COMPARATIVE ANALYSIS OF ANTIMICROBIAL EFFICACY AND ANTIBIOTIC RESISTANCE OF ENDOPHYTIC BACTERIA ISOLATED FROM *ZINGIBER OFFICINALE* AND *CURUCUMA LONGA*

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE AWARD OF THE DEGREE

OF

MASTER OF SCIENCE
IN
BIOTECHNOLOGY

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CANDIDATES'S DECLARATION

I, **VARSHA**, [**2K22/MSCBIO/55**], student of M.Sc. Biotechnology, hereby declare that the project Dissertation title **"Comparative Analysis of Antimicrobial Efficacy and Antibiotic Resistance of Endophytic Bacteria Isolated from *Zingiber officinale* and *Curucuma longa"*** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is authentic and not copied from any source without proper citation, and carried out during a period from 10th Janurary,2024 to 30th April, 2024, under the guidance of Prof. Jai Gopal Sharma. I hereby ensure that based on this work, no prior degree, certificate, associateship, fellowship, or other title or honor has been given.

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To the best of my knowledge, this is to certify that, the project work, named **"Comparative Analysis of Antimicrobial Efficacy and Antibiotic Resistance of Endophytic Bacteria Isolated from Ginger and Turmeric"** submitted by **Varsha, [2K22/MSCBIO/55],** M.Sc. Biotechnology, has never been submitted anywhere else, in whole or in part, for any Degree, Diploma at this University or anywhere.

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15th INTERNATIONAL CONFERENCE ON RECENT ENGINEERING & TECHNOLOGY

ORGANIZED BY

SRI VENKATESHWARA COLLEGE OF ENGINEERING

IN ASSOCIATION WITH

ORGANIZATION OF SCIENCE & INNOVATIVE ENGINEERING TECHNOLOGY

IN COLLABORATION WITH

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This is to certify that Dr./Mr./Ms. Varsha from Delhi Technological University has presented a paper titled Exploring the Antimicrobial Efficacy of Endophytic Bacteria Isolated from Ginger and Turmeric: A Comparative Analysis

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Name of the Conference: **15Th International Conference on Recent Engineering and Technology**

Conference Date : **30th May 2024**

ACKNOWLEDGEMENT

I would like to take this opportunity to thank my mentor and supervisor, Prof. Jai Gopal Sharma, of Department of Biotechnology at Delhi technological University, from the bottom of my heart, for his immense help unwavering encouragement, and inspiring ideas, suggestions throughout my research phase, without which I could not have completed my dissertation within the time limit.

I want to express my gratitude to entire biotechnology department faculty for their steadfast support. Additionally, I would like to thank Miss. Megha, my lab seniors, for their unwavering support and advice, during the courses of the completed work.

At last, I would like to express my gratitude to my family and friends for their support, love and best wishes on a personal level.

VARSHA

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ABSTRACT

The purpose of this study is to evaluate the antibacterial and antibiotic properties of endophytic bacteria that have been isolated from ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*). Bacterial isolates from both plants, which are famous for their therapeutic properties, were tested for salt tolerance, catalase activity, antibacterial potential, and antibiotic resistance. The salt tolerance varied between the bacterial isolates obtained from ginger and those obtained from turmeric. Observations from Gram staining showed that most bacteria were Gram-negative. Among the tested isolates, only one did not show catalase activity, that is, G3. Ginger's bacterial isolate, G2 had lipase activity shown by the presence of bacterial growth in the nutrient agar. The findings of further ginger isolate G2 showed promising antibacterial effects on *E.Coli* with a zone of inhibition measuring 2mm. When looking at the drug sensitivity patterns of bacteria against Drotvine M, it was realized that only one bacterium isolate G2 showed resistance by forming 1.5mm diameter of zone inhibition. The significance of plant-derived sources in combating antibiotic resistance are underscored by these findings because they suggest that endophytic bacteria from ginger may act as natural antibacterial agents.

Key words – *Zingiber officinale*, *Curcuma longa*, endophytic bacteria, salt tolerance, catalase, phytochemical tests, lipase, antimicrobial

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CHAPTER 1
INTRODUCTION

1.1 BACKGROUND

Endophytic bacteria which are fascinating microorganisms which are known to live inside the tissues of plants without harming their hosts. Rather, they establish mutually beneficial interactions with the plants, which help to promote disease resistance, stress tolerance, and growth. These bacteria settle down and interact with the physiology of the plant in a variety of plant organs, such as the roots, stems, leaves, and even the seeds. Endophytic bacteria have the potential to produce growth-promoting hormones, improve nutrient uptake, and strengthen a plant's resistance to environmental stressors including salinity, drought, and infections through complex pathways [1].

Turmeric and ginger are within the Zingiberaceae family of medicinal plants and spices. Both were chosen in order to show that the isolates encompassed a genus of bacteria and had potent antibacterial and antibiotic resistance qualities. The isolates would be chosen based on their morphological similarity for additional analysis. This will serve as a beacon, encouraging the exploration of the mysteries concealed in the bacteria' natural healers. Every colony has a possible source of newly discovered, undeveloped antibacterial agents nearby. It's an exciting journey to learn more and evaluate the traditional healers included in the pharmacopoeia of the botanical world [2].

SCIENTIFIC NAME: ***Zingiber officinale***

FAMILY: Zingiberaceae

The Sanskrit phrase singabera is the source of the name "ginger" (*Zingiber officinale*), which is derived by the Greek word zingiberis. It is a blooming plant which has been used from thousands of years for spice, for treatment of a great number of ailments such as, cold, nausea, arthritis, migraine, hypertension, cough and so on.

