COMPARATIVE EVALUATION OF THE ANTIBACTERIAL ACTIVITIES OF *OEDOGONIUM, MORINGA OLEIFERA* AND *AMARANTHUS VIRIDIS*

Project submitted

TO

MAHATMA GANDHI UNIVERSITY

In partial fulfillment of the requirements in degree of

BACHELOR OF SCIENCE IN BOTANY

Submitted by

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Register no: 210021022654

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DEPARTMENT OF BOTANY

BHARATA MATA COLLEGE

THRIKKAKARA

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CERTIFICATE

This is to certify that this project work entitled **COMPARATIVE EVALUATION OF THE ANTIBACTERIAL ACTIVITIES OF *OEDOGONIUM, MORINGA OLEIFERA AND AMARANTHUS VIRIDIS*** is a bonafide piece of project work done by **KAMALESH. U (Register no: 210021022654)** in the Department of Botany, Bharata Mata College, Thrikkakara under my guidance and supervision for the award of Degree of Bachelor of Science in Botany during the academic year 2021-2024. This work has not previously formed the basis for the award at any other similar title of any other university or board.

Place : Thrikkakara

Date :

Department of Botany

Bharata Mata College

Name & signature of the

Supervising teacher

DECLARATION

I hereby declare that this project entitled entitled **COMPARATIVE EVALUATION OF THE ANTIBACTERIAL ACTIVITIES OF *OEDOGONIUM, MORINGA OLEIFERA AND AMARANTHUS VIRIDIS*** is the result of work carried out by me under the guidance of Newby Joseph Mam, Department of Botany, Bharata Mata College, Thrikkakara. This work has not formed on the basis for the award at any other similar title of any other university of board.

KAMALESH. U

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CONTENTS

CHAPTER 1

INTRODUCTION

Plants and plant products have been a source of foods and medicines from the dawn of human civilization. The plants used by human to cure the illness and to relieve the sufferings are called “Medicinal Plants”.

Infectious diseases are still a major health concern accounting for 41% of the global disease burden measured in terms of Disability – Adjusted Life Years (DALYS). One of the main causes of this problem is the widespread of acquired bacterial resistance to antibiotics in such a way that the world is facing today, a serious threat to global public health in the form of not only epidemics, but also pandemics of antibiotic resistance.

From the very beginning of human civilization, use of plants and plant products for medicinal purposes can be traced back. It's the most ancient collection of knowledge in history, according to legend. Various fields of life sciences and the art of healing, as well as some medicinal qualities, are addressed in its eight divisions by Ayurveda, which is based on Hinduism.

After a period of neglect and decline, this ancient practice of "green medicine" is returning to the forefront of our health care programs. As a result, the demand for so called herbal medicines is constantly increasing and these systems are now widely accepted by scientists around the world: Ayurveda, Unani or Chinese Medicine.

Despite great advances in medical science, infectious diseases such as those caused by bacteria, fungi, viruses and parasites are a major concern for public health. They have a particularly important effect on the use of medicinal products and on the development of drug resistance in general. Resistance to antimicrobial therapy is increasing both for new and existing antibiotics, while a variety of defenses are emerging from bacterial and fungus pathogens. Due to the increased failure of chemotherapeutics as well as antibiotics resistant to pathogenic microbiologic agents, a number of medicinal products have been evaluated for possible antibacterial activity.

In contrast to synthetic drugs, natural products derived from higher plants may provide a new source of antimicrobial agents with potentially unique mechanisms of action. Antimicrobials derived from plants also have a low incidence of side effects and a great deal of therapeutic potential to treat a variety of infectious diseases. In addition to this issue, antibiotics can also cause hypersensitivity, immunosuppression, allergic reactions, and the loss of good gut and mucosal microorganisms when taken by the host. Thus, the development of substitute antibacterial medications is imperative.

Algae are a diverse spectrum of aquatic, autotrophic, photosynthetic creatures that include enormous, sophisticated seaweeds and single-celled bacteria known as microalgae. Because algae are cheap to harvest and grow only in water, they have a good effect on both food security and the environment by reducing the need for arable land. In addition, they contribute oxygen to the atmosphere and can lower methane emissions if fed to ruminants. The technology used in algal culture is comparable to that used in terrestrial plant agriculture, however algae are more productive.

• In 1860, W. Hilse found the green algae, *Oedogonium*, which is free-living, in Poland's freshwater. This type of algae lives in freshwater environments and is both planktonic and benthic. It reproduces both sexually and asexually. Oedogonium is frequently found in peaceful freshwater bodies. They frequently float freely or are affixed to other plants. Typically, *Oedogonium* filaments have a single cell thickness and are unbranched. With the exception of the basal cell, which functions as a holdfast resembling a root, every cylindrical filament cell has a sizable central vacuole and a net-like chloroplast. Algal diversity is enormous, and because different compounds grow at different intensities and produce different kinds of bioactives, these organisms can be used as a source of enrichment.

Oedogonium have shown cosmopolitan in distribution and it is found in almost all water resourses that have been left undisturbed for long time. If this growing algal mass shows the properties of eliminating or killing bacteria and other pathogenic microorganisms, it can be used to produce antibiotics which is natural and of less side effects than the chemical antibiotic that give adverse side effects.

Plants contain a plethora of biologically active compounds with antibacterial qualities. The evaluation of plants with antibacterial activity against a range of diseases has increased recently on a global scale.

- ***Moringa oleifera***: The "tree of life" or "miracle tree," *Moringa oleifera*, is regarded as an important herbal plant because of its many health benefits, both medicinal and non-medical. It is also referred to as the "horseradish tree" or the "drumstick tree." The plant has historically been used to treat inflammation, cancer, heart disease, liver disease, wounds, and pain. Because of their high nutrient content, moringa leaves have been used for centuries as a valuable source of nourishment. By consuming the powdered leaves of *Moringa oleifera*, people all over the world can take advantage of its many health benefits.
- ***Amaranthus viridis:*** Known by most names as green amaranth or slender amaranth, *Amaranthus viridis* is a widely distributed species in the Amaranthaceae family of plants. An annual herb with an upright, light green stem is *Amaranthus viridis*. In many parts of the world, *Amaranthus viridis* is consumed as a vegetable or as a boiled green. Nut-filled edible seed clusters that are edible as snacks or added to biscuits can also be found in green amaranth. It's also a great way to replace spinach. Protein content in green amaranth can reach up to 38% by dry weight. An important amino acid called lysine can be found in the leaves and seeds.

These two plants can be assessed for their antimicrobial activity because they both exhibit active ingredients that have been shown to have antibacterial qualities.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Medical technologists, also known as clinical laboratory scientists, use antimicrobial susceptibility testing, or AST, in the lab to determine which antimicrobial regimen is most effective for a given patient. It supports the assessment of medical care provided by clinics, hospitals, and federal initiatives for the larger-scale management and prevention of infectious diseases.

Agar disk diffusion testing: The official method for conducting routine antimicrobial susceptibility testing in many clinical microbial science research centers is agar disk diffusion testing, which was developed in 1940. The Clinical and Laboratory Standards Institute (CLSI) has

released several accepted and approved standards in recent years for the testing of bacteria and yeasts. With particular culture media, different incubation conditions, and interpretive criteria for inhibition zones, the testing of some discerning bacterial pathogens, such as *streptococci, Haemophilus influenzae, Haemophilus parainfluenzae, Neisseria gonorrhoeae*, and *Neisseria meningitidis,* has been standardized.

The agar well diffusion One of the most popular techniques for determining whether plant or microbial extracts have antimicrobial qualities is the agar well diffusion method. The disk diffusion method and this method are comparable. A quantity of microbial inoculum will be applied to the entire surface of the agar plate. Next, a sterile cork bore or tip is used to aseptically punch a hole (6 to 8 mm) into the agar surface. The well is filled with a volume (20–100 µL) of an agar extract solution or an agar antimicrobial agent at the appropriate concentration. After that, the agar plates are incubated in the proper environment for the test microorganisms. The antimicrobial agent diffuses through the agar medium, preventing the test microbial strain from growing.

Agar plug diffusion, cross-streak method, poisoned food method, and others are other types of diffusion methods

In Kerala, the majority of people rely on the widespread use of plants in traditional medicine. Keralans use herbs extensively for medicinal purposes, but very few species have had their biological activity thoroughly studied. For this reason, it is worthwhile to examine Kerala plants' activities in more detail.

The current study is done for the comparative evaluation of antibacterial activities of algae (*Oedogonium sp*.) and plants (*Moringa oleifera* and *Amaranthus viridis*) against *Escherichia coli* and *Staphylococcus saprophyticus* using agar well diffusion method which are used for treatment of some infectious diseases.

CHAPTER 2

SIGNIFICANCE AND OBJECTIVES

SIGNIFICANCE

1. Extracts from plants and algae show great promise as antimicrobial agents against microbes. As a result, they can be applied to treat infectious diseases brought on by microorganisms with resistance.

2. Antibiotics and plant extracts work synergistically to combat resistant bacteria, opening up new treatment options for infectious diseases. When the appropriate antibiotic loses its efficacy on its own during therapeutic treatment, this effect permits the antibiotic to be used.

3. Plant and algal extracts can be used to produce antibiotics which is natural and of less after effects than the chemical antibiotic that give adverse side effects.

4. The extraction of plant compounds from nature bring back the ethnic and traditional ways of treating and curing diseases, pains and wounds, thus recovering olden days.

5. The current study provide a greater scope in industries especially, in pharmaceutical, medical and ayurvedic industries by achieving the property of preparing antibiotics and medicines with the naturally obtaining ingredients.

OBJECTIVES

- **General Objective**

  Comparative evaluation of antibacterial activities of *Oedogonium sp.* (algae), *Moringa oleifera* and *Amaranthus viridis* (higher plants)

- **Specific Objective**

  1. Gathering plants and algae to test two clinical strains of bacteria that frequently cause secondary infections in vitro
  2. To extract crude compound from the above selected algal (*Oedogonium sp*.) and plant (*Moringa oleifera* and *Amaranthus viridis*) samples using distilled water.
  3. To prepare nutrient agar plates for the screening of antibacterial properties of the samples collected.
  4. To inoculate Gram-negative *(Escherichia coli*) and Gram-positive (*Staphylococcus saprophyticus*) bacteria on agar plates.
  5. Utilizing the well diffusion method to ascertain the antibacterial properties of various extracted concentrations of plants and algae against both Gram-positive and Gram-negative bacteria.
  6. Add information and knowledge to the scientific research done on antibacterial activity of algae and higher plants.

CHAPTER 3

REVIEW OF LITERATURE

This chapter presents the review of related literature and studies of the sub – topics of this research; *Oedogonium*, *Moringa oleifera*, *Amaranthus viridis*, antimicrobial activity testing, Well diffusion method.

1. OEDOGONIUM ALGAE

P. Negrete Redondo et al (2006) analyzed the antibacterial activity of *Oedogonium capillare* against pathogenic bacteria in fish. The research demonstrated in vitro the antibacterial activity of an extract from the freshwater green algae *Oedogonium capillare* against 23 distinct bacterial species belonging to the Enterobacteriaceae, Pseudomonadaceae, Aeromonadaceae, and Vibrionaceae families, which are significant aquaculture pathogens. High correlation coefficients were found between the antibiotics used in this investigation and the activity of the algae extract.

