

<https://africanjournalofbiomedicalresearch.com/index.php/AJBR>

Afr. J. Biomed. Res. Vol. 28(1s) (January 2025); 2879-2894

Research Article

Assessment Of Antibiotic Susceptibility And Antibacterial Evaluation Of Bacterial Endophytes Isolated From *Andrographis Paniculata* And *Oroxylum Indicum*

Ms. Tisha Liza Tomy^{1*}, Dr. Edward A², Dr. Sr. Meena K Cheruvathur³

^{1*}Research scholar, Bharathidasan University

²Assistant Professor, St. Josephs College, Trichy

³Assistant Professor, St. Marys College, Thrissur

Received: 2025-01-02 Accepted: 2025-01-07

DOI: <https://doi.org/10.53555/AJBR.v28i1S.7295>

© 2025 The Author(s).

This article has been published under the terms of Creative Commons Attribution-Noncommercial 4.0 International License (CC BY-NC 4.0), which permits noncommercial unrestricted use, distribution, and reproduction in any medium, provided that the following statement is provided. "This article has been published in the African Journal of Biomedical Research"

INTRODUCTION

Native plants are used as traditional medicines in many nations to cure illnesses and injuries. It is a standard practice to employ leaves, roots, seeds, and fruits as alternative remedies in the Indian traditional medical system of Ayurveda and other related disciplines. The kokum tree, or *Garcinia indica*, yields fruits that are utilized in Ayurvedic medicine for their antibacterial, antiulcer, anticancer, and antiobesity qualities as well as its ability to reduce inflammation and discomfort. According to Deokate and Khadabadi (2012), the roots of the *Salacia chinensis* herb tree has also been used for its therapeutic qualities in the treatment of skin disorders, ulcers, tooth decay, and obesity. The Indian devil tree, *Alstonia scholaris*, has a variety of components that exhibit strong antibacterial and antioxidant qualities, including leaves, follicles, and latex. These antimicrobial and bioactive properties through the metabolites they produce have recently been attributed to the medicinal plant-associated microbiome, and in particular to the interaction between the complex community of endophytic microorganisms. Upon screening for bioactivity, endophytic bacteria extracted from conventional Chinese medicinal herbs utilized for anticancer treatment were shown to possess cytotoxic, antibacterial, or antifungal properties in a minimum of one assay.

Endophytes

Endophytes are endosymbionts, which means that they live inside a plant with no risk to the plant's life, nor does it cause any diseases. They are found inside most of the plant species. Some species of endophytes have found to increase host growth, nutrient acquisition and ability to withstand stresses like drought. Endophytes are generally either bacterium or fungi.

Recently, endophytic archaea are also studied for their plant growth promoting properties. Most of the endophytes are generally considered as mutualists. The endophytes are dependent on plants for their carbon requirements, well in return the plants are provided with resistance to issues like poor soil, quality, water and heat stress, salinity or pathogens and diseases. This is also not strictly true as endophytic fungi can sometimes turn into pathogens or saprotrophs when the plant is stressed. Many of the endophytes that live inside plants are known as symbionts, having a unique and cherished relationship with the plants they coexist with, despite the fact that they were long ignored and thought of as pathogen-causing contaminations. The presence of endophytes has been verified in recent research using fluorescence in situ hybridization-confocal laser scanning microscopy investigations and a variety of cultivation-independent tests. While cultivation-independent strategies search for differences in the whole endophytic populations, cultivation-based techniques rely on the recovery and testing of isolates.

Any microbial or plant growth, including agar, potato dextrose agar, and any medium containing carbon or nitrogen, may be used to efficiently isolate endophytes. The most common technique for identifying and counting endophytes is separation from host plant tissue that has been surface-sterilized. Numerous factors can influence the process of endophyte colonization in a plant or microbial species. These factors include the plant's genotype, growth stage, physiological status, type of tissues, soil environment, sampling season, surface sterility, selective media and culture conditions, and various agricultural practices.

Endophytes in a single plant or microorganism have been observed to create a multitude of bioactive compounds. These metabolites have potential use in the food, cosmetics, pharmaceutical, and agricultural industries. They have been a valuable source of medications for treating a wide range of disorders. Many variables, including the time of year samples are collected, the local climate, and the endophyte's location, influence the extraction of metabolites from the organism. However, extraction from plants and other natural sources is now more practical, effective, and convenient thanks to a ground-breaking synthetic approach that has been created in recent years. The evolution of the host microorganisms, which may have incorporated genetic information from higher plants, has been directly linked to the production of bioactive substances by endophytes. This adaptation has allowed the microorganisms to perform various functions, such as protection from grazing animals, insects, and various pathogens.

Endophytic fungi can also lessen oxidative stress because of their capacity to release bioactive compounds. Cellular processes may be hampered by the generation of radicals. Endophytic fungus may have an anti-oxidative effect because they secrete flavonoids and phenolic chemicals into the growing media. Previous research has shown that endophytes connected to medicinal plants are capable of producing chemical compounds that are bioactive and resemble hosts.

Endophytes can spread horizontally (from one unrelated individual to another) or vertically (straight from parent to child). Fungal hyphae that penetrate the host's seeds are the means by which asexual, vertically transmitted fungal endophytes—like *Neotyphodium*—transmit. These fungi are frequently mutualistic because their reproductive health is closely linked to that of their host plant. On the other hand, fungal endophytes that spread horizontally are sexual and propagate by spores that can be dispersed by insects or the wind. Horizontally transmitted endophytes are not harmful in and of themselves, although they are frequently closely linked to pathogenic fungus because they disseminate similarly to pathogens.

Because they keep harmful organisms from invading host plants, endophytes may be beneficial to them. A "barrier effect" is produced when endophytes extensively colonize plant tissue, outcompeting pathogenic organisms and preventing their establishment. Additionally, endophytes have the ability

to create substances that stop competitors—including harmful organisms—from growing.

It has been demonstrated that some bacterial endophytes promote plant development. Higher rates of water loss in leaves may be caused by fungal endophytes. Nonetheless, certain fungal endophytes aid in the heat and drought resistance of plants. Arbuscular mycorrhizal fungi are the main culprits in the widespread phenomena of plants using endophytic fungi as a means of defense.

It has been possible to isolate endophytic microbes from various plant sections. They were separated from stem, bark, leaf blade, petiole, and buds, as well as from scale primordia, meristem, and resin ducts, leaf segments with midrib and roots and other locations.

Based on phylogeny and life history traits, endophytic fungi have been divided into two broad groups: the nonclavicipitaceous endophytes (NC-endophytes), which can be recovered from asymptomatic tissues of non vascular plants, ferns and allies, conifers, and angiosperms, and the clavicipitaceous (C) endophytes, which infect certain grasses. While the C group contains just one functional class, NC-endophytes represent three separate classes based on host colonization and transmission, plant biodiversity, and fitness benefits provided to hosts.

Multiple plant growth-promoting and biocontrol features that can be released concurrently by endophytic bacteria make them multifunctional. As an illustration, the endophytic *Paenibacillus polymyxa* exhibited biocontrol capabilities against pathogenic fungi in addition to fixing nitrogen and solubilizing phosphorus. Different physical, molecular, and biochemical methods are employed by endophytic bacteria to exhibit and carry out a range of growth and biocontrol characteristics. Certain endophytic bacteria may be further studied and cultivated for commercial applications since they possess the majority of the characteristics that promote plant development and biological control, making them dependable agents for plant growth, reproduction, and protection. Copper, iron, zinc, and magnesium are among the crucial organic acid-metal complexes that are absorbed by the majority of beneficial endophytic bacteria. Plants are able to remove these metals from the microorganisms through the entry of endophytic bacteria into the roots of the plants. Conversely, introducing endophytic bacteria into plants might prevent the onset of illness symptoms that are brought on by pathogenic microorganisms including nematodes, fungus, bacteria, and viruses.

Promotion of plant growth and development by endophytic bacteria:

Nitrogen fixation is an important function of an endophyte rhizobium sp. bacteria in leguminous plants. This property is extensively studied for developing the kind of nitrogen fixation in non-leguminous plants using the bacterium. Phosphate solubilising is another property of the endophytic bacteria. Phosphate acts as a precursor for enzyme synthesis and for aiding plants resistance to diseases. This phosphate has to be

converted to a more soluble and accessible form to be used up for the mineralization of the plant. This is done by certain endophytic bacteria. Endophytic bacteria that are capable of solubilizing potassium do so by producing and releasing organic acids, including gluconic acid, tartaric acid, malic acid, and oxalic acid. These acids liberate available soluble potassium by dissolving the insoluble minerals. There have also been reports of potassium-solubilizing endophytic bacteria reducing other environmental pressures, such as salt stresses, and enhancing overall output. Endophytic bacteria also helps in the uptake of iron nutrient elements. When the iron content is low in the soil, ceratin bacteria release siderophores [iron chelating molecules of low molecular weight]. Siderophores has highly attracted towards iron and acts as a source of iron for plants under stress. Another notable activity of certain endophytic microorganisms is the release of certain plant growth promoting hormones or phytohormones under stressed conditions. Some of this may include abscisic acid, cytokinins, ethylene and gibberellic acids. Indole 3 acetic acid released by certain organisms is used for plant defence against pathogenic organisms.

In the past, endophyte identification was accomplished using biochemical tests for actinomycetes and bacteria, as well as morphological features for fungi and bacteria. The identification of microorganisms by ribosomal DNA Internal Transcribed Spacer (ITS) sequence analysis has become commonplace with the advancement of molecular biology. Evidence from ribosomal DNA (rDNA) ITS has been shown to be useful in resolving phylogenetic connections at lower levels, such as those between genera or species.

