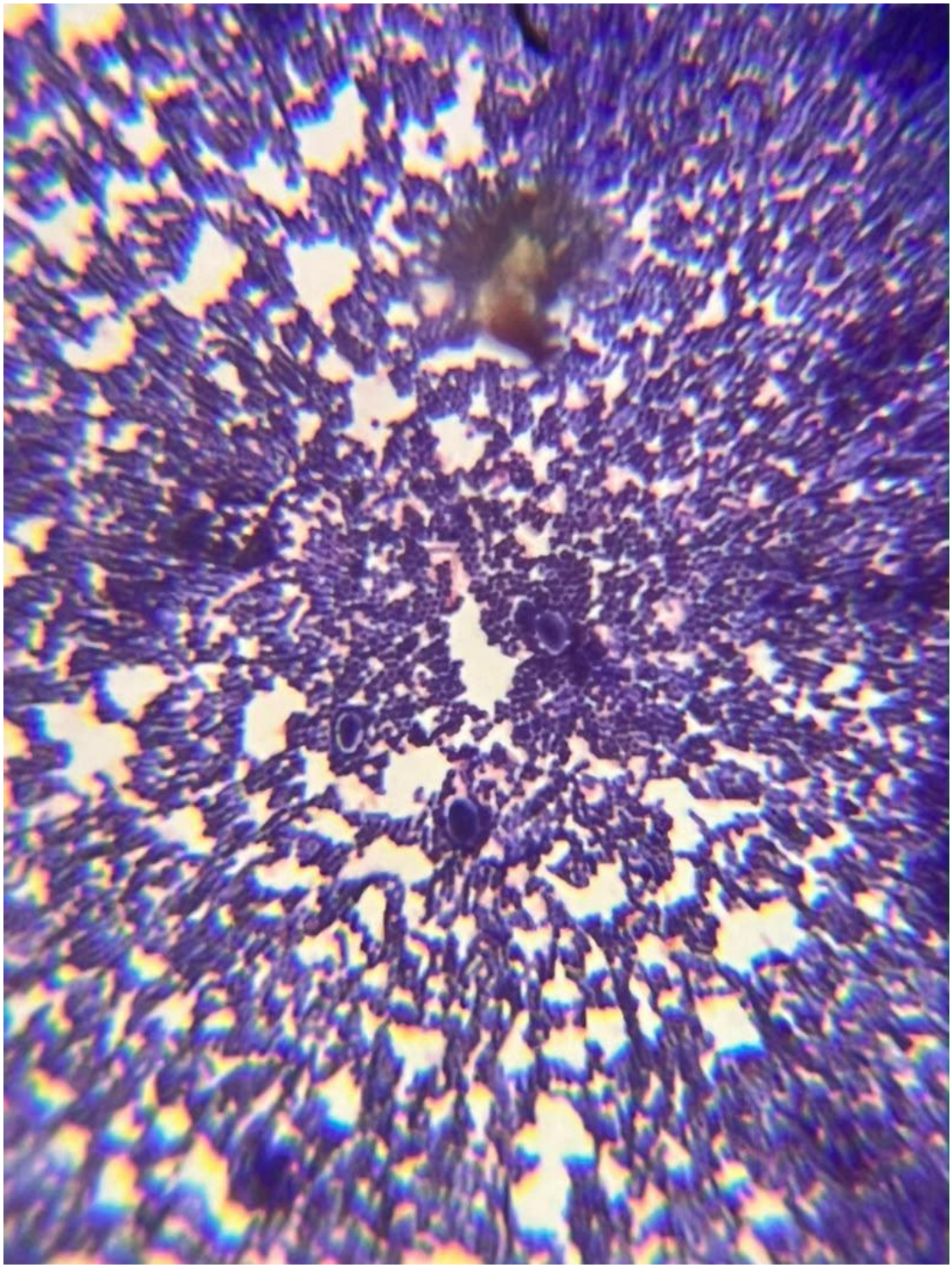

Figure 4.6: Microscopic visualization of recurring bacteria isolates from gram positive cocci shape indicating *staphylococcus aureus*