M. M. H. Chowdhury et al (2015), conducted the research in which Freshwater and marine algae were screened for their antibacterial and antifungal properties as a common natural antibiotic found in Bangladesh. The purpose of the study was to determine whether Bangladeshi algae could be used as a potent natural antibiotic to combat a range of infections. Using the disc diffusion method, in vitro screening revealed antimicrobial activity of 10 freshwater and marine algae (including *Oedogonium sp*.) organic solvent extracts against 2 Gram-positive, 4 Gram-negative, and 1 fungus.

Neelma Munir et al (2018) evaluated the antioxidant and antimicrobial potential of oil extracts of four different algae. Higher antioxidant activities were observed in methanolic extracts of Oedogonium sp. Additionally, it demonstrated that, at the concentrations employed in the investigation, the antibacterial activity of all ethanolic extracts was greater against Gram-negative bacteria than that of methanolic extracts.

Bruce C. Parker (2019) in his research about 'The Structure and Chemical Composition of Cell walls of Three Clorophycean Algae' presents that the cell wall of *Oedogonium* consist of countless lamellae of parallel microfibrils of cellulose alternating approximately at right angles with each other. These lamellae are encrusted by a spongy substance.

2. LEAFY VEGETABLES

a) MORINGA OLEIFERA

Armando Caceres et al (1991) described the antimicrobial properties of *Moringa oleifera* seeds, bark, roots, and leaves against human-pathogenic bacteria, yeast, dermatophytes, and helminths in vitro. *Pseudomonas aeruginosa* and *Staphylococcus aureus* growth is inhibited by fresh leaf juice and aqueous extracts from the seeds, and this activity is inhibited by extraction temperatures above 56 oC, as shown by a disk-diffusion method.

Anthonia Olufunke Oluduro (2012) stated the antimicrobial properties of *Moringa oleifera* leaf extract on specific bacteria and fungi that cause enteropathogenic and orthopaedic wounds. Using the paper disc diffusion method, the leaf extracts' antimicrobial properties were assessed. According to the study, moringa leaves have inhibitory qualities, making them a good source of supplemental nutrients as well as an alternate form of treatment for wounds and some fungal infections.

Nivedita Patel et al (2014) founded the antibacterial property of *Moringa oleifera*, family Moringaceae. Also, the results of the phytochemical screening revealed that the extracts contained flavonoids, tannins, steroids, alkaloids, saponins, and other substances. The well diffusion method was employed to evaluate the extracts' antibacterial potential against microorganisms. Both the ethanolic and aqueous extracts were effective against every strain; however, the ethanol leaf extract was most effective against the Streptococcus mutant, while the aqueous extract was most effective against Proteus vulgaris.

Abdull Razis et al (2014) described the health benefits of *Moringa oleifera.* It stated that the idea of using *Moringa oleifera* as a nutritional supplement or ingredient in food preparation is supported by the plant's ability to contain essential amino acids, carotenoids in its leaves, and components

with nutraceutical properties. Nearly every part of the moringa plant has nutritional and other benefits to offer.

AMARANTHUS VIRIDIS

B. S. Ashok Kumar et al (2012) investigated the whole plant methanolic extract of *Amaranthus viridis* (MEAV) exhibited antidiabetic, antihyperlipidemic, and antioxidant properties in rats with alloxan-induced diabetes. The study concluded that MEAV posses antidiabetic, antihyperlipidemic and antioxidant activities.

Muhammad Javid Iqbal et al (2012) described the Antioxidant and antimicrobial activities of Chowlai (*Amaranthus viridis* L.) leaf and seed extracts. The study evaluated the phenolics, antioxidant, and antimicrobial properties of extracts obtained from the leaves and seeds of *Amaranthus viridis* L., an edible herb. The extracts from the seeds showed better antimicrobial and antioxidant properties. The study concluded that *A. viridis* leaf and seed could be investigated as a possible source for the isolation of antimicrobial and antioxidant agents for use in pharmaceuticals and functional foods.

Rama Koyyati et al (2014) stated the antibacterial activity of silver nanoparticles synthesized using *Amaranthus viridis* twig extract. The outcomes demonstrated protein and nucleic acid leakage into LB media, confirming the damage to the treated bacterial cells. These nanoparticles are useful for the formulation of novel bactericidal materials because they are easy, quick, and affordable to make.

Md. Reyad-ul-Ferdous et al (2015) presented the biological status of *Amaranthus viridis*, a potentially medicinal plant. According to the study, this plant contains a chemical component with strong antiviral, anti-inflammatory, antihepatotoxic, and anti-ulcer properties.

Svetoslava Terzieva et al (2019) described the antimicrobial activity of *Amaranthus spp*. extracts against some mycotoxigenic fungi. Their primary goal was to evaluate the antifungal properties of various extracts derived from the three species of Amaranthus L. (*A. deflexus* L., *A. retroflexus* L., and A. hybridus L.). Plant extracts in the wells were surrounded by zones of microbial growth inhibition, which were used to measure the antimicrobial activity. The best extracts were *A. deflexus* and *A. hybriddus* ethanol flower extract, *A. retroflexus* ethanol root extract, and *A.*

retroflexus methanol leaves and stem extract. These extracts demonstrated activity against all tested strains of microorganisms.

3. ANTIMICROBIAL TESTING

R. Valsaraj et al (1997) described the antimicrobial screening of selected medicinal plants from India. 78 plants were chosen from the Indian traditional medicines based on their efficacy in treating infectious diseases. Using the agar-well diffusion method, different concentrations of 80% ethanol extracts were tested against two fungi, *Aspergillus niger* and *Candida albicans*, and four bacteria, *Bacillus subtilis, Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*, using the agar dilution method.

Zaidan et al (2005) stated the In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. The study demonstrated the existence of antibacterial activity in the crude extracts of *Andrographis paniculata, Vitex negundo, Morinda citrifolia, Piper sarmentosum*, and *Centella asiatica*—some of the frequently used medicinal plants in Malaysia. With the exception of *A. paniculata* and *P. sarmentosum*, which demonstrated activity against *P. aeruginosa*, they concluded that none of the five plant extracts tested exhibited antibacterial activities against gram-negative *E. coli* and *K. pneumoniae*.

Jigna Parekh and Sumitra Chanda (2007) evaluated the ability of a few chosen Indian medicinal plants to inhibit the growth of certain bacterial strains, including *Klebsiella pneumoniae, Bacillus cereus, Staphylococcus aureus, Enterobacter aerogenes*, and *Escherichia coli*. Methanol and water were the solvents employed in the plant extraction process. The agar disc diffusion and agar well diffusion method were used to measure the in vitro antibacterial activity. They employed *Caesalpinia pulcherrima* as a plant. According to the results, *Caesalpinia pulcherrima* may be used to treat illnesses brought on by the test organisms.

N. Gandhiraja et al (2009) described the Phytochemical Screening and Antimicrobial Activity of the Plant Extracts of *Mimosa pudica* L. Against Selected Microbes. Using the well diffusion method, the antimicrobial activity of Mimosa was investigated. The activity was evaluated at varying concentrations of 50, 100, and 200µg/disc against *Aspergillus fumigatus, Citrobacter*

divergens, and *Klebsiella pneumonia*. The outcomes are presented in an illustrative manner. Using phytochemical analysis, the study identified the active phytocomponents of *Mimosa pudica.*

Jehan Bakht et al (2011) stated the *Eclipta alba*'s antimicrobial potential using the well diffusion method. The well diffusion assay was used to screen nine different microbial species for susceptibility to an antimicrobial extract from *Eclipta alba*. There were three different volumes tested: 24, 30, and 36 µl/well. All of the *Eclipta alba* extracts exhibited antimicrobial activity, according to the data analysis. The findings indicated that Bacillus cereus was the most resistant Gram-positive bacterium, while *Bacillus subtilis* was the most susceptible, as it was inhibited by all six extracts from *Eclipta alba*. Of the Gram-negative bacteria, Escherichia coli and *Salmonella typhi* were the most resistant, while *Erwinia carotovora* was the most susceptible.

CHAPTER 4

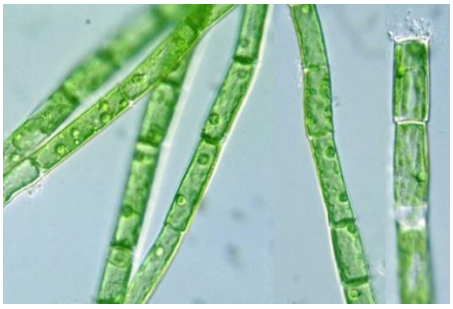
MATERIALS AND METHODS

This study is aimed at investigating the antibacterial activity of extracts from selected 2 medicinal angiosperm plants and one algal species against 2 common bacterial pathogens. Given the variety of approaches used in the literature, choosing strategies and methods for examining the in vitro antibacterial activity of plants can be difficult. The selection of the research methods was guided by the distinct needs of the chosen bacteria as well as the customary applications of the medicinal plants.

MATERIALS USED

1. ALGAL SPECIMEN

1.1. OEDOGONIUM SP.



Systematic Position:

Division : Chlorophyta

Class : Chlorophyceae

Order : Oedogoniales

Family : Oedogoniaceae

Genus : *Oedogonium*

Green algae known as *Oedogonium* are free-living and were initially found in Poland's freshwater by W. Hilse in 1860. This type of algae lives in freshwater environments and is both planktonic and benthic. It reproduces both sexually and asexually. *Oedogonium* is frequently found in peaceful freshwater bodies. They frequently float freely or are affixed to other plants. Typically, *Oedogonium* filaments have a single cell thickness and are unbranched. Under a microscope, *Oedogonium* cells have a narrow, cylindrical shape. With the exception of the topmost (apical) and bottommost (holdfast) cells, every single filament cell is identical. The apical cell has a cap and is rounded at the tip, despite its width. The elongated growth on both unattached sides of the holdfast cell aids in the filament's tight grip on the substrate. Furthermore, the holdfast is the filament's only colorless cell. How many times a cell has divided is indicated by the number of caps on the cells in a filament. Within the central vacuole are inorganic compounds, secretions, and excretions that make up the cell sap. The big, oval-shaped nucleus is located in the middle of the cell.

2. PLANT SPECIMEN

2.1. MORINGA OLEIFERA

Systematic Position:

Division: Magnoliophyta

Class: Magnoliopsidsa

Order: Capparales

Family: Moringaceae

Genus: *Moringa*

Species: *Moringa oleifera* Lam.

The "tree of life" or "miracle tree," *Moringa oleifera*, is regarded as an important herbal plant because of its many health benefits, both medicinal and non-medical. Thirteen species make up the Moringa family; *M. oleifera*, which is native to India, is widely recognized for its applications in biogas production, fertilizer, and other applications. *Moringa oleifera* grows in tropical and subtropical climates worldwide. It is also referred to as the "horseradish tree" or the "drumstick tree." The plant has historically been used to treat inflammation, cancer, heart disease, liver disease, wounds, and pain. Within 6 to 8 weeks of planting, moringa leaves are ready for harvesting; however, once harvested, they quickly regenerate, making further harvesting possible within the same time frame. A single moringa plant can be harvested year after year because these trees are also perennial. Because of their high nutrient content, moringa leaves have been used for centuries as a valuable source of nourishment. By consuming the powdered leaves of *Moringa oleiefera,* people all over the world can take advantage of its many health benefits.