SCIENTIC NAME: ***Curcuma longa***

FAMILY: Zingiberaceae

Within the Zingiberaceae family of ginger plants, turmeric is a flowering plant. This hardy herbaceous plant, native to Southeast Asia with the Indian subcontinent, does well in temperatures between 20 and 30 °C (68 and 86 °F) and significant yearly precipitation. These plants are harvested each year for their rhizomes; some are kept for human use and others are multiplied for use in subsequent growing seasons.

In China and India, folk medicine has traditionally employed ginger. In particular, India uses a lot of ginger, both wet and dry, in its culinary and medical industries. *Zingiber officinale* subterranean stem, when wet, is a common culinary component. It is an essential ingredient in many different cultures' pastries, sweets, gingerbread, savoury foods, and non-alcoholic drinks.It was historically utilized in traditional medicine for the treatment of asthma, colds, sore throats, joint pain, and the stimulation of the appetite. Additionally, ginger is a good source of several nutrients, including calcium, potassium, and phosphorus, all of which are crucial for human physiological functions [3].

Turmeric (***Curcuma longa***), is a common cooking ingredient that enhances food flavors and colors. Apart from its culinary use, turmeric has numerous health advantages. The strong anti-inflammatory and antioxidant qualities of curcumin, the chemical that is active in it, could potentially help lower the risk of chronic illnesses while enhancing general health.

Turmeric has been associated with better digestion, increased immunity, and improved cognitive health when taken regularly [4] .

The antibacterial qualities of turmeric and ginger against bacteria producing clinically relevant infections are being evaluated in light of the current surge in antibiotic resistance. Primarily, ginger is grown in Southeast Asia and cultivated using farming practices of many nations. Additionally, turmeric is relevant because many people use it for medicinal purposes and food as well. Therefore, the antibacterial activity of the spices creates the clue for alternative therapy given the rising concerns on antibiotic resistance in microorganisms [5] .

Due to their bioactive ingredients, gingerol in ginger and curcumin in turmeric, both spices have antimicrobial effects. These include well-known antibacterial substances that have the power to eradicate a variety of germs, including those responsible for gastrointestinal and respiratory disorders[6]. In addition, research has been done to see if ginger and turmeric can inhibit the growth of bacteria associated with oral problems, including dental plaque formation. Apart from their well-established health benefits, incorporating turmeric and ginger into your diet or supplementing them with natural products may be advantageous in avoiding bacterial infections and improving oral health [7].

Curcuma longa and ***Zingiber officinale*** contain special bacteria inside them. These bacteria help plants grow strong and healthy. The bacteria make plant hormones that control how cells divide and get longer. This causes plants to grow bigger. Some hormones they make are gibberellin, cytokinin, and indole-3-acetic acid. The bacteria also use nitrogen to make proteins, helping plants develop properly. Additionally, they break down phosphate, so plants can absorb more nutrients easily. They activate defense against diseases in the whole plant [8]. These beneficial microbes tolerate plant stress and plant pathogens, in addition to competing for resources since biological management aids plant

illnesses by suppressing them rather than completely blocking them, these advantageous bacteria compete for resources, withstand plant stress, and withstand plant infections. Therefore, there is a significant deal of potential for applying endophytic bacteria's salt tolerance, antifungal activity, and plant growth promotion to sustainable agriculture and the extraction of medicinal plants [9].

In this study, we focused on isolating and categorising endophytic bacteria that are present in ginger and turmeric plants, particularly in those that thrive in saline environments. We employed oxidase, lipase, gramme staining, and a particular phytochemical test that included phlobotannins, terpenoids, and saponins. The medication's antimicrobial impact was also assessed. The purpose of the study was to learn more about the unique functions of these bacterial endophytes in plant health and potential applications for their use in medicine [10] .

1.2 LITERATURE REVIEW

Overview of Endophytic Bacteria

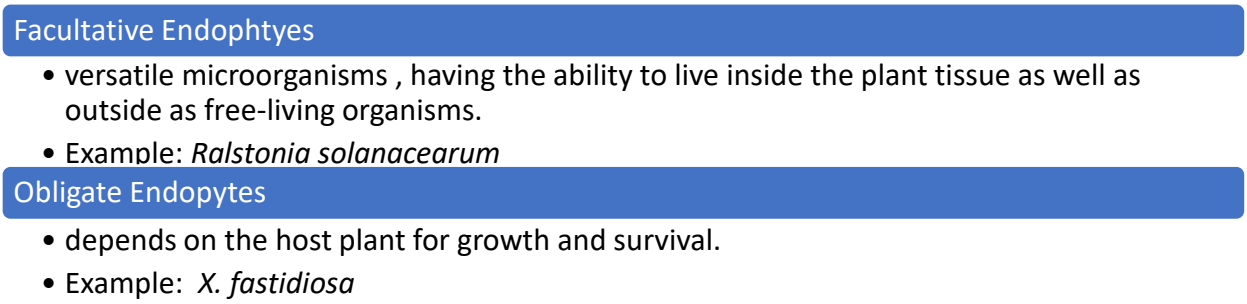
Plant-associated bacteria live in a variety of plant parts, such as the soil around roots (rhizobacteria), the surface on leaves (epiphytes), and internal tissues (endophytes). Endophytes have been found in a variety of plant parts, including fruits, flowers, leaves, stems, roots, and seeds. The aforementioned bacteria are widely distributed throughout plant tissues and derive advantages from the host plant's defence against external stresses and microbial competitors [11].