Andrographis paniculata

A commercially significant plant in the *Andrographis* genus is *A. paniculata*. It is an annual blooming plant that grows upright and branched. This plant grows well in roadside hedgerows, damp habitats, waste grounds, hillsides, plane lands, and farms. It may be cultivated in a garden as well. Forests, wastelands, and damp, shaded areas are ideal for their good development. Due to its sensitivity to salt, this plant's development is restricted under stressful situations, especially salinity stress, which has a significant negative impact on crop yield and plant growth. When grown, this plant may grow to a height of 30 to 110 cm. Its dark green stem measures 30 to 110 cm in length and 2 to 6 mm in diameter. It is quadrangular in shape, with wings at the angles of the juvenile sections and longitudinal furrows; the nodes are somewhat expanded. The petiole is extremely short, and the leaves are dark green, glabrous, opposite, decussate, lanceolate, whole edge, and venation pinnate. They are 2–12 cm long and 1–3 cm wide. The tiny blooms have a thin tube, five linear perianth calyxes, and a white corolla that is about 6 mm long and has rose-purple markings on its petals. The time for blooming and fruiting is from December to April. The fruits are tiny, compressed, linear-oblong, two-celled, odorless, upright capsules that are 1–2 cm long and 3–5 mm broad. The leaves have a strong bitter flavor, and the seeds are many, subquadrate, and have a yellowish-brown color. Various extracts and their secondary metabolites, including andrographolide, are among the natural compounds that have been researched in great detail. Aerial portions, leaves, roots, whole plants or callus are used to create the therapeutically active extracts or isolate the metabolites.



FIGURE 1. *Andrographis paniculata*

Andrographis paniculata is an appealing medicinal plant whose metabolites have attracted significant and growing research over the course of several decades. Known by many as "King of the bitters" or "Kalmegh," this annual plant is a member of the Acanthaceae family.

Distribution and uses:

It is extensively distributed throughout Southern and Southeast Asia, including Bangladesh, China, Hong Kong, Indonesia, Malaysia, Myanmar, Philippines, and Thailand. It is native to India and Sri Lanka. Typically, *A. paniculata*'s aerial portions, roots, or leaves are

employed independently. These plant parts are traditionally used in powder, infusion, or decoction form; they can be used alone or in combination with other medicinal plants to treat a variety of conditions, including alopecia, griping, irregular bowel habits, diabetes, scabies, respiratory tract infections, scabies, boils, skin eruptions, dysentery, diarrhea, enteritis, helminthiasis, herpes, peptic ulcer, skin infections (topically applied), and snakebites (topically applied). Through comprehensive examinations, modern science is concentrating on confirming the traditional claims made about this plant. Pharmacological properties of *A. paniculata* have been studied in vitro and in vivo using various extracts (acetone, chloroform, ethanol, hexane, methanolic or aqueous extract, for example) and isolated pure metabolites. These properties include antibacterial, antiviral, antifungal, antiparasitic, choleric, hypocholesterolemia, antiinflammatory, anti-hyperglycemic, hepatoprotective, anticancer, immunomodulatory, cardiovascular, anti-snake venom, anti-platelet aggregation, anti-fertility, carminative, and antipyretic.

In Malaysia, it's known as Hempedu Bumi. This medicinal plant included flavonoids, diterpenes, lactones, aldehydes, alkanes, and ketones. Their leaves include two beneficial chemical compounds: andrographolide and kalmeghin (Firdous et al., 2020). Apart from its extensive use as an immunostimulant, Kalmegh is said to possess anti-inflammatory, antipyretic, anti-malarial, anti-snake venom, antihepatotoxic, antimalarial, antibiotic, and anti-thrombogenic properties (Nayak, 2015). Tamil speakers refer to it as "Sirunangai" or "Siriyanangai" (Arunachalam and Gayathri, 2010). According to Masi et al. (2019), *A. paniculata* contains endophytic bacteria that have the potential to regulate and stimulate plant development. The main bitter components of this plant are the labdane type diterpene lactones, 14-deoxyandrographolide, 14-deoxy-11,12-didehydroandrographolide, neoandrographolide, and andrographolide (Rashid et al., 2018). Medicinal plants have a unique microbiome that might improve the potential for interaction with microbes because of the presence of several bioactive compounds. By fighting with microbial pathogens, triggering plant defense mechanisms, and secreting compounds that promote plant development such as auxins, bacterial volatiles,

and cytokinins, microorganisms that promote plant growth increase plant growth. Studies on endophytes and medicinal plants have shown that these plants' therapeutic qualities are derived from both the endophytes that live inside the plants and the chemicals present in the plants themselves.

Human diseases are known to exist in roughly 1400 species. Despite the seemingly high quantity, they represent fewer than 1% of all microbial species on earth. Despite making up fewer than 1% of all microorganisms, a seemingly benign microbe might occasionally become hazardous in certain situations, such as in an immunocompromised patient. The medical world is always looking into the best drugs to treat these bacteria that spontaneously change their behavior. Bioactive compounds and extracts from *A. paniculata* were studied in relation to a range of infections, including several that are resistant to antibiotics, such as

Streptococcus pneumoniae, *Pseudomonas aeruginosa*, *Shigella* spp., *Salmonella* spp., and *Candida* spp. *A. paniculata* extracts and/or their extracted pure components have been tested for their antibacterial activity against 59 different invasive microorganisms that we have found. There were 33 bacterial, 4 viral, 12 fungal, and 10 parasitic species among the classified microorganisms.

Oroxylum indicum

Oroxylum indicum, sometimes referred to as the Indian trumpet flower or shattered bones tree, is a semi-deciduous flowering tree in the Bignoniaceae family. It originates from the Indian subcontinent and is mostly found in the foothills of the Himalayas, reaching elevations of 1000–1200 meters above mean sea level. It is extensively dispersed over the Eastern and Western Ghats in India, as well as a significant portion of the country's northeast. Many colloquial names for *Oroxylum indicum* exist, including Bhatghila in Assamese, Indian Trumpet flower, Tree of Damocles, Midnight Horror in English, and Tentu in Gujarati. In Sanskrit, the term is Katvanga, Tuntuka, Shyonaka; in Hindi, Sonapata, Shyonak, and Sauna; in Nepali, Tatelo; in Marathi, Tetu, Tayitu; and in Tamil, Achi, Puta- Puspam, and Vengamaram. Anthocyanins, carotenoids, polysaccharides, phenolics, terpenoids, and other bioactive substances that are advantageous to humans are said to be abundant in this plant.



FIGURE 2. *Oroxylum indicum*

The deciduous tree *Oroxylum indicum* can reach a height of 12 to 18 meters and has a branching habit at the top of the tree. The tree has corky lenticels and greenish-brown bark.

The leaves are oblong to elliptic in form, 3–7 cm long, green in color, and pinnately complex. There are many of flowers, with the inside part being pale yellowish in color and the outside part being reddish purple. In order to draw pollinators, flowers also emit a foul-smelling scent. The plant produces flat, sword-shaped, woody, resilient fruits that are filled with tiny, flat seeds that have a papery, creamy yellow color.

Oroxylum indicum has long been used to prepare a number of Ayurvedic formulations, including Amritarista, Dantadyarista, Brahma Rasayana, etc., for the treatment of a variety of ailments. The root bark of *Oroxylum indicum* is used to treat stomatitis, nasopharyngeal carcinoma, and TB. It is beneficial for fevers, asthma, intestinal worms, vomiting, dysentery, and diarrhoea. It also aids in boosting appetite. The decoction of this plant's leaves is said to be effective in treating rheumatic fever, ulcers, and coughs. Additionally, it has been shown that the seeds can help with hypertension and throat infections. Studies on the fruit of *Oroxylum indicum* have shown that it protects the heart and throat and is useful in treating conditions including bronchitis and piles. *Oroxylum indicum* blossoms are mostly used as a vegetable. They are eaten in a variety of ways, including pakoras, soups, and curries. Freshly picked flowers have an unpleasant flavor, so it is best to blanch or boil them in boiling water for a few minutes to get rid of the bitterness before cooking them to your desired consistency. During their blossoming season, some farmers make some money by selling their freshly picked flowers at the neighborhood market.

India is a multicultural nation where a wide range of people live according to different cultures, faiths, traditions, etc. *Oroxylum indicum* is said to be very significant in a number of Buddhist cultures. The dried seeds of *Oroxylum indicum* are used by the majority of Buddhist believers in their priceless cultural and traditional rites, such as marriage ceremonies and religious ceremonies, indicating the plant's significant role in a number of religious and cultural contexts.