2.2. AMARANTHUS VIRIDIS

Systematic Position:

Division: Magnoliophyta

Class: Magnoliopsida

Order: Caryophyllales

Family: Amaranthaceae

Genus*: Amaranthus*

Species*: Amaranthus viridis* L.

Green amaranth or slender amaranth are common names for *Amaranthus viridis*. *Amaranthus viridis* is an annual herb that reaches a height of 60 to 80 cm on an upright, light green stem. The plant produces small green flowers with three stamens and terminal panicles with few branches. In many parts of the world, *Amaranthus viridis* is consumed as a vegetable or as a boiled green. Nut-filled edible seed clusters that are edible as snacks or added to biscuits can also be found in green amaranth. The seeds can be boiled in water to make a porridge. It's also a great way to replace spinach. Under the Sanskrit name Tanduliya, *Amaranthus viridis* is used as a medicinal herb in traditional Ayurvedic medicine. Protein content in green amaranth can reach up to 38% by dry weight. An important amino acid called lysine can be found in the leaves and seeds.

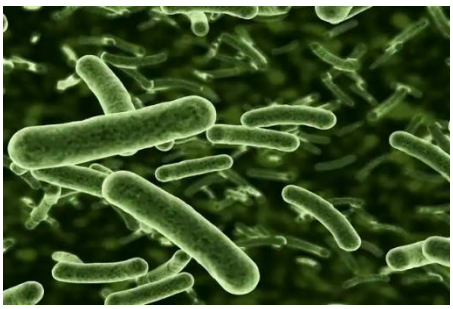
Table 1. Specimens collected for testing antibacterial activity

SI No.	Specimen collected (scientific name)	Common Name	Family	Plant part used	Country of Origin	Traditional Claims
1.	*Oedogonium sp.*	Green algae	Oedogoniaceae	Thallus	Poland	Eaten as food, used as good fertilizer
2.	*Moringa oleifera*	Drumstick tree	Moringaceae	Leaves	India	To treat paralysis, helminthiasis, sores and skin infections, used in Ayurvedic medicines.
3.	*Amaranthus viridis*	Slender amaranth	Amaranthaceae	Leaves	East Asia	Used for ulcers, diarrhea, swelling of the mouth or throat and high cholesterol

3. TEST ORGANISMS

The following organisms were used in testing the antimicrobial activity of the plant materials.

3.1. ESCHERICHIA COLI



Systematic Position:

Phylum: Proteobacteria

Class: Gamma Proteobacteria

Order: Enterobacteriales

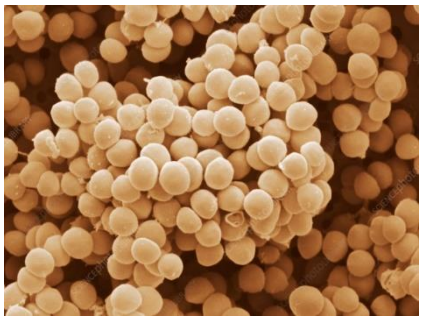
Family: Enterobacteriaceae

Genus: *Escherichia*

Species: *Escherichia coli*

Enterobacter spp. (*E. coli*) gram-negative bacteria are typically found in the intestines of animals and healthy humans. The majority of *E. coli* strains are benign or only temporarily induce diarrhea. However, some strains, like *E. coli* O157:H7, can result in vomiting, violent stomach cramps, and bloody diarrhea. The most frequent pathogen causing simple cystitis is *Escherichia coli*, which is also responsible for pneumonia, bacteremia, and infections of the abdomen such as spontaneous bacterial peritonitis. *E. coli* illnesses place a heavy burden on patients and the healthcare system, so early detection and effective treatment are essential.

3.2.STAPHYLOCOCCUS SAPROPHYTICUS



Systematic position:

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: Staphylococcaceae

Genus: *Staphylococcus*

Species: *Staphylococcus saprophyticus*

Uncomplicated urinary tract infections (UTIs) are frequently caused by the Gram-positive, coagulase-negative, non-hemolytic bacterium *Staphylococcus saprophyticus*. This is especially true for young, sexually active females. Complications such as acute pyelonephritis, urethritis, epididymitis, and prostatitis are less frequently caused by it. It is well known that *Staphylococcus saprophyticu*s, a spherical, novobiocin-resistant, catalase-positive, urease-positive bacterium, is a typical component of the perineum's flora. It has been linked to the development of struvite urinary stones and urinary tract infections. In contrast to *S. aureus, S. saprophyticus* is coagulase-negative, meaning it is devoid of the coagulase enzyme. Its resistance to Novobiocin allows it to be distinguished from other coagulase-negative staphylococci.

Table 2. Bacterial strains used in the antibacterial activity

SI No.	Bacterial Strain	Type	Diseases caused
1.	*Escherichia coli*	Gram-negative	Urinary tract infection, abdominal and pelvic infection, pneumonia, bacteremia, meningitis
2.	*Staphylococcus saprophyticus*	Gram-positive	Acute pyelonephritis, epididymitis, prostatitis, urethritis, urinary tract infections

4. MEDIUM USED

NUTRIENT AGAR MEDIUM

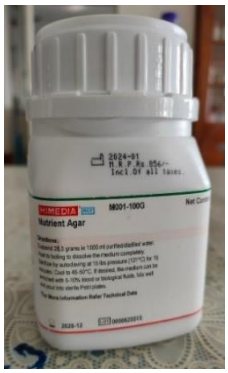


Figure 1 Nutrient agar

Nutrient agar is a versatile substance that facilitates the proliferation of various non-fibrous organisms. This medium is highly favored due to its ability to provide essential nutrients for bacterial growth, as well as support the development of diverse fungi and bacteria. The composition of nutrient agar consists of peptone, beef extract, and agar, forming a simple yet effective recipe that fulfills the nutritional needs of numerous less demanding microorganisms.

COMPOSITION OF NUTRIENT AGAR:

- Peptone (0.5%) - Peptone is an enzymatic protein digest. The main organic nitrogen source for the bacterial growth is peptone.
- beef extract/yeast extract (0.3%) - Salts, vitamins, carbohydrates, and organic nitrogen compounds are among the water-soluble materials that promote bacterial growth.
- Agar (5%) - This is the agent used to solidify.
- NaCl (0.5%) - Sodium chloride, when added to nutrient agar, keeps the medium's salt concentration comparable to that of the microorganisms' cytoplasm.
- Distilled water- Water serves as a medium for the transportation of different nutrients and is necessary for the development and reproduction of microorganisms.
- At 25^0C, the pH is adjusted to neutral 7.4

METHODOLOGY

1. Collection of sample plants

Collection of alga specimen:

The selected algae, *Oedogonium sp.* were collected from nearby fresh water source. The algae were collected with a long fork and spooled out from the water resource. The healthiest and purest algae were chosen, given that dead leaves and organic matter from various sources accumulate on the water's surface. They were meticulously washed under a continuous stream of clean water, ensuring the removal of any visible contaminants. The algae is then kept in freshwater and separated the clumps formed. As the algae resembles threads, it must be loosened to observe under the microscope. The substance was subsequently examined under a microscope to assess its purity. The algae were once more loosened using forks and transferred into individual large trays.

Collection of plant specimens:

The selected plant specimens – *Moringa oleifera* and *Amaranthus viridis*, were collected from the nearby green vegetation. It is easily available from the neighbouring areas as these plants are cosmopolitan in distribution. The plants were plucked with bare hands and stored in a plastic bag to retain its moisture and freshness as it is plucked in early morning. The plants were then washed under running water and dissected the part needed – leaves. The leaves were again washed and weighed to analyze fresh weight. The weighed leaves were then stored it trays for further processing.

Figure 3- ***Amaranthus viridis***

Figure 2 - Oedogonium

2. Identification and documentation of collected samples

The collected specimens were then identified. The algae were examined under the microscope to analyze the structure and morphology and recognized as *Oedogonium sp.* The plants collected were identified according to the description given on different books and sites. It is then recognized with the help of guide and referring other taxonomic literatures. The plants thus were identified – *Moringa oleifera* and *Amaranthus viridis*.

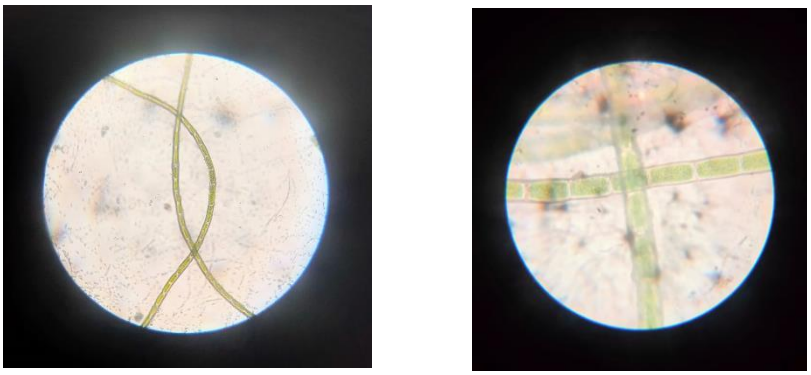


Figure 4 - Microscopic view of Oedogonium

3. Extraction of collected specimens

The collected algae (*Oedogonium sp*.) underwent a thorough washing process to eliminate any soil and foreign materials, including other plant parts, grasses, herbs, or any undesirable substances. After thoroughly washed, the algae was uniformly spread out into absorbing paper (tissue paper or filter paper) to drain out the water from the algal sample. It is then kept undisturbed and again evenly distributed on the absorbing sheet to retain the remaining water content. The solvent used for the extraction is distilled water. Extraction of the algae were done by grinding the dried algae of specific weight with appropriate amount of distilled water to get the desired concentration.

The leaves of the plant samples (*Moringa oleifera* and *Amaranthus viridis*) were carefully cleaned to eliminate any soil and foreign materials, such as other plant parts, grasses, herbs, or any undesired substances. Subsequently, the leaves were finely chopped into small pieces. The

chopped leaves were then weighed for the extraction. After taking the weights, the leaves of known weight were grinded with specific amount of distilled water to get the desired concentrations. The grinded specimens (both algal and plant) of known concentrations were then filtered using Whatman No.1 filter paper into 200ml conical flasks for each concentration. The extracts were then stored in refrigerator (2-8^{0}C) until use.

Figure 5 - Extraction of Samples Collected

Different concentrations of both algal and plant extracts have been prepared with distilled water as solvent. *Oedogonium* were extracted into 4 concentrations and the two plants were extracted into 2 concentrations each. Dilution of the pure extracts taken from the specimens determined the concentration. These solutions after sealed properly were stored in refrigerator for further use.

Table 3. Extracted concentrations of the samples collected for antibacterial activity

SI No.	Sample collected	Fresh weigh taken for extraction (mg)	Amount of distilled Water added (ml)	Concentration of the Extract (mg/ml)
1.	*Oedogonium sp.*	25 mg	100 ml	0.25 mg/ml
		25 mg	50 ml	0.5 mg/ml
		5 mg	5 ml	1 mg/ml
		5 mg	1 ml	5 mg/ml
2.	*Moringa oleifera*	10 mg	20 ml	0.5 mg/ml

		10 mg	10 ml	1 mg/ml
3.	*Amaranthus viridis*	10 mg	20 ml	0.5 mg/ml
		10 mg	10 ml	1 mg/ml

4. Collection of test organisms

Figure 6 - Bacterial strains collected

The standard cultures of two pathogenic bacterial strains such as one Gram-negative and one Gram-positive were collected from different labs.