In most plant species, endophytes are either widely distributed or often present. They are mostly spread by seeds, and as soon as a seed germinates, they start to play a positive function in fostering plant development and vigour. Similar benefits to plants are seen by other endophytes that can be sourced from the soil. Plants require endophytic bacteria to perform a variety of tasks. These tasks include increasing the amount of nutrients that plants take in, protecting them from insects and viruses, enhancing their ability to withstand stress, controlling plant development, and inhibiting the growth of weeds. The particular mechanisms through which endophytic bacteria execute diverse functions in plants likely vary based on the specific bacterium and the host plant involved [12].

A significant characteristic that facilitates interference with the host plant's physiology is the way 1-aminocyclopropane-1-carboxylate deaminase, which is produced by bacteria, modulates the amounts of ethylene in plants. Because it facilitates better colonization, endophytes possessing this ability may benefit from their relationship with the plant. In turn, decreased stress and enhanced root growth are advantageous to host plants. This process gives rise to the idea of "competent" endophytes, which are endophytes that possess genes necessary for the upkeep of plant-endophyte relationships [13].

Bacterial endophytes are categorized as "obligate" or "facultative" based on their life strategies. For growth and survival, obligatory endophytes are completely reliant on their host plant; they spread to other plants either vertically or through the use of vectors.

There is a point in the life cycle of facultative endophytes when they live apart from their host plants [13].



Why *Zingiber officinale* and *Curcuma longa* for endophytic bacteria isolation?

Traditional medicine makes use of ginger and turmeric for their antibacterial and other therapeutic qualities. Endophytic bacteria live in the tissues of plants and don't seem to inflict any damage. They may support the defense systems and general health of the plant. The antibacterial qualities of substances present in ginger and turmeric, such as gingerol in ginger and curcumin in turmeric, have been investigated. These substances may prevent non-endophytic bacteria from growing, promoting the growth of endophytic bacteria in culture media made from these plants [14].

The Zingiberaceae family include significant therapeutic herbs. Numerous substances, including turmerin, sesquiterpenes, steroids, and essential oils, have been found in several species. The ginger family includes several significant genera, including *Curcuma*,

Hedychium, Costus, Zingiber, Amomum, Alpinia, and *Kaempferia*. Each of these genera has unique chemicals that are useful in the pharmaceutical business. The ginger family includes species like white turmeric, which have long been employed in herbal therapy to treat cancer-related illnesses. These bacteria are excellent candidates for further research due to their endophytic properties. Furthermore, a number of research demonstrated the pharmacological antibacterial, anticancer, and antioxidant properties of white turmeric. White turmeric's rhizome contains essential oil that has the ability to stop the growth of cancer cells [15] .

Ginger may lower the level of lipid peroxides, which are controlled by lipoxygenase and membrane NADH oxidase activities. The latter produces hydrogen peroxide, which has been demonstrated to stimulate cyclooxygenase activity, since a peroxide tone appears to be necessary for the formation of prostaglandins. Furthermore, membrane lipid oxidation can be started by oxidase-generated H_2O_2 and Oi-, which produce peroxidized lipids in quantities high enough to activate cyclooxygenase activity. This implies that oxidants and lipid peroxides produced by phagocytic cells during immunological responses or at inflammatory areas may influence prostaglandin synthesis [16].

In Turmeric, the mature dried rhizome is a widely used spice in Indian cuisine and has long been recognised for its antibacterial and antipyretic properties. Because curcuminoid and sesquiterpenoid molecules are present, the compounds have been given therapeutic characteristics. The most significant curcuminoid, curcumin, has antibacterial, anti-inflammatory, and antioxidant properties. It also works well against HIV and cancer. Plants engage in interactions with varied colonies of microbes for a range of functions, such as enhancing growth, managing diseases, and improving production. The microorganisms then use the host plants as a source of food and shelter. The most noticeable area for microbial interaction is the rhizosphere, although certain bacterial species infiltrate plant tissue and live there as endophytes undetected [17].

Although there is still much to learn about the antibiotic resistance profiles of endophytic bacteria derived from turmeric and ginger, interest in doing so is growing because of the potential uses of these bacteria in biotechnology, agriculture, and medicine.

Turmeric was shown to have the second-highest antioxidant potency, after ginger. Numerous studies have demonstrated that spices' antibacterial properties can stop germs from growing in food. Numerous research investigations have documented the antimicrobial properties of spices and herbs against food-borne diseases. It has been demonstrated that the essential oils found in spices, which have antibacterial properties, cause gramme negative bacteria to be more resistant to them than gramme positive bacteria [18]. The study revealed that the antibacterial efficacy of these spices ranked as follows: ginger > turmeric. According to the study, spices with antioxidant and antibacterial properties include turmeric and ginger [18].

Drotvine-M

It is used for abdominal pain, it can be treated with the combination medication Drotvine-M 80mg/250mg Tablet. Through the relaxation of the stomach and gastrointestinal muscles, it effectively reduces abdominal pain, bloating, discomfort, and cramps. Moreover, it inhibits some chemical messengers that are responsible for discomfort and pain.

The combination of two medications, Drotaverine + Mefenamic Acid1 and Drotaverine + Mefenamic Acid2: Drotaverine + Mefenamic Acid1 is an anti-spasmodic medication that relieves abdominal smooth muscle contractions, or spasms. An NSAID (non-steroidal anti-inflammatory medication) is rotaverine plus mefenamic acid2. It functions by preventing the production of specific chemical messengers that result in inflammation and pain in the abdomen (swelling).

