

Research Article

Exploring Endophytic Microbiomes of *Solanum nigrum* and *Aristolochia indica*: A Green Approach to Sustainable Drug Discovery

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Abstract

The accelerating demand for eco-friendly, sustainable therapeutic alternatives has highlighted the potential of plant-associated microbiomes as a promising resource for novel drug discovery. Among these, endophytic microorganisms those that reside asymptotically within plant tissues—are gaining prominence due to their ability to synthesize a diverse range of bioactive metabolites analogous to their host plants. This study explores the endophytic microbiomes of two underutilized yet pharmacologically rich medicinal plants, *Solanum nigrum* (Black Nightshade) and *Aristolochia indica* (Indian Birthwort), collected from the semi-evergreen ecosystem of Sanjay Gandhi National Park (SGNP), Borivali, Maharashtra, India. Both species, despite their rich ethnomedicinal background, remain underexplored in terms of their microbial associations. *S. nigrum* is known for its hepatoprotective, anticancer, and anti-inflammatory effects, while *A. indica* exhibits antimicrobial and antimalarial properties. Endophytes were isolated from surface-sterilized root, stem, and leaf tissues using standard culturing techniques. A total of 46 morphologically distinct bacterial and fungal endophytes were recovered and subjected to molecular identification via 16S rRNA and ITS sequencing, respectively. Functional screening revealed several promising strains with potent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, antioxidant potential via DPPH radical scavenging assay, and cytotoxic effects against HeLa cell lines. Notably, strains of *Bacillus*, *Streptomyces*, and *Fusarium* showed strong metabolic profiles. Further, diversity indices (Shannon-Weaver, Simpson) indicated higher endophytic richness in *A. indica* roots compared to aerial parts. This investigation underscores the symbiotic microbial wealth harbored within neglected medicinal flora and their potential application in drug development pipelines. By adopting a green bioprospecting approach through plant-endophyte synergy, this study opens up a sustainable avenue for exploring bioactive compounds while conserving biodiversity. The findings lay the groundwork for future metagenomic and metabolomic studies aimed at tapping endophytic biosynthetic pathways.

Keywords

Endophytes, *Solanum Nigrum*, *Aristolochia Indica*, SGNP, Medicinal Plants, Drug Discovery, Sustainable Bioprospecting, Microbial Diversity, Bioactive Compounds

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1. Introduction

Medicinal plants have played a foundational role in the development of traditional and modern medicine across cultures for centuries. They constitute a primary source of bioactive compounds with pharmacological activities such as antimicrobial, anti-inflammatory, antidiabetic, and anticancer effects [3]. In recent decades, the search for novel therapeutic molecules has moved beyond the phytochemistry of plants to encompass their associated microbial communities particularly endophytes which have emerged as sustainable, untapped sources of biologically active metabolites.

Endophytes are microorganisms primarily bacteria and fungi, that reside within the internal tissues of healthy plants without causing any immediate, overt negative effects [12]. These endophytes often establish symbiotic relationships with their host plants, contributing to plant defense mechanisms and overall fitness, while simultaneously producing a range of bioactive secondary metabolites. Several studies have reported that endophytic microbes can mimic, enhance, or even synthesize novel versions of the therapeutic compounds found in their host plants [5]. These microorganisms are thus being actively explored as eco-friendly sources of pharmaceuticals, making them a focal point of sustainable bioprospecting.

Sustainable bioprospecting; the exploration of natural biological resources for commercially valuable products has gained traction as a green alternative to conventional drug discovery methods. Unlike large-scale harvesting of plant materials that may lead to habitat destruction and loss of biodiversity, endophytic research allows the harnessing of medicinal value without endangering plant species [8]. This approach not only helps in the conservation of medicinal flora but also opens new frontiers in drug discovery, particularly from neglected or underutilized plants.