The two bacterial strains used for the antibacterial study were:

- *Escherichia coli* – Gram-negative bacteria
- *Staphylococcus saprophyticus* – Gram-positive bacteria

These bacterial strains were collected in liquid culture forms inorder to obtain perfect cultures in petri-plates during the antibacterial screening of the collected samples. Pure cultures of *E. coli* were obtained by inoculating a full loop of the collected culture on agar plates.

5. Sterilization of glassware and medium

Sterilization of lab glassware and media is the first and most important step in performing a microbiological analysis. To get rid of or neutralize any bacteria or other microbes that might be in the glasses or other equipment, this procedure is crucial. Using a hot air oven, the glassware—which included conical flasks, test tubes, measuring cylinders, petri plates, and glass rods—was sterilized. The most widely used sterilization technique is this one, which uses dry heat for two to four hours at temperatures between 160°C and 170°C.

- The glassware for use were properly washed in running water using suitable detergent to clean it properly. It is then air dried in room temperature.
- The dried glassware were then sealed properly with aluminium foil or with paper for sterilization.

- The objects were then organized into the hot air oven in such a way that there is s\air supply.
- The temperature was reduced to 40°C before removing the sterilized objects.
- The glassware were then kept on sterile surface to cool down.

For the sterilization of agar medium, autoclave is used. Autoclaves, also referred to as steam sterilizers, are commonly utilized in healthcare or industrial settings. These machines utilize steam under pressure to eliminate harmful bacteria, viruses, fungi, and spores present on objects enclosed within a pressure vessel. The objects undergo heating to an appropriate sterilization temperature for a specific duration. The moisture in the steam effectively transfers heat to the objects, disrupting the protein structure of bacteria and spores. The medium is subjected to autoclaving at 121°C, 15 pounds for 20 minutes. Following autoclaving, the medium is sterilized and devoid of any microorganisms.

Figure 7 - Sterilization and Autoclaving of Medium and Glassware

6. Preparation of the medium

The medium used for testing the antibacterial property of the samples collected were Nutrient Agar Medium. Nutrient agar is widely used due to its ability to promote the growth of a wide range of bacteria and fungi, providing essential nutrients required for their development.. About 500ml of the agar medium were prepared by the following steps:

- Dissolve 28 grams of nutrient agar powder in 1 liter of distilled water in conical flasks.

- Continuously stir the mixture while heating it until all components are fully dissolved.

- Autoclave the heated mixture at a temperature of 121 degrees Celsius for a duration of 15 minutes to sterilize it and obtain a completely pure agar medium.

- After autoclaving the medium, the nutrient agar is allowed to cool down but not solidified.

- After sometimes, the cooled medium is poured in to each sterilized plates (about 10ml on each plates) and kept them on the sterile surface to solidify the agar.

- The solidified agar plates are then stored in refrigerator for further use.

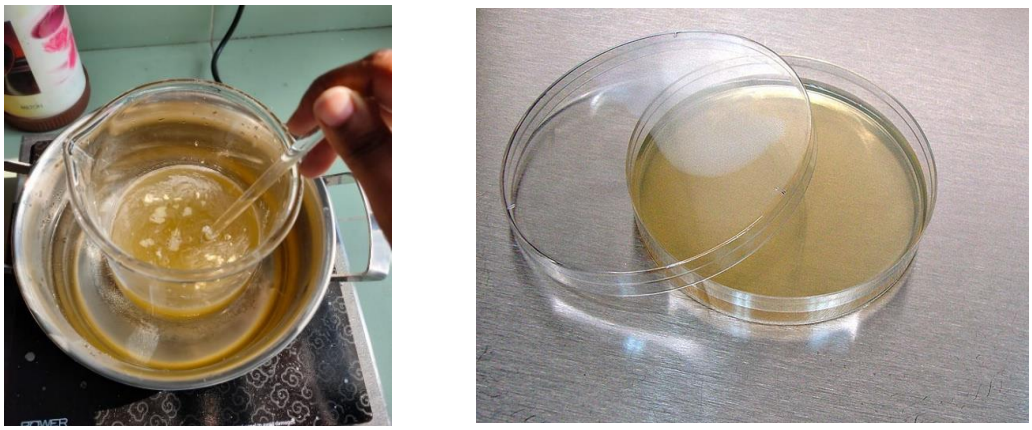


Figure 8 - Preparation of Nutrient Agar Plates

7. Screening and evaluation of antibacterial activity

The study utilized the Agar well diffusion method to screen and assess the antibacterial activity of the prepared algal and plant extracts. The diameter of the zone of inhibition (ZOI) caused by the extracts on the two bacterial strains collected was measured to determine the antibacterial effectiveness of each extract.

The antibacterial activity of the extracts of *Oedogonium sp., Moringa oleifera* and *Amaranthus viridis* were screened against the test organisms – *Escherichia coli* and *Staphylococcus saprophyticus* by Agar well diffusion method.

Nutrient Agar plates with a thickness of around 4 mm were sterilized and prepared. Prior to utilization, the plates underwent a drying process using hot air at the suitable temperature to eliminate any excess moisture from the media surface.

Next, sterile cotton swabs were extracted and immersed into the bacterial inoculum of *Escherichia coli.* The surplus of inoculum was eliminated by applying pressure and rotating against the inner upper wall of the test tube, above the liquid level. Subsequently, the swabs were meticulously swiped across the entire surface of the plates. After each swabbing, the plate was rotated at a 90^0 angle. Lastly, the swab was moved along the edges of the agar surface. The plates containing the inoculated samples were then allowed to air dry for a few minutes at room temperature, with the lid securely closed. *E. coli* was inoculated in about 9 agar plates for the proper triplicate testing of the three samples collected. Similarly bacterial inoculum of *Staphylococcus saprophyticus* were also surface swabbed in 9 agar plates for the antibacterial testing.

- Antibacterial activity of Oedogonium algae:

For *Oedogonium sp*. four concentrations of the algal extracts were prepared – 0.25 mg/ml, 0.5 mg/ml, 1 mg/ml and 5 mg/ml.

Three wells were made in each plate with the help of sterile cork borer no.8 in three agar plates inoculated with *Escherichia coli*. So, the diameter of a well was 8 mm. For the current study two concentrations of the algal extract were added to one agar plates for easy comparison of the zone formed on the plates. Therefore 100 µl of two concentrations of the *Oedogonium* extract (0.25 mg/ml and 0.5 mg/ml) and distilled water as control were added in each well with the help of micropippete. This were then repeated in the other two agar plates *of E. coli* for triplicate testing.

Similarly, 100 µl of the other two concentrations of the *Oedogonium* extract (1 mg/ml and 5 mg/ml) and distilled water as control were added to the other three agar plates inoculated with *Escherichia coli*.

The four extracts of *Oedogonium* of concentrations 0.25, 0.5, 1, 5 (mg/ml) were similarly poured into 6 agar plates, but with the plates inoculated with *Staphylococcus saprophyticus*.

This is done to examine the antibacterial property of *Oedogonium* against both the Gram-negative and Gram-positive bacteria at different concentrations. The plates were then left for half an hour with the lid closed so that the extract diffused into media. Finally, the plates were incubated overnight at 37^0C.

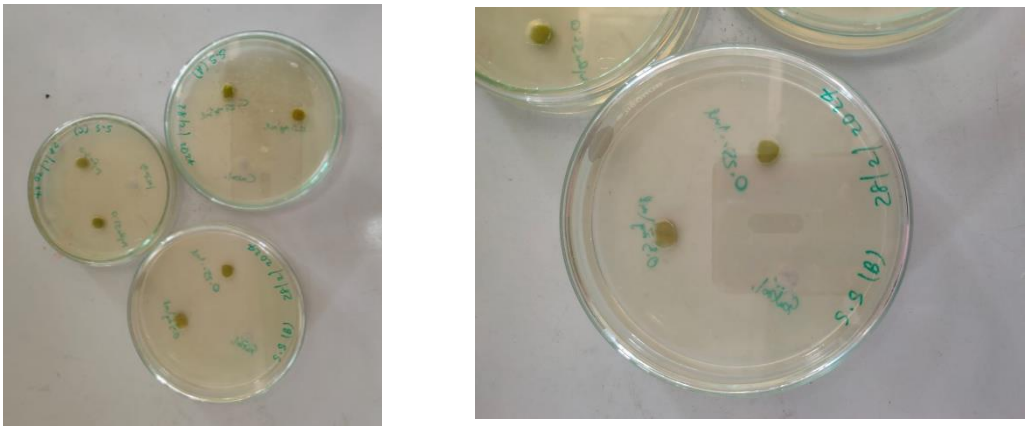


Figure 9 - Agar plates with Oedogonium extract in wells

- Antibacterial activity of plant extracts:

For the plant extracts, *Moringa oleifera* and *Amaranthus viridis,* two concentrations were prepared for each plant – 0.5 mg/ml and 1 mg/ml.

Inoculated with *Escherichia coli,* two agar plates were used to create three wells each using a sterile cork borer no.8.So, the diameter of a well was 8 mm. In this case, same concentrations of the two plant extract were added to one agar plates for easy comparison of the zone formed by the two plant extracts on the plates at same concentration. Therefore 100 µl of the same concentration (0.5 mg/ml) of both the Moringa and Amaranthus extracts and distilled Water was added as a control to each well using a micropipette. This process was then repeated on another agar plate of *E. coli* for duplicate testing.

Similarly, 100 µl of the other concentration (1 mg/ml) of both the plant extracts and distilled water as control were added to the other two agar plates inoculated with *Escherichia coli*.

The two extracts of *Moringa oleifera* and *Amaranthus viridis* of concentration 0.5 mg/ml and 1 mg/ml were similarly poured into 4 agar plates, but with the plates inoculated with *Staphylococcus saprophyticus*.

This is done to examine the antibacterial property of both Moringa and Amaranthus against both the Gram-negative and Gram-positive bacteria at different concentrations. The plates were subsequently left for 30 minutes with the lid sealed, allowing the extract to permeate the media. After that, the plates were incubated at a temperature of 37°C overnight.

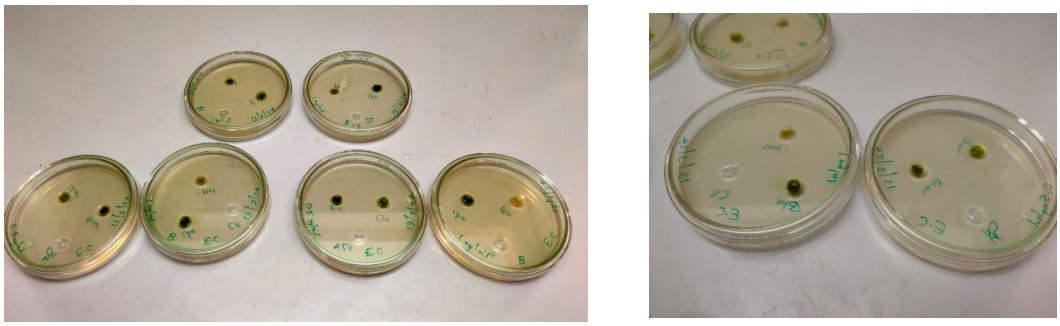


Figure 10 - Agar plates with Plant extracts in wells

After a suitable incubation period of 18-24 hours, the plates were examined to determine if there was any inhibition of bacterial growth. This inhibition was indicated by the presence of a clear zone around the wells. The size of these zones was measured, and the antibacterial activity was quantified by calculating the average diameter of the zone of inhibition in centimeters. If no zone of inhibition was observed, it was interpreted as the absence of antibacterial activity. In the case where a zone of inhibition was present, the assay was performed three times for algal specimens and twice for plant specimens. The zones of inhibition were measured using a scale, and the mean value was recorded.