Antibiotic resistance:

Antibiotic resistance is the capacity of bacteria to resist the impact of antibiotics, making these drugs ineffective against them. This resistance may develop organically as a result of genetic changes or may be acquired as a result of things like antibiotic abuse or overuse. Antibiotics can no longer efficiently kill or stop the growth of germs when the bacteria develop resistance to them. Antibiotic-resistant bacteria can therefore be harder to cure, which can result in longer illness times, a higher chance of complications, and higher medical expenses.

CHAPTER 2
METHODOLOGY OF STUDY

2.1 MATERIALS REQUIRED

A. Materials

Ginger and Turmeric Rhizomes

B. Chemicals:

Crystals violet Stain, Gram's Iodine Stain, Ethanol, Safranin: For Gram Staining

Sodium Hypochlorite: For sterilization

Nutrient Mediums: a. Nutrient Agar

b. Potato Glucose Agar

. c. Mueller Hinton Agar

d. LB Agar

Conc. H_2SO_4

Conc. HCl

Chloroform

Spirit

NaCl

70% Ethanol

Saline Solution

Peptone, CaCl, Nacl, Agar, distilled water : for Lipase Test

Tween 20

2.2 SAMPLE PREPARATION

Rhizomes of ginger and turmeric were purchased from a vegetable vendor and stored in plastic bags with a closure.

(a)

(b)

Figure 1: Sample of ginger and turmeric
(a) Ginger
(b) Turmeric

These rhizomes were washed to get rid of the dirt on their surface. After washing the rhizomes of both samples to get rid of dirt, they were surface-sterilized for one minute by immersing them in 70% ethanol, then 4% sodium hypochlorite for five minutes, and finally 70% ethanol for thirty seconds. They were then cleaned one more using clean water after being rinsed five times with distilled sterilised water. The rhizomes were cleaned, dried, and ground to a powder, and then a liquid extraction was carried out. Ginger and turmeric were then mashed with a mortar and pestle and 1% saline solution. After precisely measuring 1 ml of the ginger and turmeric extracts respectively, they were put into a test tube and serially diluted up to a 10^{-5} dilution. Onto nutrient agar plates, 0.1 ml of this was plated. Four different kinds of agar plates: Nutrient Agar, Mueller-Hinton agar, Potato Glucose agar, and LB agar, were used for this [19].

2.3 BACTERIAL GROWTH

Nutrient agar plates (all four agar were used to check if there is any hindrance in the growth of bacterial colonies because of their different composition) were plated with 1 mL of each of the two samples that had been serially diluted up to a 10^{-5} dilution. Every plate, except the control, was incubated at 30-32 degrees for 24–32 hours, and the growth of bacteria was checked regularly. Figure 2 shows the colonies used to identify comparable morphologies, and these characters were chosen for additional research. Three bacterial colonies of ginger were isolated and grown on nutrient agar plates named G1, G2, and G3. Similarly, based on the morphological similarity two bacterial colonies of turmeric were isolated and grown on nutrient agar plates named T1, and T2 [20]. Same dilution and procedure for growth of bacterial colonies was performed for turmeric extract.

(a) (b)

Figure 2: Bacterial isolates from the rhizome of (a) *Zingiber officinale* and (b) *Curcuma longa,* respectively

2.4 BACTERIAL ISOLATES CHARACTERIZATION

2.4.1 SALT TOLERANCE

The test bacterial isolates were streaked on nutrient medium for every bacterial colony to determine salt tolerance. NaCl was added to nutrient agar medium at different concentrations ranging from 2% to 10% [21].

2.4.2 GRAM STAINING

A laboratory technique called Gramme staining is used to distinguish among Gram-positive and Gram-negative bacteria according to the makeup of their cell walls. Preparing a thin smear of bacterial colonies G1, G2, G3, T1 and T2 respectively on glass slide. Apply crystal violet stain to smear for 1 min and then rinse gently. Then apply gram's iodine solution to fix the stain and rinse gently after a minute. Decolorize with alcohol or acetone. And lastly counter staining with safranin. Rinse the slide and blot it dry.

2.4.3 CATALASE ACTIVITY

The catalase test is carried out to find out if the catalase enzyme is present, which aids in the breakdown of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2). A tiny quantity of bacterial growth is transferred onto a sterile glass slide using a sterile inoculating loop, and was immediately exposed to a 3% solution of hydrogen peroxide directly to the bacterial growth. Examine, after a short while, the region where the hydrogen peroxide was added [14].

2.4.4 PHYTOCHEMCIAL EVALUATION METHOD

1. Saponins test

In a test tube, combine 5 ml of the ginger extracts with 5 ml of distilled water, and same followed with Turmeric 5ml of turmeric extract with 5 ml of distilled water. When shaken with water, the appearance of frothing indicated a presence of saponins [22].

2. Phlobotannins test

To find out if phlobotannins are present, heat the water-soluble extract from the test sample (turmeric and ginger) in a solution containing 1% hydrochloric acid [22].

3. Terpenoids test

Combine 1 ml of the extract of test samples with 2 ml of chloroform and 3 ml of concentrated sulfuric acid (H_2SO_4). Then, observe the color change at the interface [22].

2.4.5 DETERMINATION OF ANTIMICROBIAL ACTIVITY

For 48 hours at 37°C, the endophytic bacterial isolates were grown as shaking cultures in LB broth. To obtain cell-free supernatants, the culture broth from each isolate, G2 and T2, was centrifuged at 3000 rpm. Test plates (Petri dishes: 94 mm in diameter and 16 mm in height) containing biofilm-forming bacteria were made by pouring 12 mL of LB agar on top of a base layer that was then covered with 2ml of the infected seed layer once it had set. 5mm agar well was created using a sterile cork borer on both the plates with respective broth, and each well held 6µl of supernatant. In order to allow the supernatant to diffuse, the test plates were incubated at 37°C for a period of 18 to 24 hours after being left at room temperature for two hours. The diameter of the inhibitory zones was measured in millimeters after 18 to 24 hours[23].