Figure 4.7: Microscopic visualization of recurring bacteria isolates from gram-positive roda indicating *Bacillus spp*

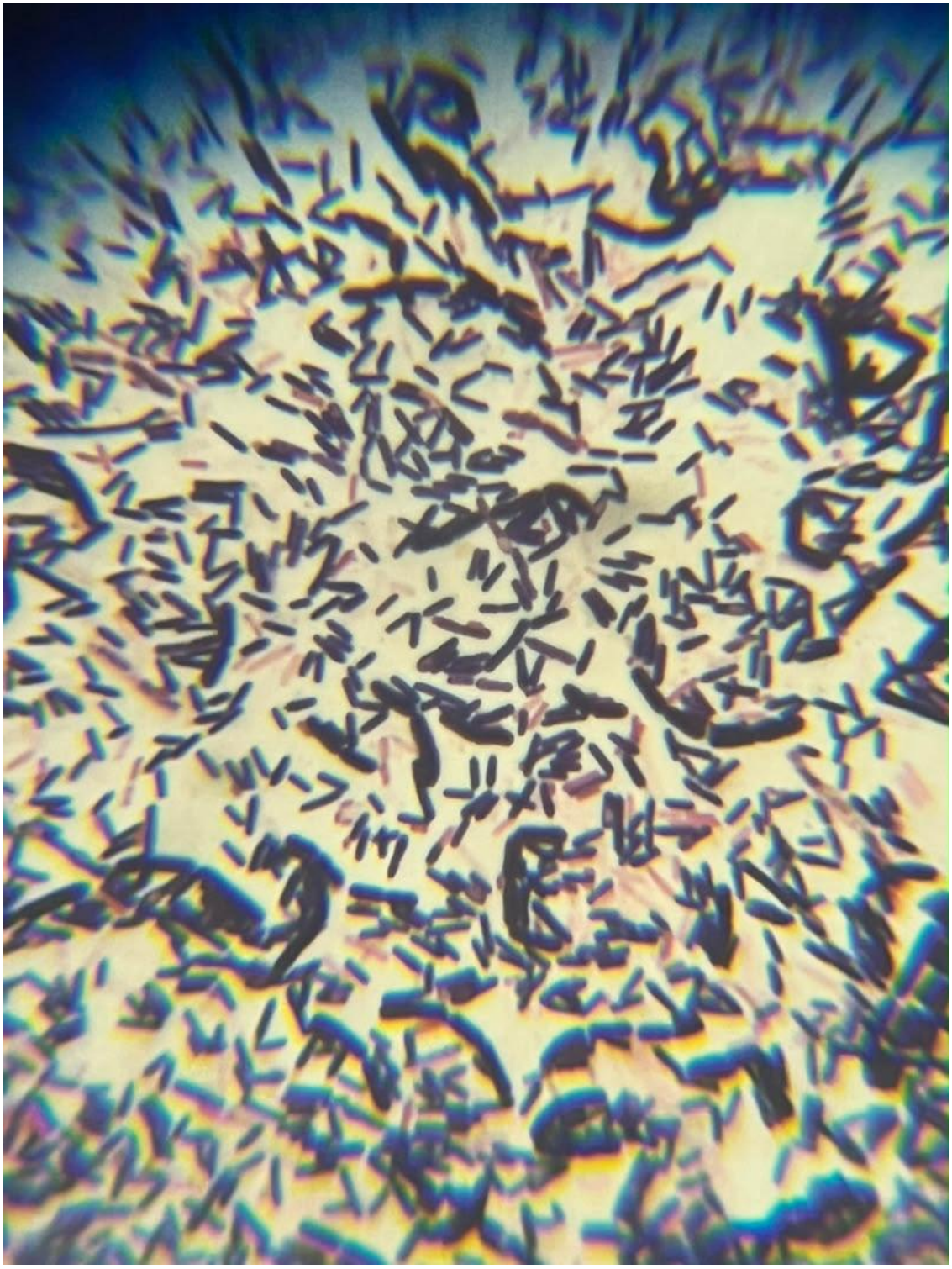

Figure 4.8: Microscopic visualization of recurring bacteria isolates from mix of gram-negative rods indicating *Enterobacter spp*. and *Klebsiella spp* before treatment