CHAPTER 5

RESULTS AND DISCUSSION

The antimicrobial properties of the chosen plant extracts in this research were tested for potential antibacterial effects using the agar well diffusion method. If a zone of inhibition was observed, three measurements of the diameter of the zone were obtained and the average was calculated.The results obtained in the evaluation of the antimicrobial activity of extracts from *Oedogonium sp., Moringa oleifera* and *Staphylococcus viridis* against *Escherichia coli* and *Staphylococcus saprophyticus* are list in the tables below.

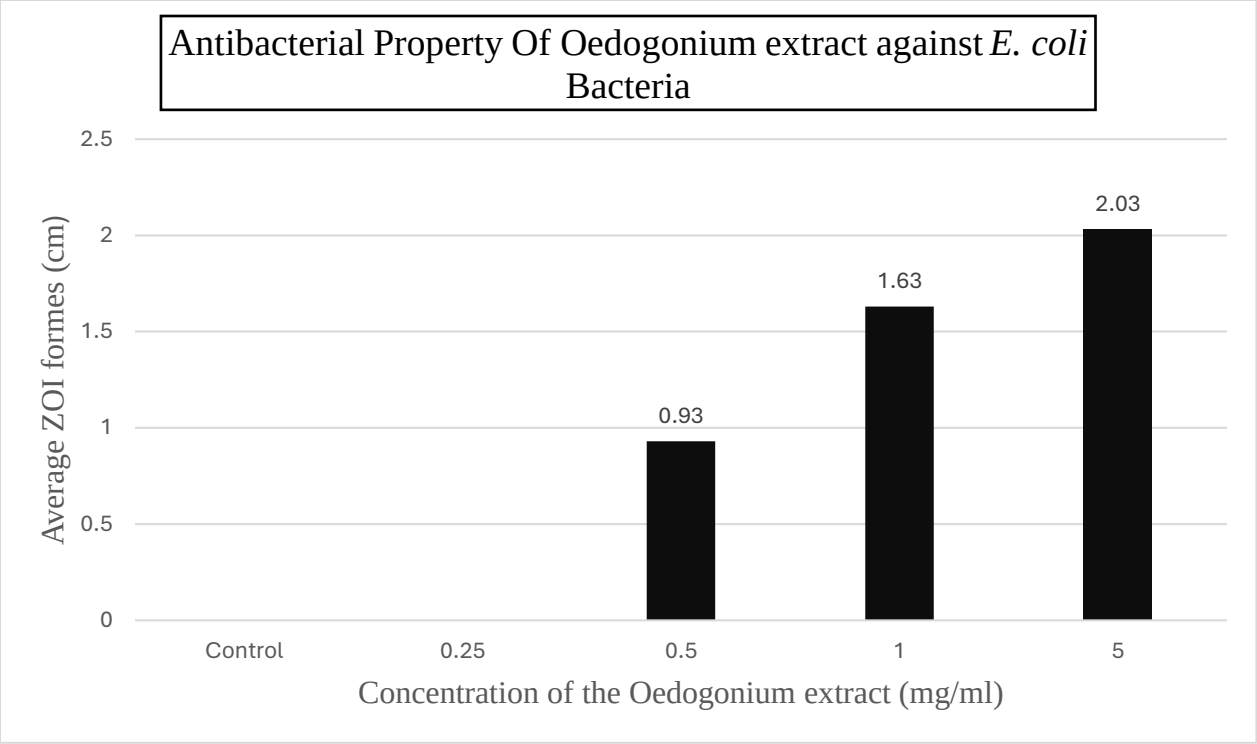
1. Evaluation of antibacterial activity of Oedogonium sp.

a. Antibacterial activity of *Oedogonium* extract against *Escherichia coli*:

Table 4. Antibacterial activity of algal extracts on E. coli

SI No.	Concentration of the algal extract (mg/ml)	Inhibition zone diameter (ZOI) (cm)			Average (cm)
		E.C 1	E.C 2	E.C 3	
1.	Control (distilled water)	-	-	-	-
2.	0.25 mg/ml	-	-	-	-
3.	0.5 mg/ml	0.9 cm	1 cm	0.9 cm	0.93 cm
4.	1 mg/ml	1.5 cm	1.6 cm	1.8 cm	1.63 cm
5.	5 mg/ml	2 cm	1.9 cm	2.2 cm	2.03 cm

Note: Absence of zone of inhibition was denoted as negative (-)



Graph 1 - Antibacterial Property Of Oedogonium extract against *E. coli* Bacteria

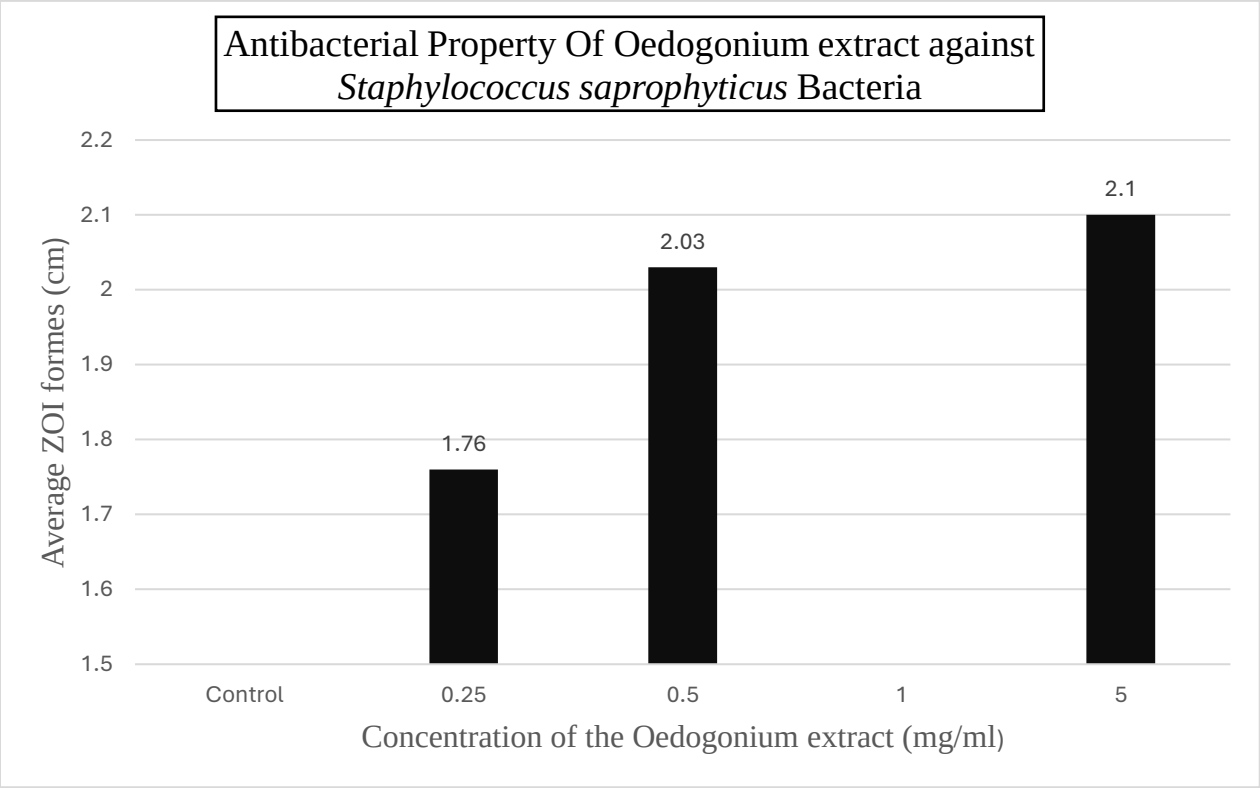
Extracts of *Oedogonium* was effective against *Escherichia coli* as it showed zone of inhibition against the bacteria. At the lowest concentration (0.25 mg/ml), the extract shows no inhibition of the bacteria. But when the concentration was increased gradually, it showed results of inhibition against *E. coli*. That means the zone of inhibition of *E. coli* is directly proportional to the concentration of *Oedogonium* extract. Highest ZOI was observed at the highest concentration (5 mg/ml) of the extract by an average of 2.03 cm. This results that the algae, *Oedogonium,* shows antibacterial property against *E. coli* bacteria, and the property increases with increasing concentration of the algal extract.

b. Antibacterial activity of *Oedogonium sp*. against *Staphylococcus saprophyticus*:

Table 5. Antibacterial activity of algal extracts on *Staphylococcus saprophyticus*

SI No.	Concentration of the algal extract (mg/ml)	Inhibition zone diameter (ZOI) (cm)			Average (cm)
		S.S 1	S.S 2	S.S 3	
1.	Control (distilled water)	-	-	-	-
2.	0.25 mg/ml	2 cm	1.8 cm	1.5 cm	1.76 cm
3.	0.5 mg/ml	1.9 cm	2.2 cm	2 cm	2.03 cm
4.	1 mg/ml	-	-	-	-
5.	5 mg/ml	2.3 cm	2 cm	2 cm	2.1 cm

Note: Absence of zone of inhibition was denoted as negative (-)



Graph 2 - Antibacterial Property of Oedogonium extract against *Staphylococcus saprophyticus* Bacteria

Extracts of *Oedogonium* was effective against *Staphylococcus saprophyticus* as it showed zone of inhibition against the bacteria. In this case, the lowest concentration (0.25 mg/ml) of the extract also showed inhibition of the bacteria by an average of 1.76 cm. When the concentration was increased gradually, it showed results of inhibition against *Staphylococcus saprophyticus*. But it showed inhibition only at 0.25, 0.5 and 5 (mg/ml) concentrations. There is no zone of inhibition observed at 1 mg/ml concentration. That means the zone of inhibition of *E. coli* is not directly proportional to the concentration of O*edogonium* extract. Highest ZOI was observed at the highest concentration (5 mg/ml) of the extract like against *E. coli* by an average of 2.1 cm which is higher than that against *E. coli* at the same concentration. This results that the algae, *Oedogonium*, shows antibacterial property against *Staphylococcus saprophyticus* bacteria only at specific concentrations of the algal extract.