2.4.6 EFFECT OF ANTIBIOTIC ON THE GROWTH OF BACTERIA IN ORDER TO CHECK IT'S ANTIBIOTIC RESISTANCE

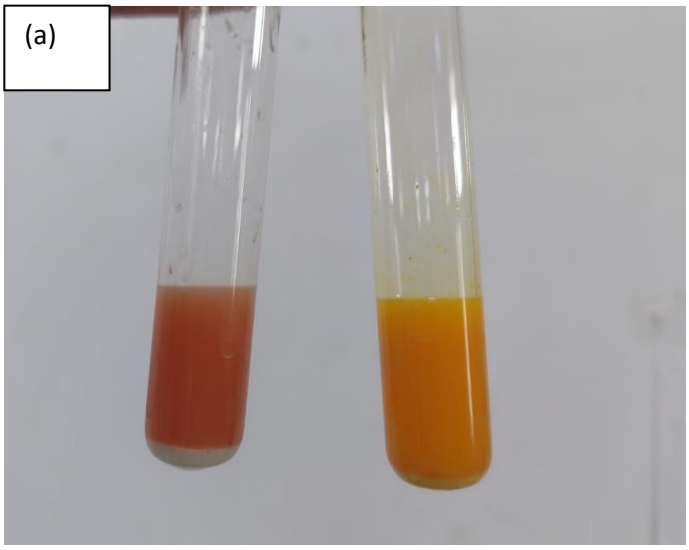
To evaluate the property of antibiotic resistance of bacterial isolate, antibiotic resistance test was conducted. To evaluate the bacterial isolates' resistance to antibiotics compared to common strains, in vitro tests were conducted on them. They were incubated for 48 hours at 28°C on nutrition plates until there was noticeable bacterial growth. As a baseline, control plates devoid of microorganisms were employed. Following the incubation time, each plate's center was treated with Drotvine-M. The breadth of the growth inhibition zone between Drotvine-M and the tested bacterial isolates was used to calculate the effectiveness of antibiotic resistance.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Phytochemical screening

Phlobotannins, saponins, and terpenoids were discovered by phytochemical screening of the plant extract, revealing a rich chemical makeup. Meanwhile, steroids had been conspicuously absent from the evaluation. In this extensive analysis, important knowledge about the bioactive substances that may have produced the plant extract's therapeutic effects and medicinal traits is presented.



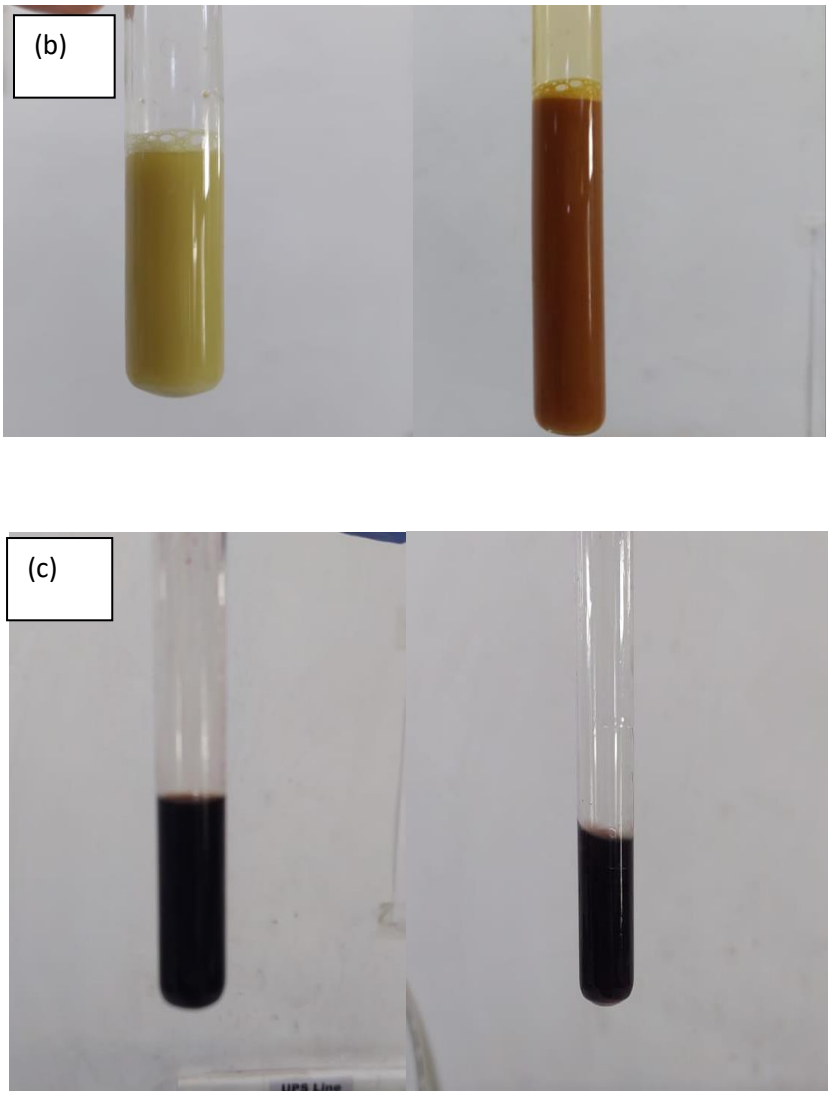


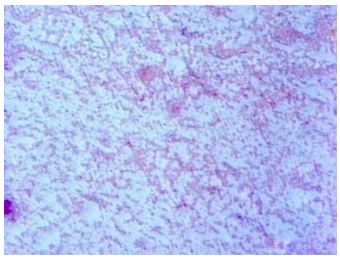
Figure 3: Summarising the phytochemical test results (a) Phlobotannins (b) Saponin