Figure 4.8 Persistent Organism *Bacillus* spp after treatment

Figure 4.9: MPN Test tube for coliform analysis before treatment

Table 4.7: Biochemical Characteristics of Bacterial Isolates from Water Samples

Biochemical Test	***E. coli***	***Staphylococcus aureus***	***Klebsiella* sp.**	***Enterobacter* sp.**	***Bacillus* sp.**
Gram Reaction	Gram – rod	Gram + cocci	Gram – rod	Gram – rod	Gram + rod
Catalase Test	+	+	+	+	+
Oxidase Test	–	–	–	–	+
Indole Test	+	–	–	–	–
Citrate Utilization	–	–	+	+	+
Urease Test	–	+	+	–	–
Coagulase Test	–	+	–	–	–
Motility Test	+	–	–	+	+
Lactose Fermentation	+	–	+	+	–
Spore Formation	–	–	–	–	+

Key: (+) Positive reaction, (–) Negative reaction

Table 4.8 Colony forming units count of microbial isolates

Water Samples ID	Dilution Factor	Colonies Counted	Colony Forming Units (CFU/mL)
A	10^2	TNTC	Nil
	10^3	281	2.81×10^6
	10^4	129	1.29×10^7
B	10^2	188	1.88×10^5
	10^3	117	1.17×10^6
	10^4	105	1.05×10^7
C	10^2	280	2.80×10^5
	10^3	230	2.30×10^6
	10^4	140	1.40×10^7
D	10^2	TNTC	Nil
	10^3	190	1.90×10^6
	10^4	139	1.39×10^7

Key: (A): Emene River Water; (B): Abakpa River Water; (C): Thinkers Corner Well Water; (D): Trans-Ekulu Well Water; (TNTC): Too Numerous to Count; (Nil): Non-viable colony count.

Table 4.9. Average microbial load count concentration of isolates and percentage bacteria reduction

Water Samples ID	Average Count for Raw Water Samples (CFU/mL)	Average Count for Treated Water Samples (CFU/mL)	Percentage Reduction (%)
A	1.40×10^9	3.23×10^3	99.997
B	1.30×10^8	3.73×10^3	99.997
C	1.24×10^8	~<1	99.999
D	1.65×10^9	~<1	99.999

Key: (A): Emene River Water; (B): Abakpa River Water; (C): Thinkers Corner Well Water; (D): Trans-Ekulu Well Water; (~): Negligible values less than 1CFU/mL and below formed colonies.