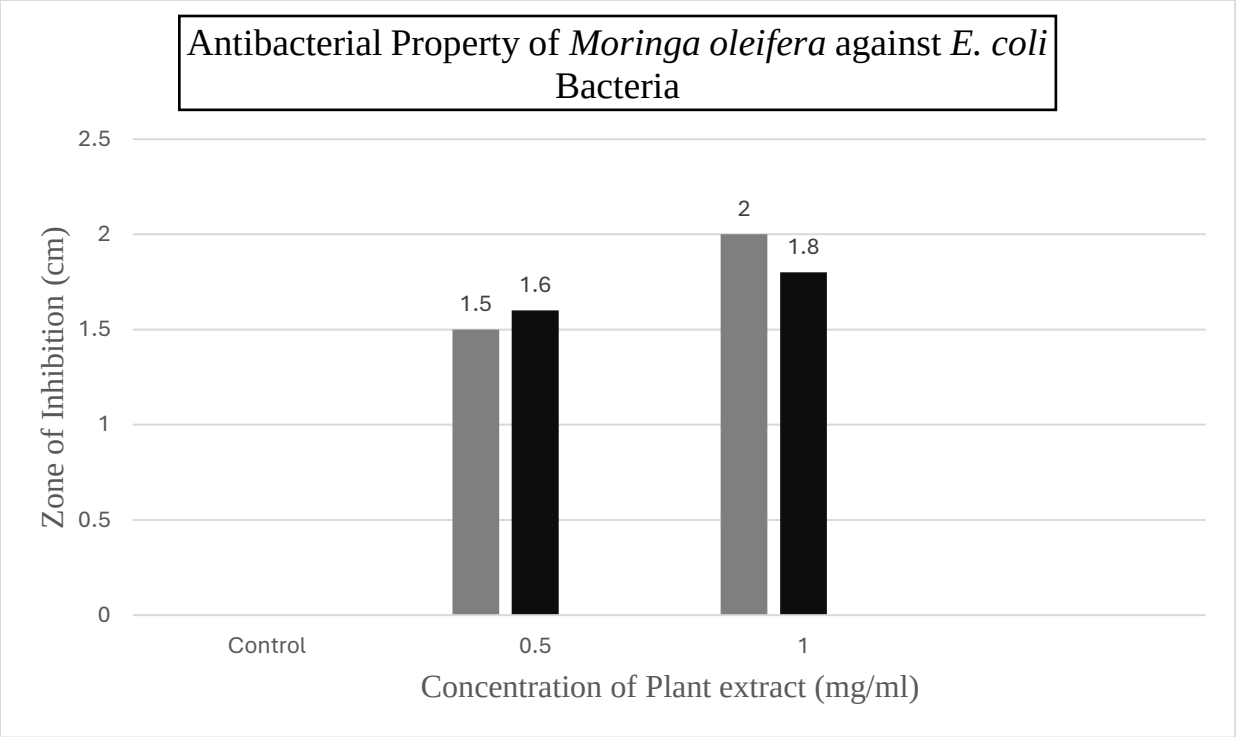
2. Evaluation of antibacterial activity of *Moringa oleifera*

Evaluation of antibacterial activity of *Moringa oleifera* extract against *Escherichia coli* and *Staphylococcus saprophyticus* is given below:

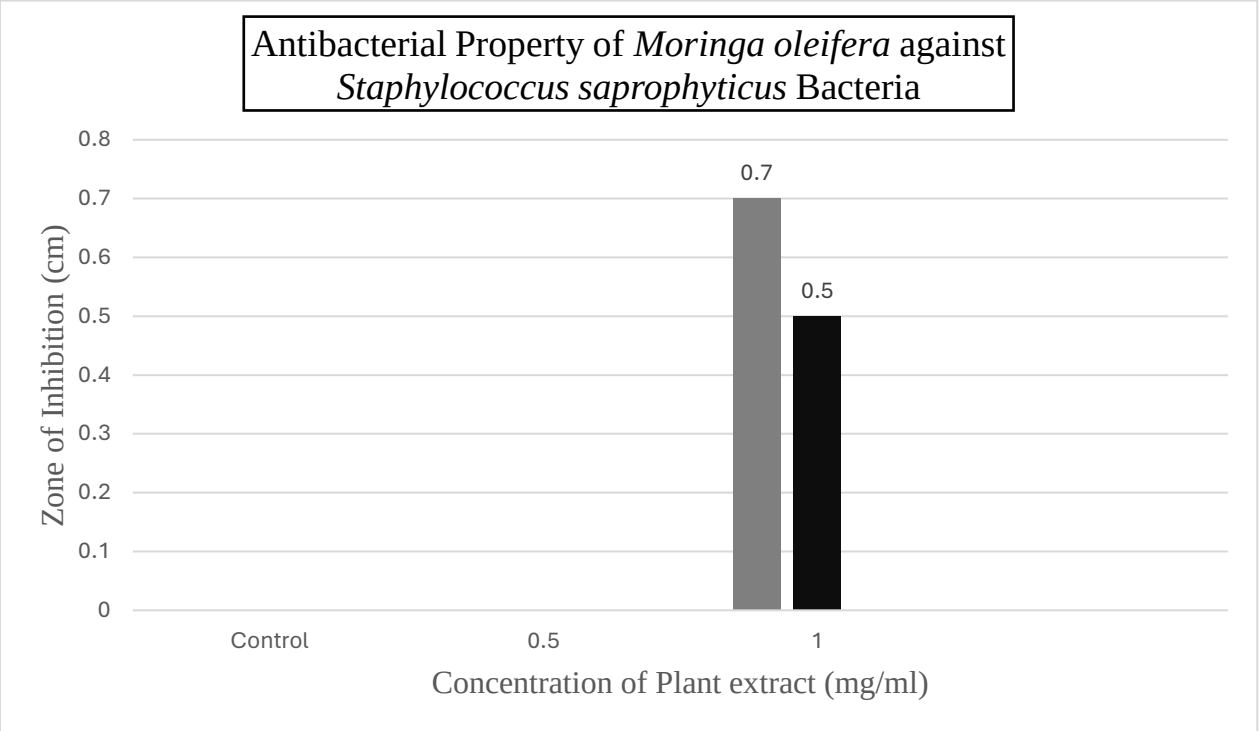
Table 6. Antibacterial activity of *Moringa sp.* against *E. coli* and *Staphylococcus*

Sl No.	Test organism	Zone of Inhibition at concentration (cm)					
		Control (water)		0.5 mg/ml		1 mg/ml	
1.	*Escherichia coli*	-	-	1.5 cm	1.6 cm	2 cm	1.8 cm
2.	*Staphylococcus saprophyticus*	-	-	-	-	0.7 cm	0.5 cm

Note: Absence of zone of inhibition was denoted as negative (-)



Graph 3 - Antibacterial Property of *Moringa oleifera* against *E. coli* Bacteria



Graph 4 - Antibacterial Property of *Moringa oleifera* against *Staphylococcus saprophyticus* Bacteria

Extracts of *Moringa oleifera* was effective against both *Escherichia coli* and *Staphylococcus saprophyticus* as it showed zone of inhibition against these bacteria. In *E. coli* bacteria both the concentrations (1.5 mg/ml and 1 mg/ml) showed inhibition. But the antibacterial property of the plant extract against *Staphylococcus saprophyticus* showed inhibition only at 1 mg/ml concentration.

In the case of *E. coli* bacteria, at 0.5 mg/ml concentration of the plant extract, the ZOI was observed by an average of 1.55 cm. when the concentration was doubled (1 mg/ml), the ZOI was seen by an average of 1.9 cm. When the concentration was increased gradually, it showed results of inhibition against E. *coli*. That means the zone of inhibition of *E. coli* is directly proportional to the concentration of Moringa extract by an increase of 1/4. This results that *Moringa oleifera* shows antibacterial property against *E. coli* bacteria at any concentrations of the plant extract.

In the case of *Staphylococcus saprophyticus* bacteria, at 0.5 mg/ml concentration of the plant extract, no zone of inhibition was observed. when the concentration was doubled (1 mg/ml), the ZOI was seen by an average of 0.6 cm. When the concentration was increased gradually, it showed slight inhibition against Staphylococcus bacteria. That means the zone of inhibition of Staphylococcus is not directly proportional to the concentration of Moringa extract. This results that *Moringa oleifera* shows antibacterial property against *Staphylococcus saprophyticus* bacteria only at high concentrations of the plant extract.

3. Evaluation of antibacterial activity of *Amaranthus viridis*

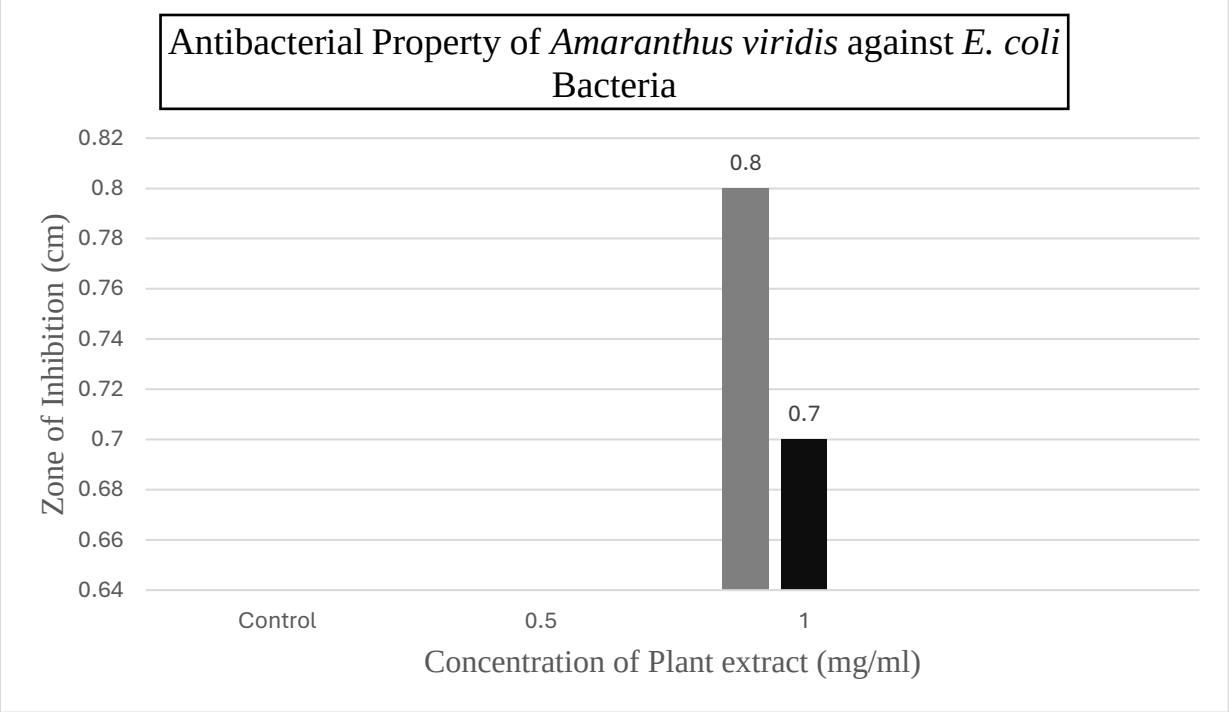
Evaluation of antibacterial activity of *Amaranthus viridis* extract against *Escherichia coli* and *Staphylococcus saprophyticus* is given below:

Extracts of *Amaranthus viridis* was less effective against both *Escherichia coli* and *Staphylococcus saprophyticus* as it does not showed zone of inhibition against these bacteria visibly. In both *E. coli* and *Staphylococcus saprophyticus* bacteria antibacterial property of the plant extract showed inhibition only at the concentration of 1 mg/ml.