(c) Terpenoid's test

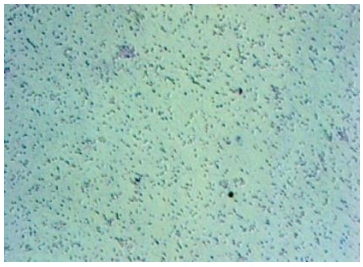
In fig. (a) Phlobotannins test ginger showed positive result by revealing a red precipitate, (b) Both Ginger and Turmeric showed positive Saponins test by showing the appearance of frothing, (c) In Terpenoids test a reddish brown coloration is observed in both samples, indicating positive terpenoid test.

3.2 Gram Staining

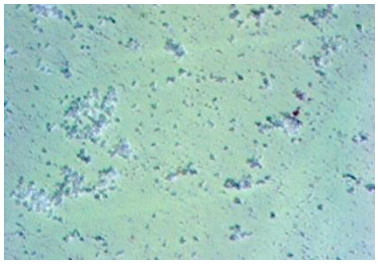
Observation made in this investigation was that when bacterial isolates obtained from ginger were stained with Gram's staining method, they yielded a pink stain mostly, which indicated the presence of Gram-negative bacteria except for G2. The same situation was observed from turmeric samples, which showed a consistent pink coloration under the microscope, also indicating that they are Gram-negative. This implies that ginger as well as turmeric could be habitats of some Gram-negative bacterial species.



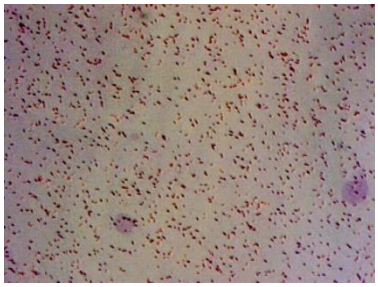
G1



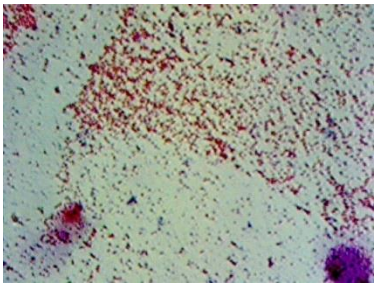
G2



G3



T1



T2

Figure 4: Picture G1, G2, G3, T1, T2 representing the result of gram staining for all the five bacterial isolates from Ginger and Turmeric respectively.

Table 1: **Gram's staining result summarized in tabular form.**

Bacterial isolates	**Gram Staining Result (Color appearance)**
G1	Pink [gram negative]
G2	Purple [gram positive]
G3	Pink [gram negative]
T1	Pink [gram negative]
T2	Pink [gram negative]

3.3 Salt Tolerance test for bacterial Colony

Three bacterial isolates that were obtained from ginger showed varying degrees of salt tolerance. The G1, G2, and G3 endophytic strains of bacteria all showed resistance up to 6% NaCl concentration; at 8% and 10% NaCl concentrations, inhibitory effects were seen. Concerning turmeric, two bacterial isolates were acquired, each displaying distinct degrees of tolerance to varying salt concentrations. T1 endophytic bacterial isolate tolerated 6% of NaCl while T2 bacterial isolate showed tolerance only at 2% of NaCl, inhibited at 4%, 6%, 8% and 10%. The result for salt tolerance test has been summarized in table 2.