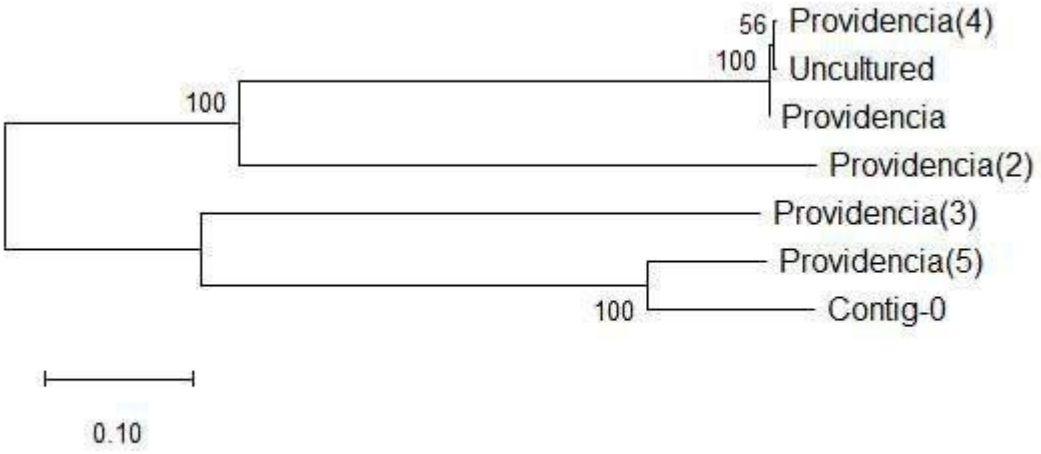

Figure 4.10: Phylo genetic tree of selected bacteria species

Interpretation

The phylogenetic analysis of the bacterial species, the isolate showed close relationship with *bacillu*s species strong bootstrap values(99-100) and short branch length indicate reliable results with minimal genetic variation.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1. Discussion

This research work examined the efficacy of extracts from the plants, *Azadirachta indica*, and *Moringa oleifera* in purifying polluted water. The findings obtained through turbidity tests, microbial testing, and Most Probable Number (MPN) analysis give clear proof of the ability of these plant extracts in purifying contaminated water.

The turbidity levels that were recorded initially, ranging from 17.8 to 20.9 NTU, suggest that the water samples were very turbid and could not be used for domestic purposes. Turbidity is usually an indicator of high amounts of suspended matter such as clay, organic matter, and microbes. Not only do such suspended materials make the water opaque, but they also serve as cover for microbes, which increases the chance of infections. The high turbidity levels confirm the pollution of the water samples being used in the study.

There were observable changes in water clarity after the use of the plant extracts. While turbidity measurements were not taken after the experiments, there was a marked decrease in the turbidity level of the solution. This phenomenon is due to the coagulating and flocculation properties of the plant extracts used. The plant extract from Moringa oleifera has certain components, specifically the cationic protein, which helps neutralize negative charges on water particles. As a result, these particles coagulate and settle. The phytochemicals in *Azadirachta indica* also contribute to the destabilization of particles.

The microbiological tests are additional evidence of efficiency of the treatment. Indeed, there were high microbial counts in the untreated samples of water. However, with the treatment, there were considerably lower numbers of CFUs, which indicates antimicrobial effect of the tested plant extracts. In particular, the aqueous extract of *Azadirachta indica* demonstrated

higher reduction of microbial content than the other samples. Thus, it may be concluded that the antimicrobial effect is higher in neem than in moringa.

In agreement with earlier reports, the present study recorded a substantial reduction in microbial load after treatment, with coliform bacteria completely eliminated (0/100 mL). Similar studies have also reported high reductions in bacterial count; however, many focused on either turbidity reduction or antimicrobial activity alone. In contrast, this study evaluated both physicochemical parameters (pH and turbidity) and microbiological quality (total bacterial count and MPN), providing a more comprehensive assessment of water purification. Furthermore, which aligns with findings from other researchers who attributed this to the stronger antimicrobial constituents present in neem. However, unlike some studies that reported complete sterilization, the presence of Bacillus species after treatment in this study highlights the resistance of spore-forming bacteria, thereby providing a more realistic representation of treatment effectiveness.

The reason for higher efficiency of neem extract can be explained by its complex chemical composition, which includes numerous bioactive substances, such as flavonoids, tannins, alkaloids, and saponins. Bioactivity of these substances implies antimicrobial potential because they are known to possess ability to destroy cell walls of microorganisms and interfere with enzymes. On the contrary, although Moringa oleifera also has numerous antimicrobial agents, its key property is coagulation rather than antimicrobial effect. That is why neem extract was more efficient in reduction of the amount of bacteria in this experiment. As a result of Most Probable Number (MPN) test, it was found that there were no coliform bacteria in treated samples, so 0 coliforms per 100 ml of solution was recorded. Such a result is very important, because presence of coliforms (mainly *E. coli* bacteria) is widely used as an indicator of fecal pollution of drinking water. Lack of coliforms means elimination of fecal contamination, so treated water is free from the possibility to cause infectious diseases (cholera, typhoid fever,

dysentery). As recommended by World Health Organization, no coliforms per 100 ml is a requirement for safe water.