MATERIALS AND METHODS

SAMPLE COLLECTION

The specimens were diligently procured from Thrissur, encompassing leaves sourced from *Oroxylum indicum* and leaves, roots, and shoots harvested from *Andrographis paniculata* plants. Following collection, each sample was carefully sealed within a plastic bag to uphold its pristine condition and safeguard against any external contaminants. Subsequently, the samples were expeditiously stored in an ice-packed container, ensuring optimal preservation of their inherent properties during transit to the laboratory. This rigorous adherence to protocol underscored the commitment to maintaining the integrity and richness of the collected specimens, thus laying a robust foundation for

subsequent analysis

SAMPLE PREPARATION

The methodology involved in this investigation comprised the systematic acquisition of *Andrographis* roots and shoots in conjunction with *Oroxylum* leaves. Each botanical specimen underwent rigorous purification, employing distilled water to eliminate any extraneous contaminants, prior to placement in distinct glass beakers. Subsequently, a precise volume of Tween-20 and distilled water was introduced into each beaker, followed by agitation for a predetermined duration spanning one to two minutes. This iterative washing procedure was executed fivefold to ensure comprehensive purification. Following this initial phase, the specimens underwent treatment with 90% ethanol, diluted to a concentration of 75%, and were subsequently subjected to rinsing for a duration ranging from one to one and a half minutes, thereby ensuring the complete removal of residual surfactant residues. Subsequent to ethanol treatment, the specimens were subjected to mercuric chloride treatment for a duration of one minute, followed by sequential rinsing with water, comprising five cycles of rinsing, followed by an additional seven rinses. Subsequent to these preparatory stages, the specimens were meticulously dissected into minute fragments, employing precision forceps, and carefully transferred onto a nutritive medium housed within individual Petri plates. In parallel, control plates were meticulously assembled for each specimen, thereby facilitating rigorous comparative analysis throughout the experimental continuum.

ISOLATION OF BACTERIA

The leaves and roots were meticulously dissected into small fragments using a sterile scalpel, minimizing the risk of external contamination. These excised plant segments were then aseptically transferred onto nutrient agar medium in Petri dishes, providing an optimal environment for the proliferation of endophytic bacteria residing within the plant tissues. Following inoculation, the Petri dishes containing the samples were securely sealed and subjected to incubation at a controlled temperature of 37°C for a maximum duration of 48 hours, facilitating the growth and development of bacterial colonies. To ascertain the absence of extraneous microbial contamination, a control plate comprising solely nutrient agar was included for reference purposes. Upon completion of the incubation period, bacterial colonies appearing on the agar plates were meticulously streaked to isolate individual colonies, employing sterile techniques to ensure the maintenance of purity. Subsequently, isolated colonies were subcultured onto fresh agar plates, thereby enabling the procurement of pure cultures and the preservation of distinct bacterial strains for subsequent characterization and analysis. This methodical approach serves as a robust framework for the systematic isolation and cultivation of endophytic bacteria from plant tissues, facilitating comprehensive investigations into their diversity, ecological significance, and potential

applications.

IDENTIFICATION OF BACTERIA

Gram Staining

Gram staining is a differential staining technique used to differentiate bacteria into two groups based on their cell wall composition. Gram-positive bacteria typically appear purple or blue, while gram-negative bacteria usually appear pink or red after gram staining.

Reagents Used

- Crystal violet (primary stain)
- Iodine solution or Gram's iodine solution (mordant)
- Decolorizer (e.g., ethanol)
- Safranin (secondary stain)
- Water

Staining Procedure

1. Prepare a slide with the cell sample to be stained.
2. Heat-fix the sample to the slide by passing it through a Bunsen burner flame three times.
3. Add the primary stain to the sample or slide and incubate for 1 minute.
4. After incubation with the primary stain, rinse the slide with water to remove excess stain.
5. Apply the mordant (iodine solution or Gram's iodine solution) to the slide and allow it to stand for 1 minute to fix the stain to the bacterial cells.
6. Decolorize the slide by gently flooding it with the decolorizer (e.g., ethanol) for a few seconds until the runoff becomes clear.
7. Quickly rinse the slide with water to stop the decolorization process.
8. Counter stain the slide with the secondary stain (safranin) and allow it to stand for 1 minute.
9. Rinse the slide with water to remove excess stain and allow it to air dry.
10. Examine the slide under a microscope to observe the coloration of the bacterial cells, distinguishing between gram-positive and gram-negative bacteria based on their characteristic colors.

BIOCHEMICAL TESTS

A series of four biochemical tests known as the IMViC tests are used to identify and distinguish members of the Enterobacteriaceae family according to their metabolic traits. The abbreviation represents the tests for the usage of citrate, methyl red, voges-proskauer, and indole. All of these assays evaluate different metabolic pathways, such as fermentation of glucose, acetoin synthesis, tryptophan metabolism, and citrate consumption. These tests allow us to differentiate between several Enterobacteriaceae species of bacteria, which helps with species identification and classification.

Indole Test:

The indole test constitutes a fundamental biochemical assay employed to ascertain the enzymatic capacity of bacteria to synthesize indole from tryptophan, an essential amino acid. Indole production is closely associated with the enzymatic action of tryptophanase,

which facilitates the hydrolysis of tryptophan into indole, pyruvic acid, and ammonia. Methodologically, bacteria are introduced into a growth medium enriched with tryptophan, such as tryptone broth, and incubated to facilitate microbial growth and enzymatic activity. Subsequently, Kovac's reagent, comprising p-dimethylaminobenzaldehyde, is introduced into the medium. The ensuing reaction between indole and Kovac's reagent yields a discernible red coloration, serving as an affirmative indicator of indole production. Conversely, the absence of a color change denotes a negative outcome. Widely employed in microbiological investigations, particularly within the Enterobacteriaceae family, the indole test assumes significance in delineating bacterial taxonomy and species differentiation.

Methyl Red (MR) Test:

The methyl red (MR) test is a pivotal diagnostic tool utilized to discern the metabolic proficiency of bacteria in generating stable acids, notably mixed acids, through glucose fermentation. The principle underpinning this assay hinges upon the distinctive pH response of methyl red, which retains its red hue at pH levels below 4.4 while transitioning to a yellow hue at pH levels exceeding 6.2. Operationally, bacteria are inoculated into MR-VP broth and subjected to a designated incubation period of 48 hours to foster fermentative processes. Following incubation, methyl red indicator is introduced into the broth, with the emergence of a red color signifying a positive outcome indicative of substantive acid production via glucose fermentation.

Voges-Proskauer (VP) Test:

The Voges-Proskauer (VP) test is a discerning analytical method employed to detect the biosynthesis of acetoin, a pivotal precursor to 2,3-butanediol, during glucose fermentation processes. Acetoin engenders a distinctive red coloration upon reacting with alpha-naphthol and potassium hydroxide (KOH) in the presence of oxygen. Operationally, bacteria are introduced into MR-VP broth and allowed to ferment for 48 hours. Subsequently, the addition of alpha-naphthol reagent and KOH solution to the broth culminates in the development of a red color within 15–30 minutes, thereby affirming the presence of acetoin and yielding a positive outcome.

Citrate Test:

The citrate test serves as a quintessential analytical modality for gauging the metabolic competency of bacteria in harnessing citrate as their solitary carbon source for proliferation. Citrate undergoes enzymatic metabolism catalyzed by citrate-permease and citrate lyase enzymes, yielding pyruvic acid and precipitating a consequent elevation in the medium's pH. Methodologically, bacteria are streaked onto a Simmons citrate agar slant and incubated for a stipulated duration of 24–48 hours. Post-incubation, observations pertaining to growth dynamics and color alterations are meticulously recorded. A positive outcome, typified by

discernible growth and a distinctive blue hue, underscores the successful utilization of citrate. Conversely, the absence of discernible growth or color changes post- incubation denotes a negative outcome, indicative of the incapacity to exploit citrate as a carbon source.

MOLECULAR IDENTIFICATION

The molecular identification of endophytic bacteria through 16S rRNA sequencing utilizing Sanger sequencing encompassed a series of methodological steps, including the isolation of bacteria from plant tissues, the extraction of genomic DNA, PCR amplification targeting the 16S rRNA gene region, the purification of resulting PCR products, Sanger sequencing, and subsequent comparative analysis against reference databases.

SECONDARY METABOLITES ISOLATION

The systematic extraction of secondary metabolites from bacterial subcultures, obtained from *Andrographis* root and shoot in addition to *Oroxylum* leaf, follows a rigorously structured protocol. Initially, the bacterial subculture is initiated by inoculating the desired bacterial strain into nutrient broth, fostering growth under optimal conditions until the desired phase is achieved. Subsequent centrifugation at 6000 rpm for 10 minutes effectively segregates bacterial cells, forming a distinct pellet, while retaining a supernatant enriched with secondary metabolites. Post- centrifugation, precise removal of the supernatant yields the bacterial cell pellet, facilitating the isolation of extracted secondary metabolites using ethyl acetate as the solvent. This extraction method allows for subsequent analysis or downstream applications, including purification, characterization, or biological assays, thereby propelling advancements in diverse research domains.

Gas chromatography – Mass Spectroscopy

For compound identification, Gas Chromatography-Mass Spectrometry (GC-MS) analysis is a cornerstone technique. Gas chromatography effectively separates individual sample components based on their volatility, while mass spectrometry discerns compounds by scrutinizing their molecular mass and fragmentation pattern. In this process, the sample undergoes vaporization and injection into the gas chromatograph, where it traverses a column propelled by an inert gas. Subsequently, as the components navigate through the column, they segregate contingent upon their interactions with the column material. These separated compounds then enter the mass spectrometer, where they undergo ionization and fragmentation. The ensuing mass spectra undergo meticulous scrutiny for compound identification, leveraging characteristic fragmentation patterns and retention times delineated in the chromatogram.

ANTIBIOTIC SUSCEPTIBILITY TEST

Antibiotic susceptibility testing serves as a crucial method employed to ascertain the efficacy of antibiotics

against bacterial isolates. This investigation utilized two distinct agar media: Muller-Hinton agar (MHA) and nutrient agar. Bacterial cultures sourced from *Andrographis* root and shoot, alongside *Oroxylum* leaf samples in nutrient broth, served as the experimental substrate. Notably, bacterial cultures from leaf, root, and shoot samples were diligently inoculated onto separate agar plates to discern any potential variation in antibiotic susceptibility across plant parts. Antibiotic discs encompassing ampicillin, gentamicin, penicillin, and tetracycline were administered on the nutrient agar, while amoxicillin, chloramphenicol, ciprofloxacin, gentamicin, and tetracycline discs were applied on MHA agar.