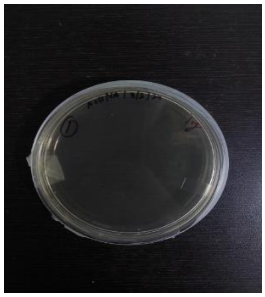
[i] Salt Tolerance of G1

G1 at 2% NaCl

G1at 4% NaCl

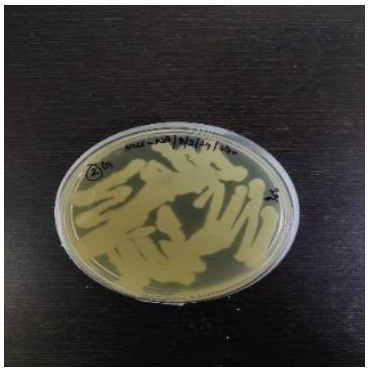
G1 at 6% NaCl

G1 at 8% NaCl



G1 at 10 % NaCl

[ii.] Salt tolerance of G2

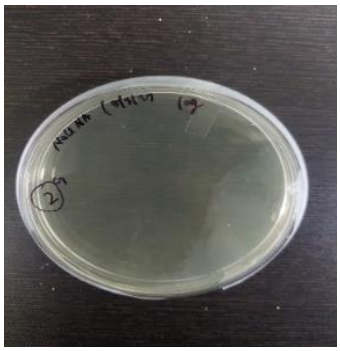


G2 at 2% NaCl

G2 at4% NaCl

G2 at 6% NaCl

G2 at 8% NaCl



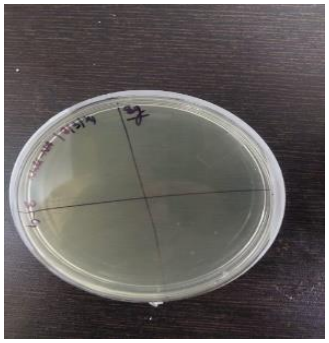
G2 at 10 % NaCl

[iii.] Salt tolerance of G3

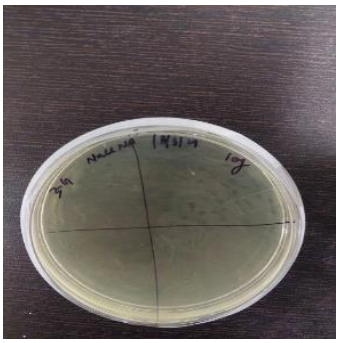
G3 at 2% NaCl

G3 at 4% NaCl

G3 at 6% NaCl



G3 at 8% NaCl



G3 at 10 % NaCl

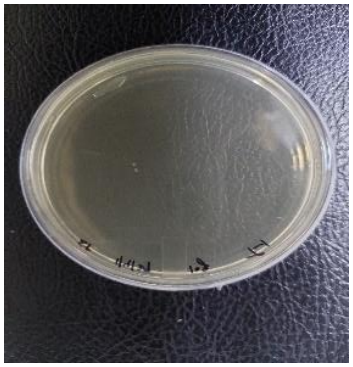
[iv.] Salt Tolerance of T1

T1 at 2% NaCl

T1 at 4% NaCl

T1 at 6% NaCl

T1 at 8% NaCl

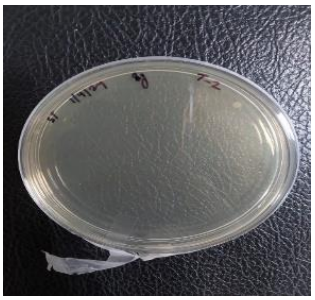


T1 at 10 % NaCl

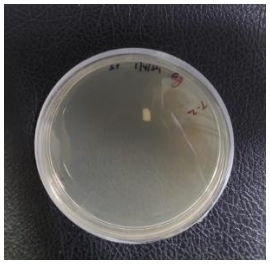
[v.] **Salt Tolerance of T2**

T2 at 2% NaCl

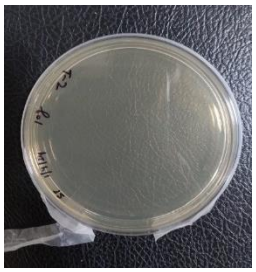
T2 at 4% NaCl



T2 at 6% of NaCl



T2 at 8% of NaCl



T2 at 10% of NaCl

Figure 5: I, ii, iii, iv, v, Showing salt tolerance of ginger and turmeric bacterial isolates at different conc. of NaCl respectively.

TABLE 2. SHOWING BACTERIAL ISOLATE'S TOLERANCE AT DIFFERENT SALT LEVELS

1. **For Ginger**

Bacteria	2% of NaCl	4% of NaCl	6% of NaCl	8% of NaCl	10% of NaCl
G1	+	+	+	-	-
G2	+	+	+	+	-
G3	+	+	+	-	-

2. **For Turmeric**

Bacteria	2% of NaCl	4% of NaCl	6% of NaCl	8% of NaCl	10% of NaCl
T1	+	-	-	-	-
T2	+	+	-	-	-

3.4 CATALASE ACTIVITY

All of the tested bacterial isolates showed strong bubbling and effervescence at the site of inoculation within seconds of administering the hydrogen peroxide except for the endophytic bacteria isolate G3. The quick breakdown of hydrogen peroxide into water and oxygen during this effervescence suggested that the endophytic bacteria have catalase activity, indicating that these endophytic bacteria have the catalase enzyme, which is essential for preventing oxidative damage to bacterial cells by hydrolyzing harmful hydrogen peroxide molecules.

G1 G2 G3 T1 T2

Figure6: G1, G2, T1, T2 showing positive catalase activity whereas G3 is the only isolate which showed negative test

3.5 LIPASE ACTIVITY

The medicinal plants Z yielded five distinct bacterial isolates. *officinale* and *Curcuma longa*; and were screened for enzyme activity i.e., Lipase activity and only G2 showed a positive test for lipase.

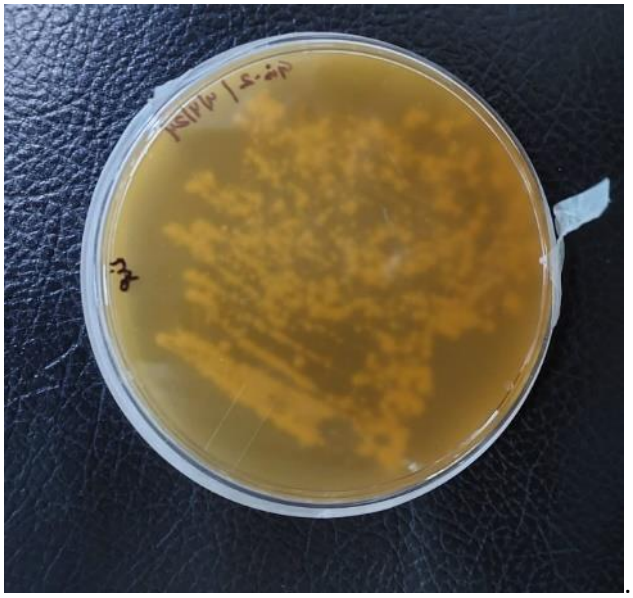


Figure7: Positive lipase test showed by ginger bacterial isolate G2.