These structures are known for being highly resistant, enabling bacteria to survive even in the toughest environmental conditions, including being exposed to any form of antimicrobial substance. This characteristic was the most probable factor behind the survival of Bacillus bacteria. Such findings bring up an essential drawback of the used approach, as it cannot provide for the survival of any form of bacteria that possess the mentioned characteristics. The results show that while plant extracts successfully reduced the number of microorganisms and eliminated coliform bacteria, they could not be used to fully sterilize water. Hence, further techniques of treatment like boiling, filtering, or chemical disinfection might need to be employed in order to make the water completely safe for consumption. Another significant finding of this experiment is the link that exists between the reduction of turbidity and the reduction in microbes. The reduction in turbidity was accompanied by a reduction in the presence of microbes. The reason for this phenomenon is due to the fact that several microbes get attached to the suspended matter in water. Consequently, their removal results in the removal of the attached microbes.

In addition, the efficacy of the method depended upon the kind of extract employed. Indeed, the aqueous extract from the neem was proven to be much more effective in comparison to the other treatment options considered. Thus, the choice of an extraction method can be considered an essential factor influencing the efficiency of purification using plants. For example, aqueous extraction might contribute to the effective separation of the required active substances in charge of antimicrobial properties. As far as other studies go, it appears that the findings of this paper coincide with previous conclusions concerning the efficiency of plant coagulants and antimicrobial agents for improving the quality of water. Nevertheless, most of the studies note

that this purification method is recommended to be used as a preliminary one rather than a comprehensive one.

5.2 Conclusion

- This study demonstrates that plant extracts from Moringa and neem are effective in improving the quality of contaminated water. The treatment resulted in a significant reduction in turbidity and microbial load, as well as complete removal of coliform bacteria.
- Among the extracts tested, aqueous neem extract exhibited the highest antimicrobial activity. Despite these positive outcomes, the persistence of Bacillus species indicates that the treatment does not achieve complete sterilization. Therefore, while the method is effective in reducing contamination and improving water quality, it may not be sufficient for direct consumption without additional treatment.
- Overall, the use of plant-based extracts represents a promising, cost-effective, and environmentally friendly approach to water purification, particularly in low-resource settings.

5.3 Contribution to knowledge

- The experiment clearly shows that water contaminated with harmful substances can be treated successfully by using plant extracts obtained from *Azadirachta indica* and *Moringa oleifera.*
- This method proved to decrease the turbidity, microbial content, and coliform presence significantly. Out of the various extracts used, the aqueous extract of the neem plant showed the maximum antibacterial potential. However, since the presence of bacillus could be detected, it can be said that sterilization was not accomplished completely.

- This shows that although the process has proven successful in terms of reducing the microbial load in contaminated water, it cannot be relied upon when it comes to making such water suitable for consumption.

5.4 Recommendations

Few recommendation can be drawn based on this project:

- Treated water must undergo further purification through processes such as boiling or filtration prior to consumption.
- More studies are needed in order to determine the exact bioactive substances that contribute to the anti-microbial effect of both neem and moringa.
- Molecular methods could be considered for identification of the various microorganisms in future studies.
- Efficacy testing for plant extract can be done under varied conditions and at larger levels.
- Treatment conditions can be optimized for better results in the process.
- Public enlightenment on the natural means of preliminary water treatment using plants is needed.

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APPENDIX

APPENDIX I: ARRANGEMENT OF BEAKERS FOR TREATMENT

APPENIDIX II: SOURCE OF WATER SAMPLE

APPENDIX III: RESEACHER AT THE SAMPLE BENCH