The antibiotic susceptibility test entails inoculating the agar medium with the bacterial culture from the samples, followed by the placement of antibiotic discs onto the agar surface. Subsequent incubation permits bacterial growth, and assessment post-incubation focuses on the presence or absence of zones of inhibition encircling the antibiotic discs. The zone of inhibition demarcates the area surrounding the antibiotic disc wherein bacterial growth is impeded or diminished. The emergence of a zone of inhibition signifies the antibiotic's effectiveness in hindering bacterial growth within the sample. Moreover, the magnitude of the zone of inhibition serves as a qualitative indicator of the bacterial isolate's susceptibility to the antibiotic. A larger zone heralds enhanced susceptibility, while a diminutive or non-existent zone indicates potential resistance of the bacterial isolate to the antibiotic. Notably, the absence of a zone of inhibition underscores the bacterial isolate's resistance, underscoring the antibiotic's ineffectiveness in suppressing bacterial growth.

ANTIMICROBIAL SUSCEPTIBILITY TEST

The antimicrobial susceptibility assay, a pivotal technique in microbiological research, elucidates the susceptibility of microorganisms to specific antimicrobial agents such as antibiotics or antifungals. This assay was conducted using meticulously prepared nutrient agar plates designed to evaluate microbial responses to various antimicrobial compounds. Bacterial isolates derived from root, shoot, and leaf samples were tested against *Escherichia coli*, *Bacillus*, *Pseudomonas*, and *Proteus*. Sterile Whatman filter paper discs, saturated with each microbial broth, were meticulously placed onto the Petri plates using forceps. The absence of bacterial growth surrounding these discs delineated discernible zones of inhibition, serving as a quantitative measure of the antibacterial efficacy of the tested agents.

RESULTS

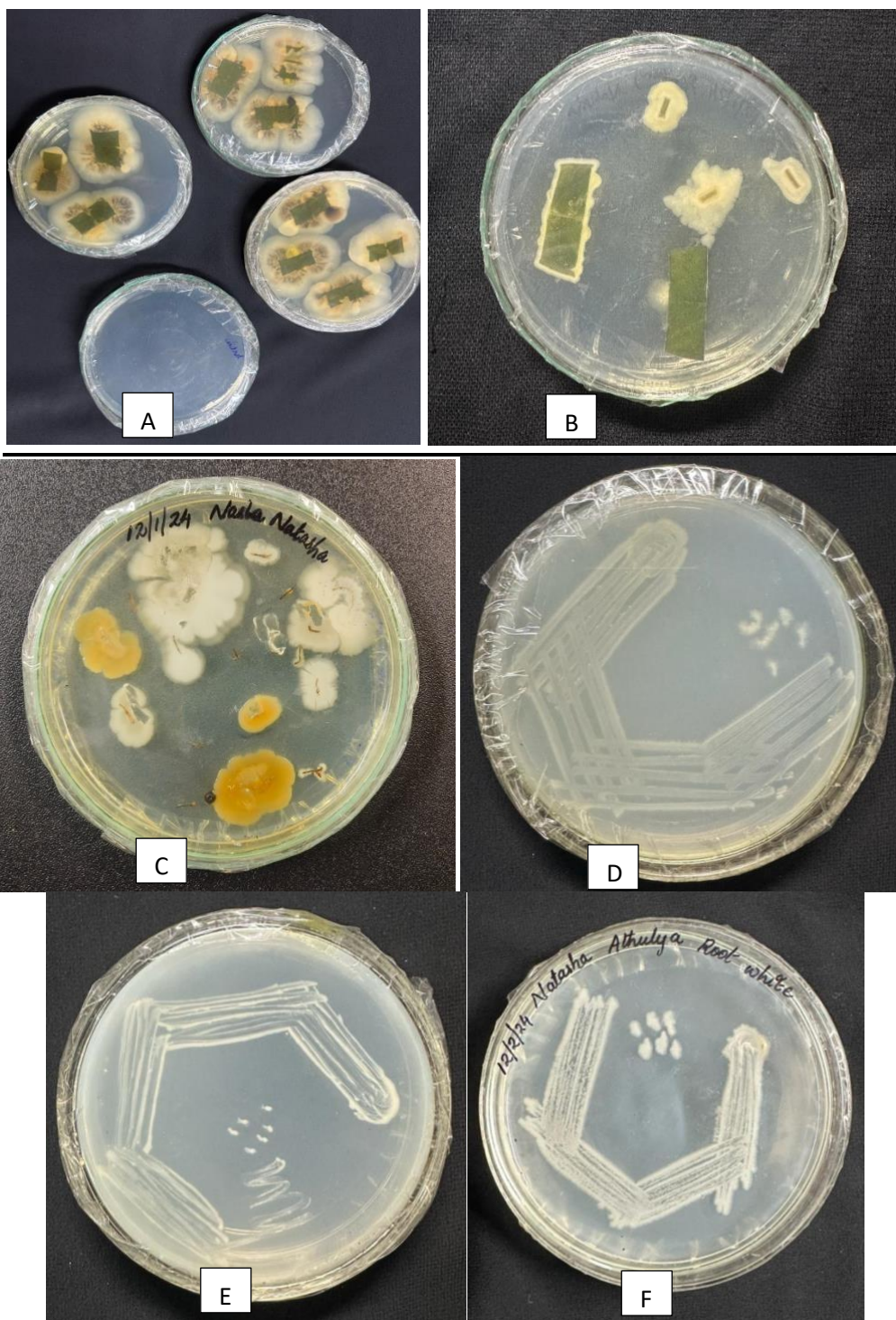
ISOLATION OF ENDOPHYTES

The leaves of *Oroxylum indicum*, and shoot and root of medicinal plant *Andrographis paniculata* were utilized for the isolation of bacterial and fungal endophytes. The surface sterilization protocol followed successfully eradicated epiphytic microbes as the control plate

Assessment Of Antibiotic Susceptibility And Antibacterial Evaluation Of Bacterial Endophytes Isolated From *Andrographis Paniculata* And *Oroxylum Indicum*

remained devoid of growth till nine days thus confirming the isolated strains were endophytic microorganisms. In this study, both bacterial and fungal strains were isolated. However, the research focused primarily on the bacterial endophytes despite the presence of fungal isolates on the medium. One bacterial isolate was obtained from leaf of *O.indicum* and shoot

of *A.paniculata*. Similarly two endophytic bacterial isolates were obtained from the roots of *A.paniculata* in which one of them was pigmented (orange) while the other remained non-pigmented/ white. The obtained bacterial endophytes were maintained by subsequent sub-culturing using quadrant streaking method.



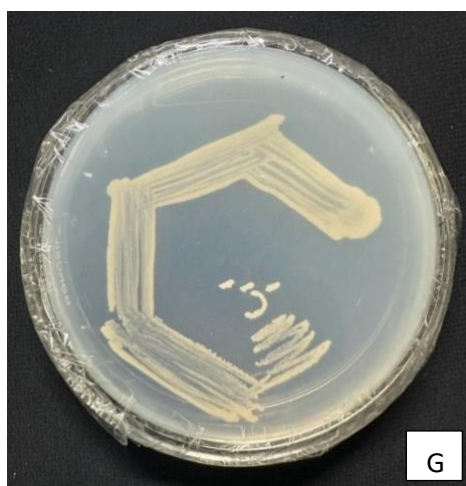


FIGURE 3. Isolation of endophytes (A) Fungal and bacterial growth on plant parts, (B) Bacterial endophytic growth on leaves of *O.indicum* and shoot of *A.paniculata*, (C) Pigmented and Non-pigmented bacterial endophytic growth on root of *A.paniculata* (D) Streak plate result of leaf of *O.indicum* (E) Streak plate result of shoot of *A.paniculata* (F) Streak plate result of root of *A.paniculata* (G) Streak plate result of root of *A.paniculata*

IDENTIFICATION OF ENDOPHYTIC BACTERIA

IDENTIFICATION USING GRAM STAINING

Gram staining was employed to classify the isolated bacterial endophytes based on their morphological and physiological characteristics. Results yielded a distinctive pattern of non-motile, non-spore forming, and gram-negative bacteria. The microscopic examination revealed that all four of the isolates

exhibited a consistent gram negative staining pattern indicating the presence of thin peptidoglycan layer in their cell wall. In addition, the pink coloured gram-negative bacterial isolate from the shoot of *A.paniculata* revealed a diverse array of rod-shaped structures with variations in sizes and cellular arrangements. They also produced a white milky colony with a smooth surface on nutrient agar medium.