TABLE3. SUMMARIZING THE RESULTS OF ACTIVITY SHOWED BY THE BACTERIAL ISOLATES

Bacterial isolates	Gram staining	Lipase activity	Catalase activity
G1	G-	-	+
G2	G+	+	+
G3	G-	-	-
T1	G-	-	+
T2	G-	-	+

3.6 ANTIMICROBIAL SCREENING

In the biological screening for antimicrobial activity against biofilm formers, promising antimicrobial activity against the test organism E. coli was exhibited by the endophytic isolates of ginger and turmeric, designated as G2 and T2, respectively. A zone of inhibition measuring 2mm was recorded for ginger, while no antimicrobial activity was detected for turmeric.

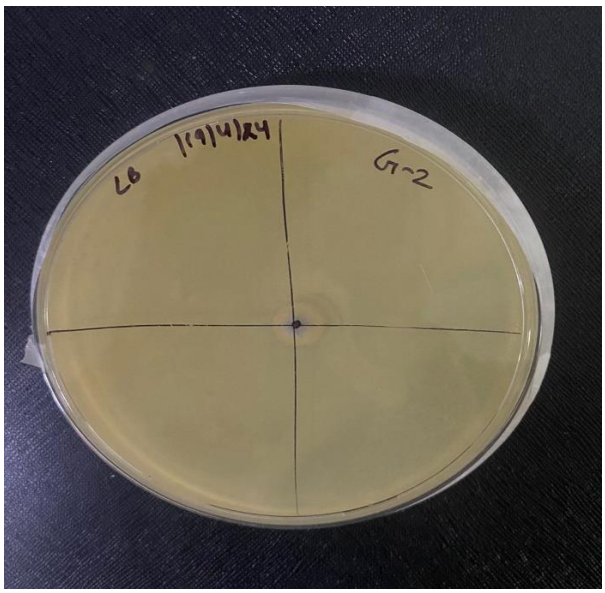


Figure8 : G2 showing antimicrobial activity against the test organism *E.coli*

3.7 Effect of Antibiotic on the growth of bacteria in order to check it's Antibiotic Resistance

The area where Drotvine-M was administered showed notable inhibitory zones for isolates G2 and not for T2. The Zone of Inhibition of 1.5mm was observed which signifies that the bacteria is not resistance to the Antibiotic. The fact that only a little amount of growth suppression was seen suggests that isolate have some resistance to the antibiotic. This demonstrates the isolates capacity for resistance and raises the possibility that they could resist the effects of Drotvine-M and perhaps other antibiotics. To tackle antibiotic resistance in bacterial populations, more research into the mechanisms underlying this resistance may yield important insights.

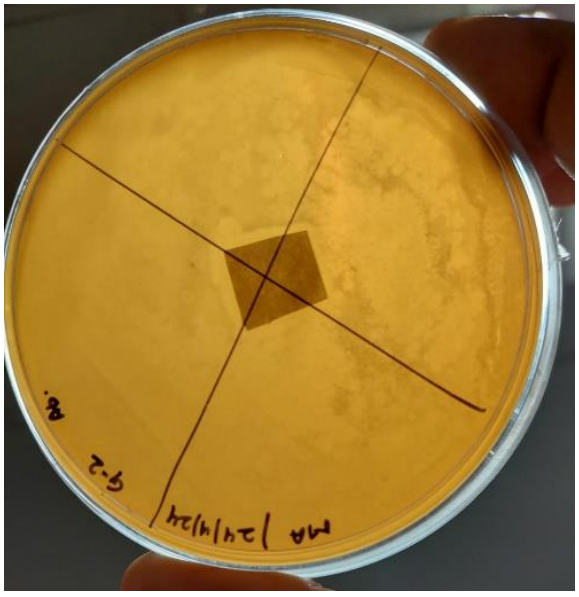


Figure 9: Result showcasing inhibitory effect of Drotvine-M against bacterial isolate of Ginger, G2.

CHAPTER 4

CONCLUSION

In conclusion, the study revealed significant findings regarding the antimicrobial potential of endophytic bacterial isolates from ginger and turmeric. Although salt tolerance, as well as catalase activity was varied among individual isolates, all of them showed great antimicrobial activity against *E. coli*. Thus, G2 isolate which is a constituent of ginger inhibited the colony by 2 mm demonstrating its properties of a natural antibacterial compound against *E.Coli*. However, plain T2 type isolate did not show the antimicrobial possibility.

The significant inhibition zones found in the region where Drotvine-M was applied suggest that isolate G2 was susceptible to the antibiotic, as demonstrated by a 1.5mm Zone of Inhibition. Conversely, isolate T2 exhibited negligible growth suppression, indicating a level of Drotvine-M resistance. These results highlight isolate G2's ability to respond to antibiotic therapy, but they also raise questions regarding isolate T2's possible resistance mechanisms. Because isolate T2 has shown resistance to Drotvine- M and maybe other antibiotics, it may be able to resist their effects. More investigation into the underlying mechanisms of resistance is necessary to address the problem of antibiotic resistance in bacterial populations. Understanding these pathways better will help us create more potent plans to fight antibiotic resistance and guarantee that antibiotics will always be effective against bacterial infections. The mentioned study is a proof that the solving of this problem could be started with investigating the plants for any new source of antimicrobial agents at the same time plant-based substitutions are being promoted due to the emergence of a strong antibiotic resistance. Additional studies are necessary to identify the very molecular pathways and possible beneficial health effects of these plant-based compounds.

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