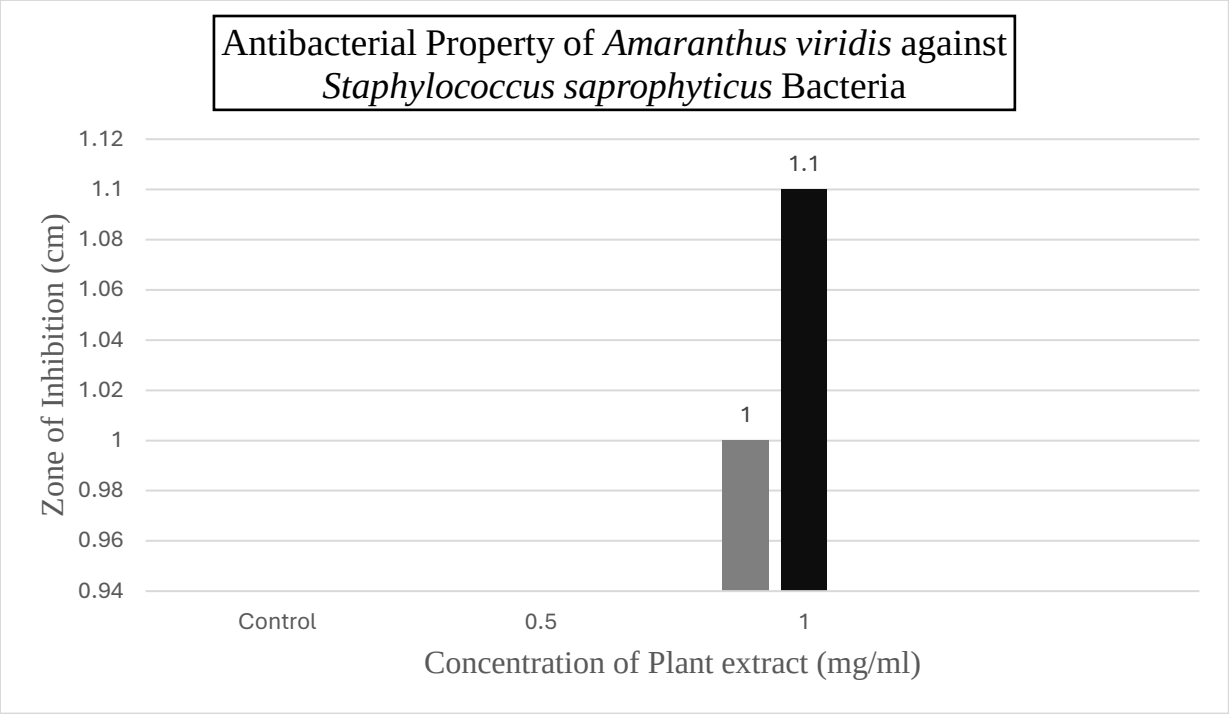
Table 6. Antibacterial activity of *Amaranthus viridis*. against *E. coli* and *Staphylococcus*

Sl No.	Test organism	Zone of Inhibition at concentration (cm)					
		Control (water)		0.5 mg/ml		1 mg/ml	
1.	*Escherichia coli*	-	-	-	-	0.8 cm	0.7 cm
2.	*Staphylococcus saprophyticus*	-	-	-	-	1 cm	1.1 cm

Note: Absence of zone of inhibition was denoted as negative (-)



Graph 5 - Antibacterial Property of *Amaranthus viridis* against *E. coli* Bacteria



Graph 6 - Antibacterial Property of *Amaranthus viridis* against *Staphylococcus saprophyticus* Bacteria

In the case of *E. coli* bacteria, at 0.5 mg/ml concentration of the plant extract, no inhibition of the bacteria was observed. Hence shows no antibacterial property at lower concentrations. But when the concentration was doubles, the Zone of Inhibition (ZOI) was observed by an average of 0.75 cm. This results that *Amaranthus viridis* shows minimum antibacterial property against *E. coli* bacteria only at high concentrations of the plant extract.

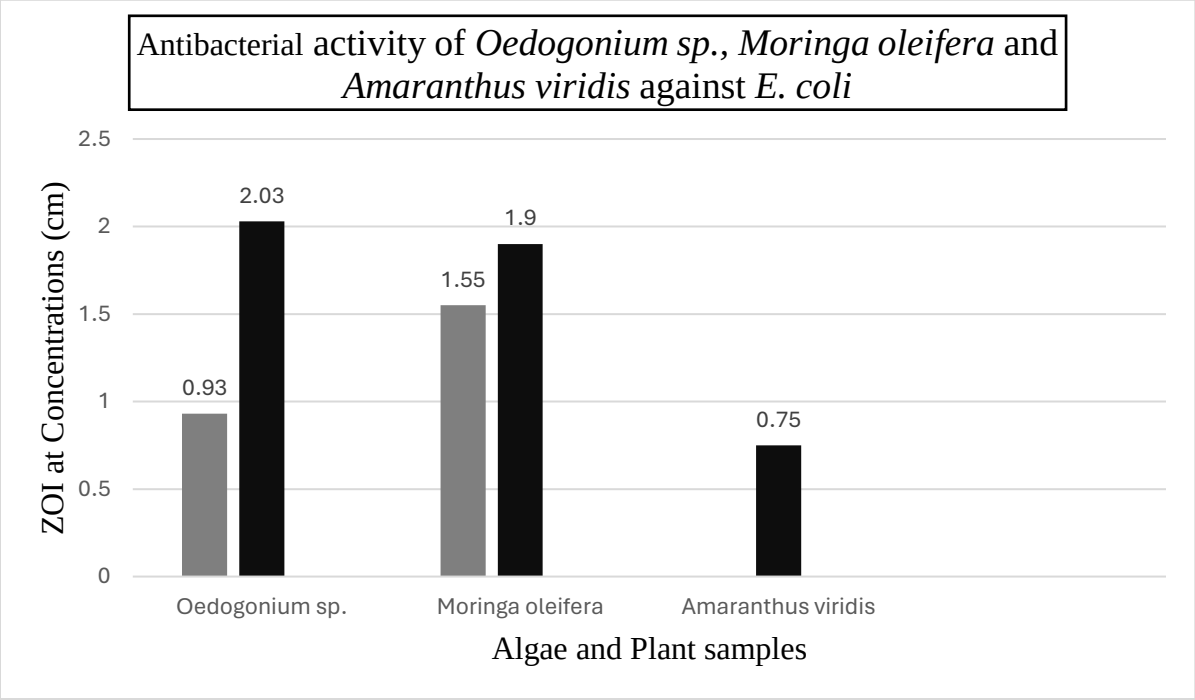
Similarly, in the case of *Staphylococcus saprophyticus* bacteria, at 0.5 mg/ml concentration of the plant extract, no inhibition of the bacteria was observed. Hence shows no antibacterial property at lower concentrations. But when the concentration was doubles, the Zone of Inhibition (ZOI) was observed by an average of 1.05 cm. But the ZOI of Amaranthus against Staphylococcus bacteria is higher than that against *E. coli* bacteria. This results that *Amaranthus viridis* shows minimum antibacterial property against *Staphylococcus saprophyticus* bacteria only at high concentrations of the plant extract. It is somewhat more effective than *E. coli* bacteria.

4. **Comparative evaluation of the antibacterial activity of *Oedogonium*, *Moringa oleifera* and *Amaranthus viridis* against *Escherichia coli***

Table 7. antibacterial activity of *Oedogonium*, *Moringa oleifera* and *Amaranthus viridis* against *Escherichia coli*

SI No.	Algae and plant samples	Average Zone of Inhibition at concentration (cm)	
		0.5 mg/ml	**1 mg/ml**
1.	*Oedogonium sp.*	0.93 cm	2.03 cm
2.	*Moringa oleifera*	1.55 cm	1.9 cm
3.	*Amaranthus viridis*	-	0.75 cm

Note: Absence of zone of inhibition was denoted as negative (-)



Graph 7 - Antibacterial activity *of Oedogonium sp., Moringa oleifera* and *Amaranthus viridis* against *E. coli*

From the above table, it is clear that *Moringa oleifera* shows more antibacterial property than *Oedogonium* and *Amaranthus viridis* against *Escherichia coli* at both 0.5 mg/ml and 1 mg/ml concentrations. *Oedogonium* shows high antibacterial property at 1 mg/ml by an average ZOI of 2.03 cm. But Amaranthus was only effective at 1 mg/ml by an average ZOI of 0.75 cm which is the lowest among the three.

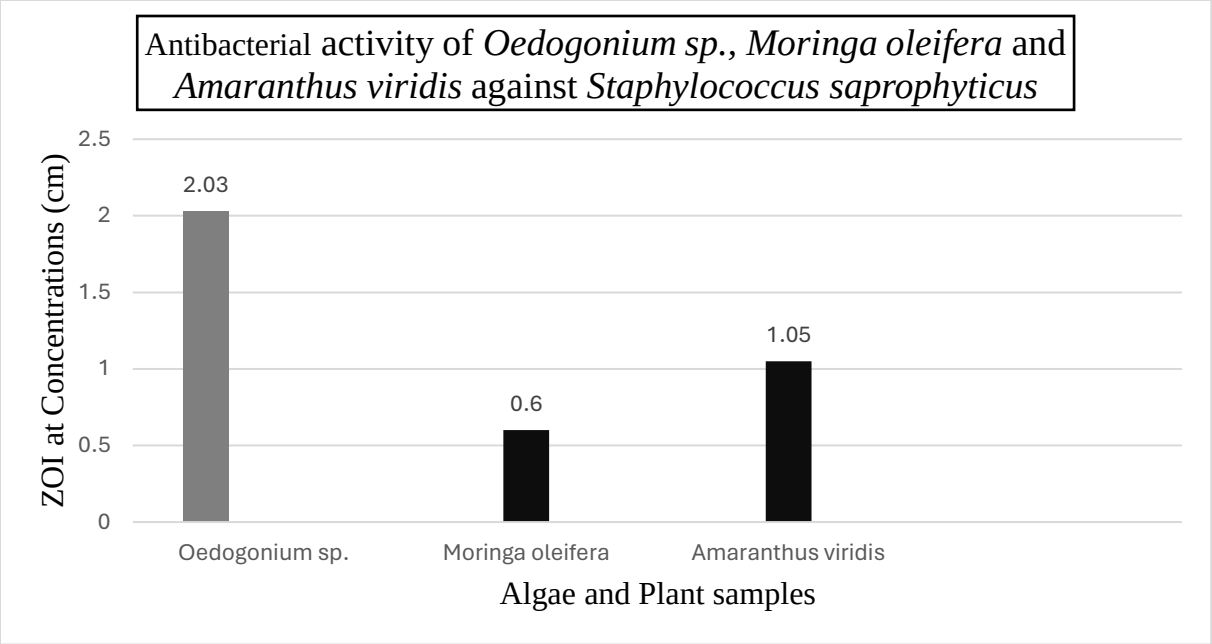
2. **Comparative evaluation of the antibacterial activity of *Oedogonium*, *Moringa oleifera* and *Amaranthus viridis* against *Staphylococcus saprophyticus***

Table 8. antibacterial activity of *Oedogonium*, *Moringa oleifera* and *Amaranthus viridis* against *Staphylococcus saprophyticus*

SI No.	Algae and plant samples	Average Zone of Inhibition at concentration (cm)	
		0.5 mg/ml	**1 mg/ml**
1.	*Oedogonium sp.*	2.03 cm	-
2.	*Moringa oleifera*	-	0.6 cm
3.	*Amaranthus viridis*	-	1.05 cm

Note: Absence of zone of inhibition was denoted as negative (-)

In the case of *Staphylococcus saprophyticus, Oedogonium* shows highest zone of inhibition at 0.5 mg/ml with an average of 2.03 cm. Both Moringa and Amaranthus shows no inhibition at 0.5 mg/ml. But when the concentration was doubled (1 mg/ml), they showed inhibition. For Moringa, it is 0.6 cm and for Amaranthus it is 1.05 cm. Therefore *Oedogonium* shows antibacterial property at lowest concentrations. Whereas Moringa and *Amaranthus viridis* shows inhibition at higher concentrations.