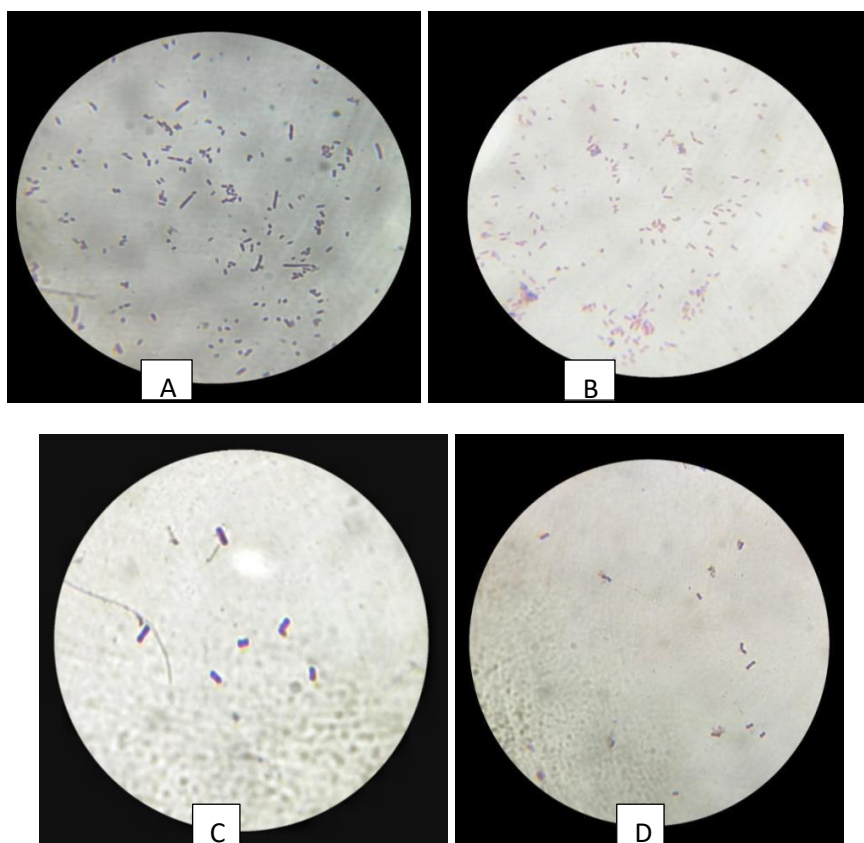


FIGURE 4. Result of gram staining under microscope with magnification 100x from (A) Leaf of *O.indicum*, (B) shoot of *A.paniculata* (C) Root of *A.paniculata* (Non-pigmented), (D) Root of *A.paniculata* (Pigmented)

BIOCHEMICAL IDENTIFICATION

IMViC TEST

In the biochemical characterization process, leaf of *O.indicum* and shoot of *A.paniculata* demonstrated positive results for citrate utilization test and negative for all the other three biochemical tests but bacterial isolates from root of *A.paniculata* demonstrated negative results for all four of the IMViC test. The negative findings suggest that the bacteria lack the metabolic pathways associated with those particular tests.

INDOLE TEST

Indole test carried out demonstrated negative results for all four endophytic bacterial isolates as there was no colour change observed even after the addition of Kovac's reagent to the culture media. This result suggests that the organisms present lack the enzyme tryptophanase which are required to degrade tryptophan into indole.

METHYL RED TEST

The isolated bacteria from leaf shoot and root tissues exhibited no colour change in the medium indicating a negative result. Typically, methyl red turns red in acidic environment or in low pH (<4.4) due to the presence of stable acidic end products from glucose fermentation. If the pH of the medium remains above 4.4, methyl red will not change color. This absence of color change suggests that the organism does not produce sufficient

amounts of mixed acids from glucose fermentation to lower the pH of the medium to the required level for a positive methyl red reaction.

VOGES-PROSKAUER TEST

No color change was observed after the addition of alpha-naphthol and potassium hydroxide reagents thereby suggesting a negative result. This absence of color change to red indicates that the organism being tested does not produce sufficient acetoin from glucose fermentation to yield a positive Voges-Proskauer reaction.

CITRATE UTILISATION TEST

The isolated endophytes from the leaf of *O.indicum* and shoot of *A.paniculata* changed the colour of the medium from green to blue which indicates a positive result. However the non-pigmented and pigmented bacterial isolates from the root of *A.paniculata* exhibited no colour change in the medium thereby indicating a negative result. A positive result suggests that the organism is capable of utilizing citrate as its sole carbon source, it will grow on the medium containing citrate, and the pH of the medium will increase due to citrate metabolism, resulting in a color change from green to blue due to the production of alkaline byproducts whereas a negative result indicates indicate that the organism lacks the enzymes necessary for citrate metabolism or that it prefers other carbon sources.

Table 1 Biochemical characterization of endophytic bacteria

Plant part	Indole test	Methyl red test	Voges- Proskauer test	Citrate utilization test
Leaf	-	-	-	+
Shoot	-	-	-	+
Root (white)	-	-	-	-
Root (Orange)	-	-	-	-

+indicates positive result; - indicates negative result.

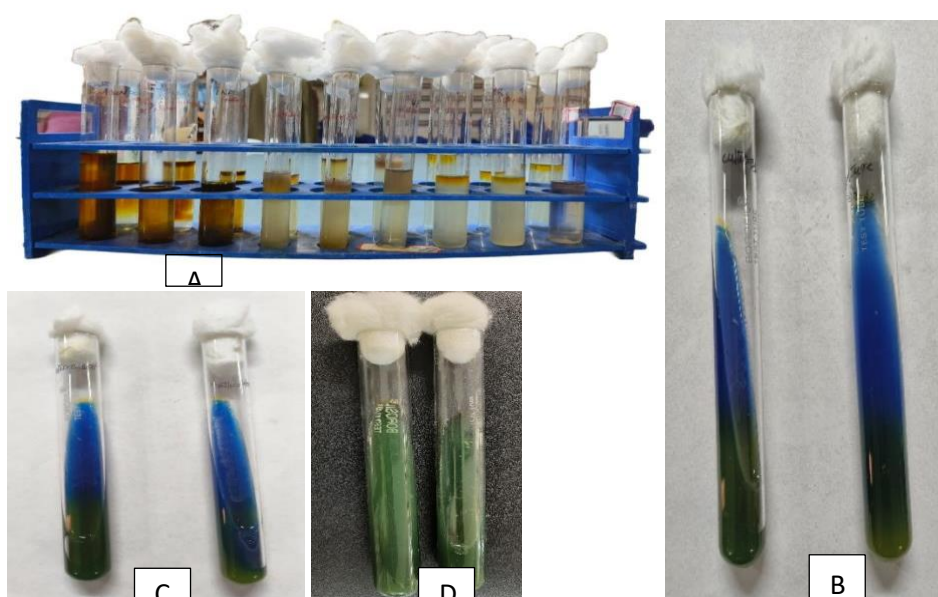


FIGURE 5. Results of IMViC tests (A)Negative results of Indole test, Methyl red test, and Voges-Proskauer test of all four endophytes, (B)Positive Citrate test of leaf of *O.indicum*, (C)Positive citrate test of shoot of *A.paniculata* (D)Negative citrate test of root of *A.paniculata*

MOLECULAR IDENTIFICATION

The molecular identification of a bacterial isolate from the shoot of *A. paniculata* using 16S rRNA sequencing revealed a close alignment with *Agrobacterium pusense*. This finding suggests a potential symbiotic relationship or colonization pattern in the specific plant tissue, emphasizing the presence of *Agrobacterium pusense* in *A. paniculata* shoot tissues. Furthermore, this result opens avenues to explore the pathogenic potential and mechanisms underlying plant tumor induction by *Agrobacterium pusense*. The unique accession number obtained from GenBank after submitting sequence is **PP461838**. The provided FASTA base pair sequence related to this research is as follows:

```
>T94_BACFEB_12_24
GCCCCGCAAGGGGAGTGGCAGACGGGTGAGTA
ACGCGTGGGAACATACCTTTCCTGCGG
AATAGCTCCGGGAAACTGGAATTAATACCGCA
TACGCCCTACGGGGGAAAGATTTATCGG
GGAAGGATTGGCCCGCGTTGGATTAGCTAGTTG
GTGGGGTAAAGGCCTACCAAGGCGACG
ATCCATAGCTGGTCTGAGAGGATGATCAGCCA
CATTGGGACTGAGACACGGCCCAAACCTC
TGCGAGACTTAACCCAAACATCTCACGACACGA
GCTGACGACAACCATGCACCACCT
GTCACCTCTGTCCCCCGAAGGGGAACGCTCTATC
TCTAGAGTTGTCTAGAGGATGTCAAGAC
CTGGTAAGGTTCTTCGCGTTGCTTCGAATTA
CCACATGCTCCACCGCTTGTCGGGGC
AAATCCCGCAGCTCAACTGCGGAACGCTTTG
ATACTGGGTATCTTGAGTATGGAAGAG
GTAAGTGGAATTCCGAGTGTAGAGGTGAAATT
CGTAGATATTCGGAGGAACACCAAGTGGC
GAAGGCGGCTTACTGGTCCATTACTGACGCTGA
GGTGCGAAAGCGTGGGGAGCAAACAGG
ATTAGATACCCTGGTAGTCCACGCCGTAAACGA
TGAATGTTAGCCGTCGGGCAGTATACT
GTTTAAACATTCCGACGGTCGCAAGATTAAAC
TCAAAGGAATTGACGGGGGCCCAACGCG
```

CAGAACCCTTACCAGCTCTTGACATTCGGGGTA
TGGG

This sequence represents genetic information that aligns with the research findings on *Agrobacterium pusense* and its potential interactions with *A. paniculata* shoot tissues.

ANTIBIOTIC SUSCEPTIBILITY TEST

The antibiotic susceptibility patterns of all endophytic bacterial isolates were determined using the disc diffusion method, specifically the Kirby Bauer method, which involved testing seven antibiotics: Ampicillin, Amoxicillin, Chloramphenicol, Ciprofloxacin, Gentamicin, Penicillin, and Tetracycline. The results revealed that the endophytic bacterium isolated from the leaf of *O. indicum* was resistant to four antibiotics namely; Ampicillin, Amoxicillin, Gentamicin, and Penicillin, showing no zone of inhibition. However, it exhibited susceptibility to Chloramphenicol, Ciprofloxacin, and tetracycline with a zone of inhibition measuring 7mm, 26mm and 7mm respectively.

The endophytic bacterial isolate from the shoot of *A. paniculata* demonstrated susceptibility to Amoxicillin, Ciprofloxacin, Gentamicin, Tetracycline, and Penicillin while exhibiting resistance to Ampicillin. Additionally, it showed intermediate resistance to Chloramphenicol, with a zone of inhibition measuring 14mm.

The non-pigmented/white bacterial isolate from root tissue of *A.paniculata* exhibited susceptibility to Chloramphenicol, Ciprofloxacin, Gentamicin, Penicillin and Tetracycline, but showed resistance to Ampicillin and Amoxixillin. On the other hand, the pigmented/orange bacterial isolate from the root tissue of *A.paniculata* exhibited susceptibility to Amoxicillin, Chloramphenicol, Ciprofloxacin, Gentamicin, and Tetracycline, while demonstrating resistance to Ampicillin. However, it displayed intermediate resistance to Penicillin, with a zone of inhibition measuring 13mm.

Table 2 Antibiotic susceptibility pattern of endophytic bacteria (zone size in mm)

Plant part	Antibiotic sensitivity (inhibition zone in mm)						
	Ampi cillin	Amox icillin	Chloramph enicol	Ciproflo xacin	Genta micin	Penici llin	Tetracy cline
Leaf	0	0	7	26	0	0	7
Shoot	0	27	14	29	22	8	15
Root (White)	0	0	19	27	26	7	18
Root (Orange)	0	14	27	28	21	13	20

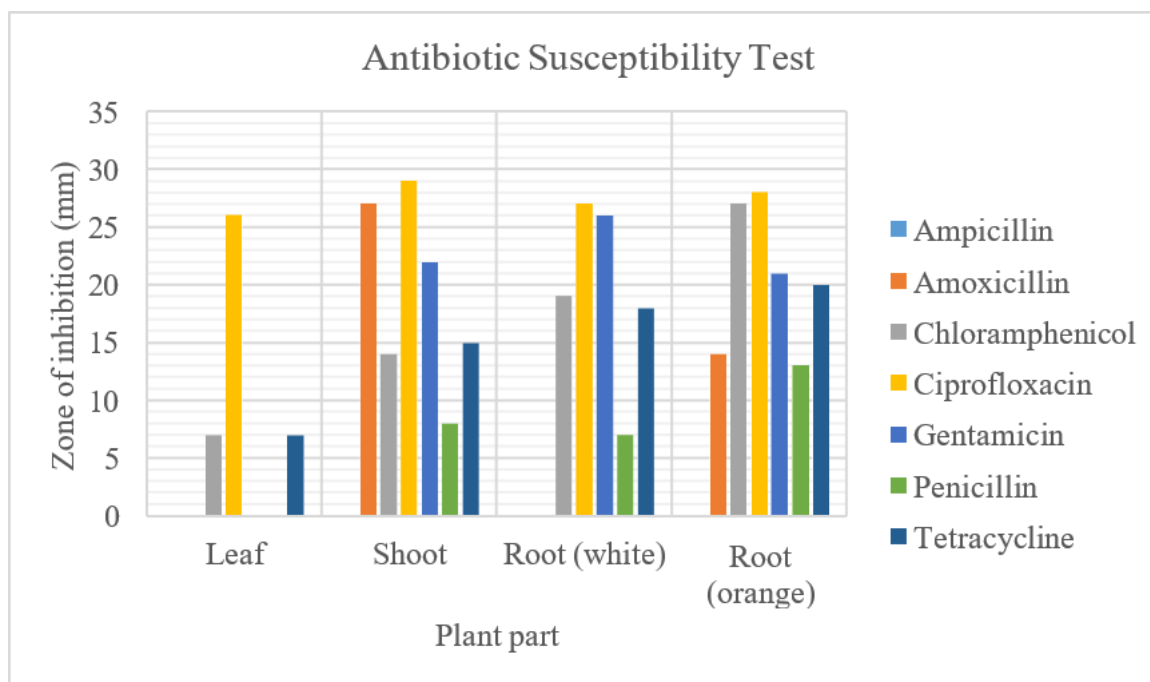
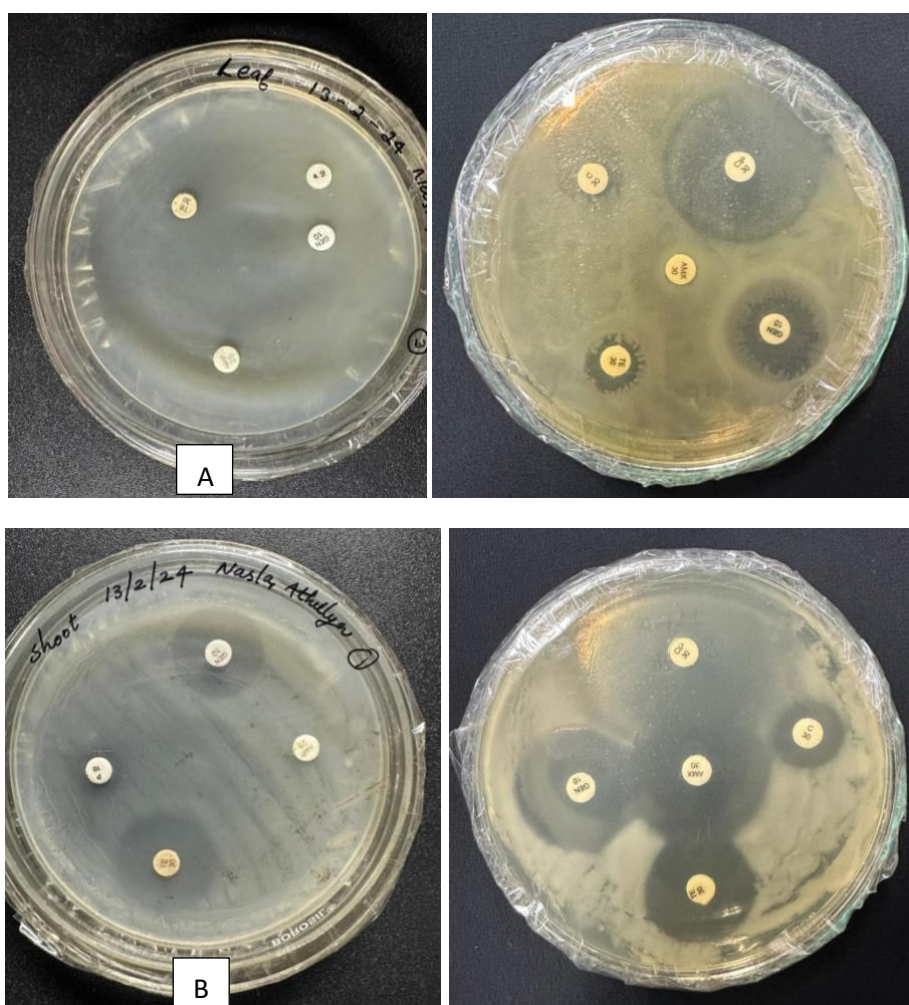


FIGURE 6. Antibiotic susceptibility pattern of various bacterial isolates



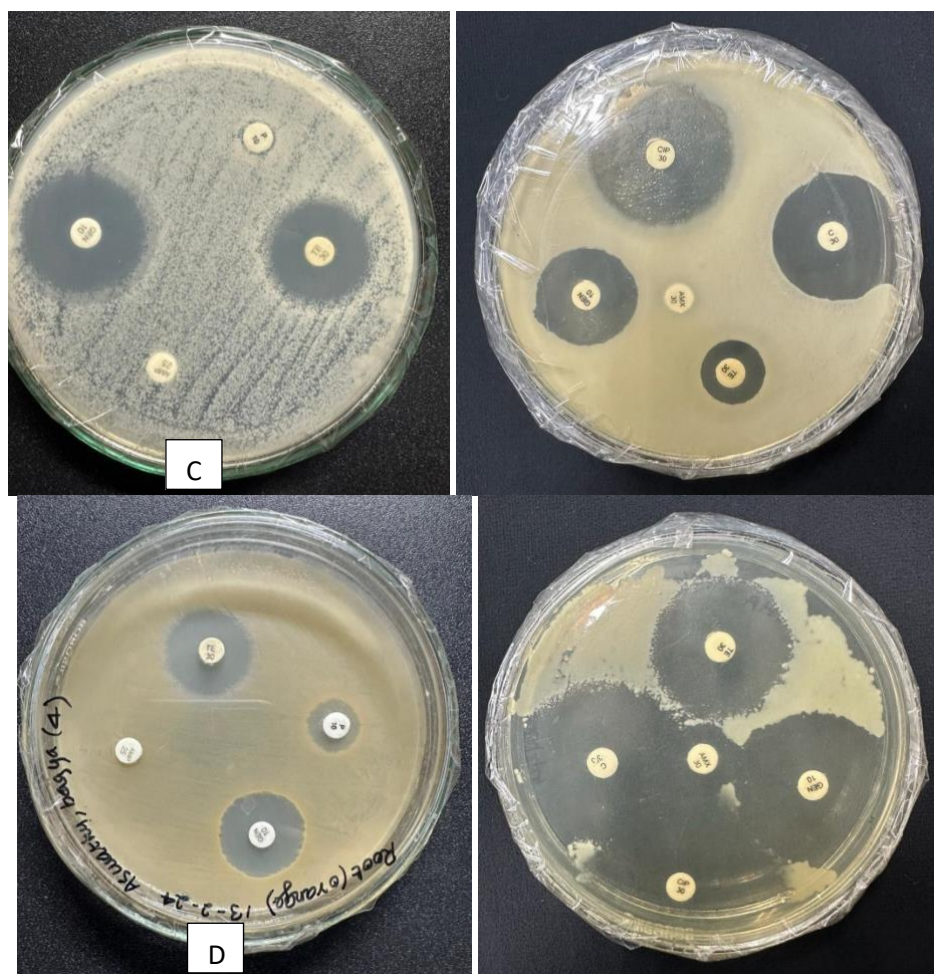


FIGURE 7. Antibiotic susceptibility test results of (A) leaf of *O.indicum*, (B)shoot of *A.paniculata*, (C)root of *A.paniculata*(non-pigmented), (D)root of *A.paniculata* (pigmented)