Graph 8 - Antibacterial activity of *Oedogonium sp., Moringa oleifera* and *Amaranthus viridis* against *Staphylococcus saprophyticus*.

Figure 11 - Agar plates showing ZOI against E. coli bacteria by *Oedogonium*

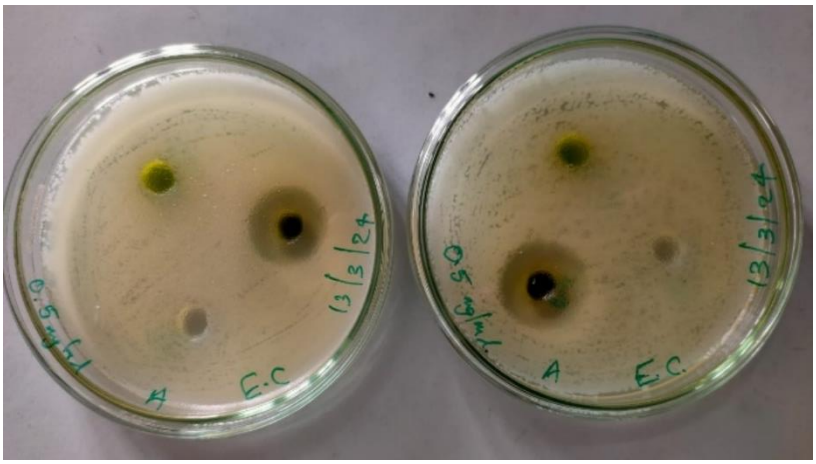


Figure 12 - Agar plates showing ZOI against *E. coli* by plant extracts at 0.5 mg/ml

Figure 13 - Agar plates showing ZOI against *E. coli* by plant extracts at 1 mg/ml

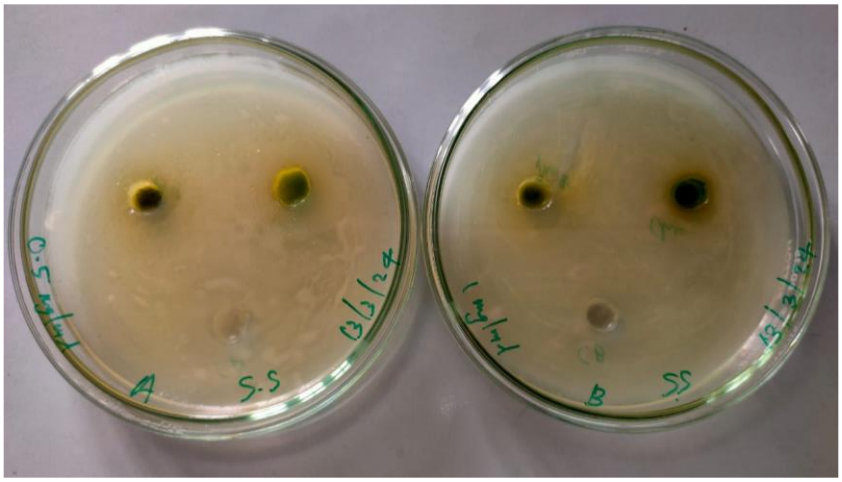


Figure 114 - Agar plates showing slight ZOI against *Staphylococcus saprophyticus* at 0.5 and 1 mg/ml

DISCUSSION

The increasing demand and popularity of medicinal plants in both rural and urban areas pose a significant threat to the survival of numerous medicinal plant species, as highlighted by Matsiliza and Barker in 2001. This alarming trend puts many valuable plants at risk of extinction. For those living in urban areas, the therapeutic use of medicinal plants is an alternative to health care, but the only means of healing for some people in rural areas.

There is a rise in the investigation of traditional medicinal plants as substitutes, driven by the escalating risk and dissemination of antibiotic resistance among numerous common pathogens. In fact, antibiotic resistance is not selective because it can affect people living in urban and rural areas all over the world with similar consequences. In particular, misuse of antibiotics and the development of antibiotic resistance is reducing the usefulness of antibiotics that once were easily treated for a range of infections (Nostro et al., 2000).

The main objective of the study was to examine whether certain conventional uses of algae and two plants for wound healing are scientifically valid. The use of effective natural plant remedies may be strengthened by information on the presence or absence of antibacterial activity.

In this study one algal species and 2 plant species were collected from the freshy bound vegetations around the locality. The selection of these items was based on their unique characteristics and their documented effectiveness in treating various ailments such as cough, fever, wounds, diarrhea, dysentery, and more as mentioned in literature and folklore. The algae collected was *Oedogonium sp.* and the other two plant species was leafy vegetables such as *Moringa oleifera* and *Amaranthus viridis.* Identification of the collected samples were done by referring various literature and herbarium of the college.

These samples there thoroughly washed and dried by draining out all the water content from the plant parts. It is then weighed and chopped. The Oedogonium thallus and plants leaves were used in this study. Different concentrations of the samples were prepared by grinding the samples with specific amount of water. No other solvent were used since this is only a preliminary study on the plant antibacterial properties. In order to obtain compounds from the plant, Thapa (2006) and

Baidya (2001) used hexane, chloroform, n-butanol and aqueous solvent at a higher polarity. The purpose of this study is to support our study.

Two strains of bacteria, one Gram-negative and other Gram-positive were used in this study. Escherichia coli was taken as Gram-negative bacteria and Staphylococcus saprophyticus was taken as Gram-positive bacteria. Sileshi Degu (2021) in the study used 13 strains of bacteria for evaluating the antibacterial activity of the root extracts of *Impatiens tinctoria.*

The antibacterial assay was conducted by the agar well diffusion method of Dingle et al. (1953). In this study different extracts obtained by continuous extraction from the collected plants using water as solvent were studied for their antimicrobial property. These extracts were tested against 2 different bacterial species using agar well diffusion method.

The results in table 7. indicates that Oedogonium algae, Moringa oleifera and Amaranthus viridis, all three showed effective antibacterial property against the Gram-negative bacteria, Escherichia coli. But in Table 8. all three extracts showed less antibacterial property against Staphylococcus saprophytics.

In classifying the antibacterial activity as Gram-positive or Gram-negative, it would generally be expected that a much greater number would be active against Gram-positive than Gram-negative bacteria (Cutcheon et al; 1992). Nevertheless, the algae and plant species exhibited activity in the research against both positive and negative Gram-negative bacteria. This dual activity suggests a potential wide spectrum of antibiotics present.

CHAPTER 6

SUMMARY

- A total of one algae and two plants were chosen and assessed for their antibacterial effectiveness against two bacterial species.
- The algae selected were *Oedogonium sp*. and the other two plant species were *Moringa oleifera* and *Amaranthus viridis*.
- The two bacterial strains used were- one is Gram-negative bacteria, *Escherichia coli* and the other is Gram-positive bacteria, *Staphylococcus saprophyticus*.
- The solvent used in this study was normal water. This solvent is used instead of other chemicals, as it is just a preliminary test on the antibacterial property of the selected samples. Different concentrations of the samples were prepared by grinding with appropriate amount of water.
- For Oedogonium sp., four concentrations were prepared – 0.25 mg/ml, 0.5 mg/ml, 1 mg/ml and 5 mg/ml. Both the plants, Moringa and Amaranthus were extracted in two concentrations – 0.5 mg/ml, 1 mg/ml.
- The high inhibition of the bacteria was shown by the two plant extracts. However, the algal extract's zone of inhibition demonstrated greater effectiveness against Gram-negative bacteria compared to Gram-positive bacteria.
- The medium for the inoculation of bacteria used were nutrient agar medium. It was prepared and sterilized before use. The medium was then poured into sterile petri-plates and stored for further use.
- Agar well diffusion was used for the evaluation of the three sample extracts against the two bacterial strains. The agar plates were inoculated with the collected bacterial strains.
- Three wells were made per agar plates using cork borer and with the help of micropipette two wells were filled with two different concentrations of one sample and the other well were filled with water as control.

- Following the appropriate incubation period of 18-24 hours, the plates were examined for any signs of bacterial growth inhibition, which was identified by the presence of a transparent zone surrounding the wells. The size of the zones of inhibition was measured and the antibacterial activity expressed in terms of the average diameter of the zone of inhibition in centimeters. The absence of a zone of inhibition was interpreted as the absence of activity. The triplicate assay was performed in the case of presence of zone of inhibition. The ZOI were measured using scale and mean was recorded.
- *Oedogonium sp.* and *Moringa oleifera* showed high antibacterial activity by inhibiting the both bacterial strains while *Amaranthus viridis* showed least antibacterial activity as it inhibited only the Gram-positive bacteria, *Staphylococcus saprophyticus*.
- With all the results obtained, it is clear that all the three samples collected showed antibacterial properties as they inhibited the growth of the common bacterial species – *E. coli* and *Staphylococcus saprophyticus*.
- The algal extract and the two plant extracts showed significant antibacterial property against both the Gram-negative and Gram-positive bacteria.

CHAPTER 7

CONCLUSION

Based on the findings of this research, it can be inferred that the algae and plants chosen for this study exhibited antibacterial properties against various types of bacteria. *Oedogonium algae* and *Moringa oleifera* shows more effective antibacterial property than *Amaranthus viridis*. Gram negative bacteria was more sensitive to selected medicinal plants than Gram positive bacteria.

In the treatment of wound infections, such as wound healing properties and pain relief, plants chosen in this study may have other characteristics which are favourable for skin infection. The use of ethnobotanical methods to choose plants for scientific studies is supported by the evidence that the traditional uses of these plants have proven effective in treating bacterial wound infections.

Thus, it is concluded that *Oedogonium, Moringa oleifera* and *Amaranthus viridis*, all three shows effective antibacterial property against the Gram-negative bacteria, *Escherichia coli*. On the other hand, these extracts showed less antibacterial property against *Staphylococcus saprophytics* which is Gram-positive bacteria.

The property of antibacterial activity thus can be utilized in treating a wide range of bacterial infections that generally caused by *E. coli*. This natural obtains can be included in the production of antibiotics that are free from any type of harmful chemicals. Thus, obtaining a better alternative for the harmful antibiotics that gives intense side effects.

This study thus shows the clear visible inhibition zones of the three various plant extracts against the wide common bacterial strains – *Escherichia coli* and *Staphylococcus saprophyticus*. But the effect is high against plants than against the algae*, Oedogonium sp*. Apart from that all the three samples collected showed antibacterial property against Gram-negative and Gram-positive bacterial strains.

CHAPTER 8

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