ANTIMICROBIAL SUSCEPTIBILITY TEST

The antimicrobial properties of isolated endophytic bacteria were evaluated against various strains of gram-positive Bacilli, gram-negative *E.coli*, *Proteus*, and *Pseudomonas*. The isolated bacterial endophytes demonstrated a broad spectrum of antibacterial activity against these microbes, which were identifiable by the formation of zones of inhibition. Specifically, the bacterial isolate from the leaves of *O.indicum* exhibited

resistance to all four strains of microorganisms as there was no formation of zone of inhibition. In contrast, the bacterial isolate from the shoots of *A.paniculata* was susceptible to all four strains with a zone of inhibition measuring 8mm. Furthermore, both bacterial isolates from the roots of *A.paniculata* showed resistance against most tested strains. Notably, the pigmented endophyte displayed intermediate resistance to the *Bacillus* strain with the zone of formation measuring 8mm.

Table 3 Antimicrobial activity of isolated endophytic bacteria

Plant Part	Inhibition zone in mm			
	Test organisms			
	<i>Bacillus</i>	<i>Escherichia coli</i>	<i>Proteus</i>	<i>Pseudomonas</i>
Leaf	0	0	0	0
Shoot	7	7	8	7

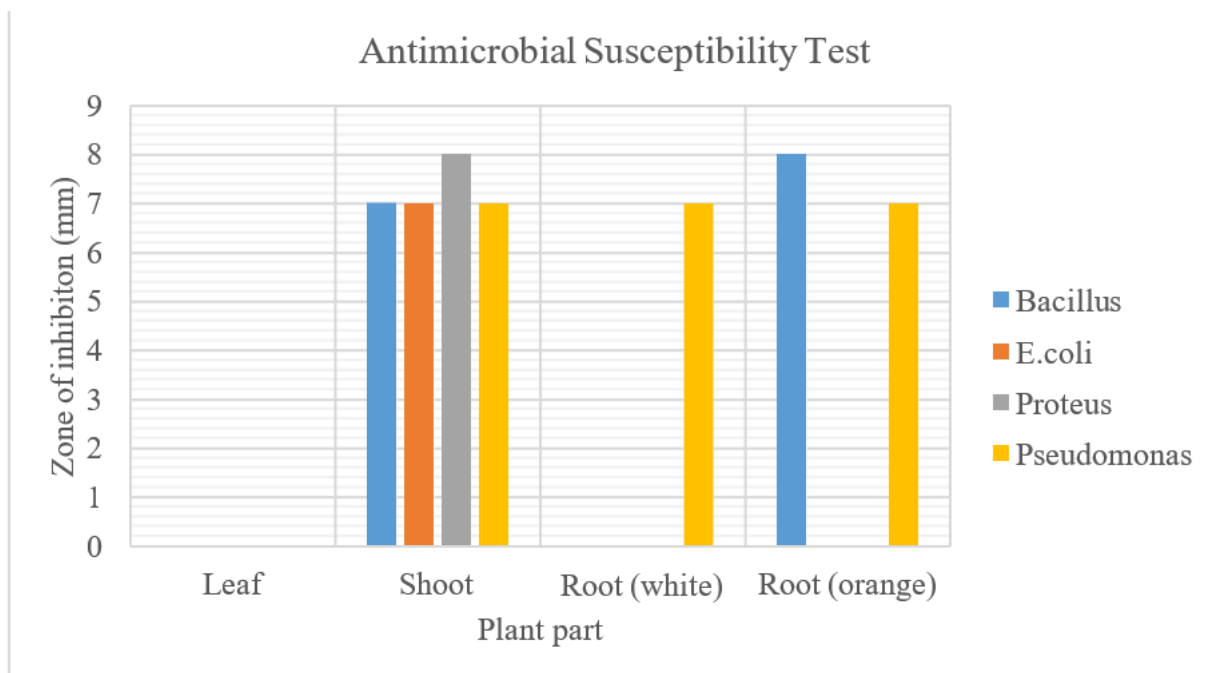
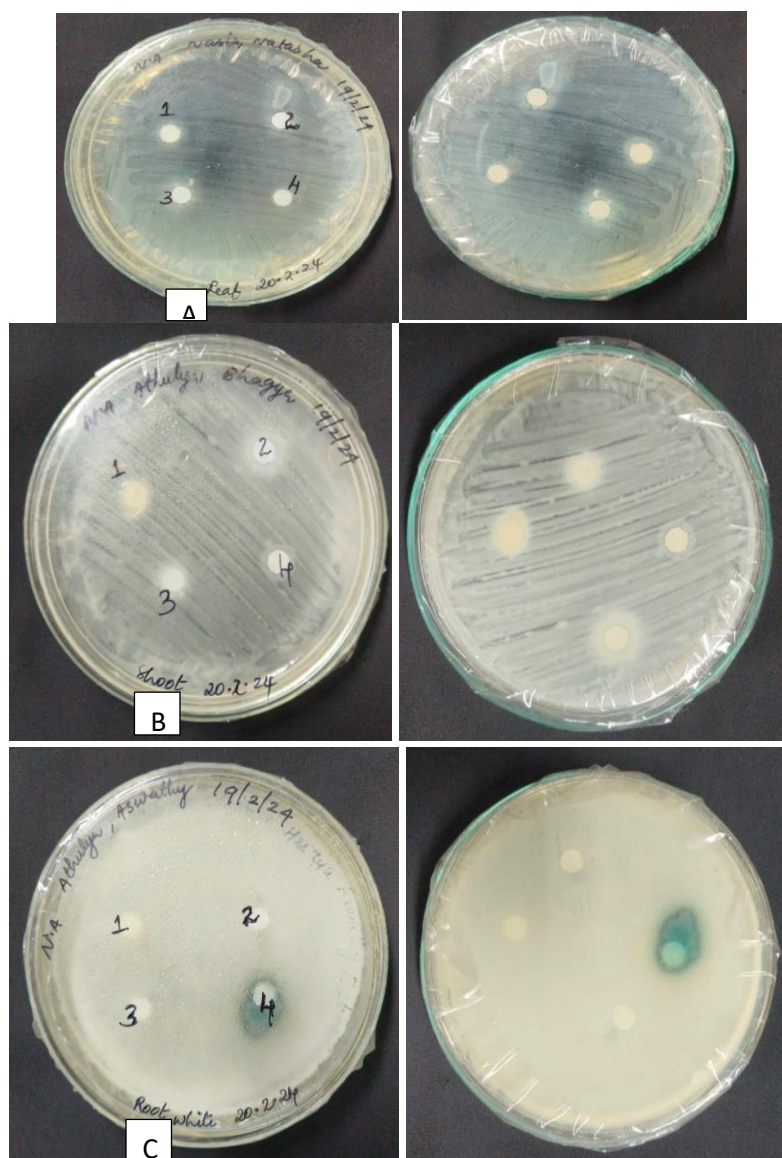


FIGURE 8. Antimicrobial activity of bacterial isolates from various plant parts showing zone of inhibition



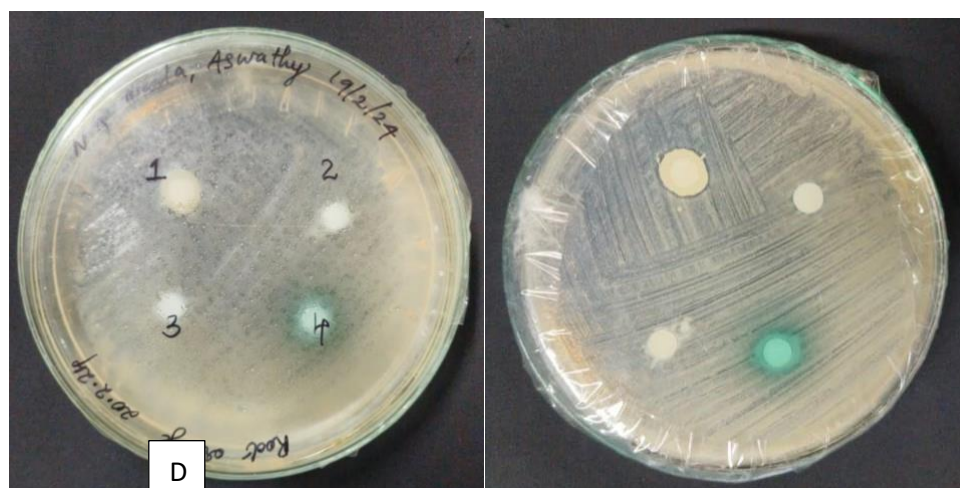


FIGURE 9. Antimicrobial activity of selected strains of endophytic bacteria (A) Leaf of *O.indicum*, (B) Shoot of *A.paniculata*, (C) Root of *A.paniculata* (Non-pigmented), (D) Root of *A.paniculata* (Pigmented) against test organisms such as 1: *Bacillus*, 2: *E.coli*, 3: *Proteus*, and 4: *Pseudomonas*

REFERENCE

1. Banihashemian, S. N., Jamali, S., Golmohammadi, M., & Ghasemnezhad, M. (2022). Isolation and identification of endophytic bacteria associated with kiwifruit and their biocontrol potential against *Meloidogyne incognita*. *Egyptian Journal of Biological Pest Control*, 32(1). <https://doi.org/10.1186/s41938-022-00601-y>
2. Beiranvand, M., Amin, M., Hashemi-Shahraki, A., Romani, B., Yaghoubi, S., & Sadeghi, P. (2017). Antimicrobial activity of endophytic bacterial populations isolated from medical plants of Iran. *Iranian Journal of Microbiology*, 9(1), 11–18. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5533999/>
3. De Oliveira Costa, L. E., de Queiroz, M. V., Borges, A. C., de Moraes, C. A., & de Araújo, E. F. (2012). Isolation and characterization of endophytic bacteria isolated from the leaves of the common bean (*Phaseolus vulgaris*). *Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology]*, 43(4), 1562–1575. <https://doi.org/10.1590/S1517-838220120004000041>
4. Dinda, B., SilSarma, I., Dinda, M., & Rudrapaul, P. (2015). *Oroxylum indicum* (L.) Kurz, an important Asian traditional medicine: From traditional uses to scientific data for its commercial exploitation. *Journal of Ethnopharmacology*, 161, 255–278. <https://doi.org/10.1016/j.jep.2014.12.027>
5. Duan, J.L., Li, X.J., Gao, J.M. Et al. Isolation and identification of endophytic bacteria from root tissues of *Salvia miltiorrhiza* Bge. And determination of their bioactivities. *Ann Microbiol* 63, 1501–1512 (2013). <https://doi.org/10.1007/s13213-013-0614-0>
6. Duhan, P., Bansal, P., & Rani, S. (2020). Isolation, identification and characterization of endophytic bacteria from medicinal plant *Tinospora cordifolia*. *South African Journal of Botany*. <https://doi.org/10.1016/j.sajb.2020.01.047>
7. Gayathri Segaran, & Mythili Sathivelu. (2023). Fungicidal and plant growth- promoting traits of *Lasiodiplodia pseudotheobromae*, an endophyte from *Andrographis paniculata*. *Frontiers in Plant Science*, 14. <https://doi.org/10.3389/fpls.2023.1125630>
8. Gusmaini, Aziz, S. A., Munif, A., Sopandie, D., & Bermawie, N. (2013). Isolation and Selection of Endophytic Bacteria Consortia From Medicinal Plant (*Andrographis paniculata*) As Plant Growth Promoting Agents. *Journal of Agronomy*, 12(3), 113–121. <https://doi.org/10.3923/ja.2013.113.121>
9. Hossain, S., Urbi, Z., Karuniawati, H., Mohiuddin, R. B., Moh Qrimida, A., Allzrag, A. M. M., Ming, L. C., Pagano, E., & Capasso, R. (2021). *Andrographis paniculata* (Burm. F.) Wall. Ex Nees: An Updated Review of Phytochemistry, Antimicrobial Pharmacology, and Clinical Safety and Efficacy. *Life*, 11(4). <https://doi.org/10.3390/life11040348>
10. Intharuksa, A., Arunotayanun, W., Yoo-in, W., & Sirisa-ard, P. (2022). A Comprehensive Review of *Andrographis paniculata* (Burm. F.) Nees and Its Constituents as Potential Lead Compounds for COVID-19 Drug Discovery. *Molecules*, 27(14), 4479. <https://doi.org/10.3390/molecules27144479>
11. Khan, A. L., Gilani, S. A., Waqas, M., Al-Hosni, K., Al-Khiziri, S., Kim, Y.-H., Ali, L.,

- Kang, S.-M., Asaf, S., Shahzad, R., Hussain, J., Lee, I.-J., & Al-Harrasi, A. (2017). Endophytes from medicinal plants and their potential for producing indole acetic acid, improving seed germination and mitigating oxidative stress. *Journal of Zhejiang University-SCIENCE B*, 18(2), 125–137. <https://doi.org/10.1631/jzus.b1500271>
12. Kumar, V., Kumar, A., Pandey, K. D., & Roy, B. K. (2014). Isolation and characterization of bacterial endophytes from the roots of *Cassia tora* L. *Annals of Microbiology*, 65(3), 1391–1399. <https://doi.org/10.1007/s13213-014-0977-x>
13. Lee, C.-W., Efetova, M., Engelmann, J. C., Kramell, R., Wasternack, C., Ludwig-Müller, J., Hedrich, R., & Deeken, R. (2009). *Agrobacterium tumefaciens* Promotes Tumor Induction by Modulating Pathogen Defense in *Arabidopsis thaliana*. *The Plant Cell*, 21(9), 2948–2962. <https://doi.org/10.1105/tpc.108.064576>
14. Nair, D. N., & Padmavathy, S. (2014). Impact of Endophytic Microorganisms on Plants, Environment and Humans. *The Scientific World Journal*, 2014, 1–11. <https://doi.org/10.1155/2014/250693>
15. Nalini, M. S., & Prakash, H. S. (2017). Diversity and bioprospecting of actinomycete endophytes from the medicinal plants. *Letters in Applied Microbiology*, 64(4), 261–270. <https://doi.org/10.1111/lam.12718>
16. Nxumalo, C. I., Ngidi, L. S., Shandu, J. S. E., & Maliehe, T. S. (2020). Isolation of endophytic bacteria from the leaves of *Anredera cordifolia* CIX1 for metabolites and their biological activities. *BMC Complementary Medicine and Therapies*, 20(1). <https://doi.org/10.1186/s12906-020-03095-z>
17. Ramzy Ali Mahdi, Bahrami, Y., & Elham Kakaie. (2022). Identification and antibacterial evaluation of endophytic actinobacteria from *Luffa cylindrica*. *Scientific Reports*, 12(1). <https://doi.org/10.1038/s41598-022-23073-4>
18. Rat A, Naranjo HD, Krigas N, Grigoriadou K, Maloupa E, Alonso AV, Schneider C, Papageorgiou VP, Assimopoulou AN, Tsafantakis N, Fokialakis N and Willems A (2021) Endophytic Bacteria From the Roots of the Medicinal Plant *Alkanna tinctoria* Tausch (Boraginaceae): Exploration of Plant Growth Promoting Properties and Potential Role in the Production of Plant Secondary Metabolites. *Front. Microbiol.* 12:633488. Doi: 10.3389/fmicb.2021.633488
19. Rat A, Naranjo HD, Krigas N, Grigoriadou K, Maloupa E, Alonso AV, Schneider C, Papageorgiou VP, Assimopoulou AN, Tsafantakis N, Fokialakis N and Willems A (2021) Endophytic Bacteria From the Roots of the Medicinal Plant *Alkanna tinctoria* Tausch (Boraginaceae): Exploration of Plant Growth Promoting Properties and Potential Role in the Production of Plant Secondary Metabolites. *Front. Microbiol.* 12:633488. Doi: 10.3389/fmicb.2021.633488
20. Salo, E. N., & Novero, A. (2020). Identification and Characterisation of Endophytic Bacteria from Coconut (*Cocos nucifera*) Tissue Culture. *Tropical life sciences research*, 31(1), 57–68. <https://doi.org/10.21315/tlsr2020.31.1.4>
21. Sharma, D., Pramanik, A., & Agrawal, P. K. (2016). Evaluation of bioactive secondary metabolites from endophytic fungus *Pestalotiopsis neglecta* BAB- 5510 isolated from leaves of *Cupressus torulosa* D.Don. *3 Biotech*, 6(2). <https://doi.org/10.1007/s13205-016-0518-3>
22. Sharma, M., & Mallubhotla, S. (2022). Diversity, Antimicrobial Activity, and Antibiotic Susceptibility Pattern of Endophytic Bacteria Sourced From *Cordia dichotoma* L. *Frontiers in Microbiology*, 13. <https://doi.org/10.3389/fmicb.2022.879386>
23. Singh, R., Pandey, K. D., Singh, M., Singh, S. K., Hashem, A., Al-Arjani, A.-B. F., Abd Allah, E. F., Singh, P. K., & Kumar, A. (2022). Isolation and Characterization of Endophytes Bacterial Strains of *Momordica charantia* L. and Their Possible Approach in Stress Management. *Microorganisms*, 10(2), 290. <https://doi.org/10.3390/microorganisms10020290>
24. Webster, G., Mullins, A. J., Cunningham-Oakes, E., Renganathan, A., Aswathanarayan, J. B., Mahenthiralingam, E., & Vittal, R. R. (2020). Culturable diversity of bacterial endophytes associated with medicinal plants of the Western Ghats, India. *FEMS Microbiology Ecology*. <https://doi.org/10.1093/femsec/fiaa147>
25. Zinniel, D. K., Lambrecht, P., Harris, N. B., Feng, Z., Kuczmarski, D., Higley, P., Ishimaru, C. A., Arunakumari, A., Barletta R. G., & Vidaver, A. K. (2002). Isolation and Characterization of Endophytic Colonizing Bacteria from Agronomic Crops and Prairie Plants. *Applied and Environmental Microbiology*, 68(5), 2198–2208. <https://doi.org/10.1128/aem.68.5.2198-2208.2002>