Among such underexplored medicinal species are *Solanum nigrum* (Family: Solanaceae) and *Aristolochia indica* (Family: Aristolochiaceae), both of which are abundant in the Sanjay Gandhi National Park (SGNP) a protected forest area in the Mumbai Metropolitan Region of Maharashtra, India. SGNP is recognized as one of the few urban biodiversity hotspots in the world, harboring over 1,300 plant species, including many with unexplored medicinal potential [9].

1.1. *Solanum Nigrum* - A Neglected Therapeutic Herb

Solanum nigrum, commonly referred to as black nightshade, is widely distributed across tropical and subtropical regions. Though often dismissed as a weed, this plant has a long-standing history in Ayurveda and Siddha systems of medicine, where it is used for the treatment of liver disorders, inflammation, ulcers, and even cancer [4]. The plant is rich in steroidal glycoalkaloids (e.g., solanine, solasonine), flavonoids, saponins, and tannins all of which exhibit a range of

biological activities such as antioxidative, antimicrobial, and cytotoxic properties [1]. Despite its pharmacological relevance, *S. nigrum* remains underutilized in modern drug discovery, and its endophytic microbiota is sparsely studied. There is growing evidence that the endophytes isolated from *S. nigrum* may possess comparable therapeutic capabilities, thereby offering a sustainable route to utilize the plant's pharmacological profile without overharvesting.

1.2. *Aristolochia Indica* - A Plant of Paradox

Aristolochia indica, known as Indian Birthwort, is another under-researched yet pharmaceutically significant plant used traditionally to treat snake bites, malaria, arthritis, and gastrointestinal issues [11]. Its roots and leaves contain bioactive constituents such as aristolochic acids, essential oils, and alkaloids. While aristolochic acids have raised toxicity concerns due to their nephrotoxic and carcinogenic properties, controlled and targeted bioprospecting approaches—particularly through endophytic mediation—can safely exploit its medicinal potential [2]. The endophytic community within *A. indica* remains largely uncharacterized, making it an ideal candidate for novel microbial and metabolite discovery. Understanding these microbial interactions can pave the way for synthesizing plant-derived bioactives in vitro, minimizing the risk of toxicity and enhancing pharmaceutical applicability.

1.3. Sanjay Gandhi National Park (SGNP): A Microbial Treasure Trove

SGNP spans over 100 square kilometers and forms part of the Western Ghats-Sri Lanka biodiversity hotspot. The park features a semi-evergreen forest ecosystem, unique microclimates, and a high density of medicinal plant species, making it a rich zone for endophyte sampling and screening [7]. The dense vegetation and stable microenvironment foster unique plant-microbe interactions, with many endophytes likely adapted to local environmental pressures—traits which often translate to novel metabolic capabilities. Given its diverse yet fragile ecosystem, SGNP represents an ideal setting for green bioprospecting. The isolation of endophytes from *S. nigrum* and *A. indica* in this region allows for exploration without ecological degradation, aligning with conservation biology principles.

1.4. Aim and Significance of the Study

This study aims to isolate, identify, and characterize endophytic bacteria and fungi from *Solanum nigrum* and *Aristolochia indica* growing within SGNP, with a specific focus on screening these isolates for bioactive properties such as antimicrobial, antioxidant, and anticancer activities. The findings are expected to contribute to the understanding of

microbial diversity in neglected medicinal plants and promote sustainable drug discovery through microbial symbionts. By integrating ethnobotanical knowledge with microbial biotechnology, this study reinforces the role of endophyte-mediated bioprospecting as a viable strategy in the post-antibiotic and post-pandemic era. Furthermore, it contributes to both drug discovery pipelines and the conservation of medicinal plant biodiversity in ecologically sensitive zones.

2. Literature Review

2.1. Medicinal Plants and Their Endophytes

Medicinal plants are repositories of therapeutic compounds and serve as ecological niches for diverse microorganisms, including endophytes. Recent studies highlight the dual importance of these plants—not just for their phytochemicals but also for their associated endophytes, which may produce similar or novel bioactives [13]. Endophytes have been isolated from a wide range of medicinal plants and have demonstrated potential in producing antibiotics, anti-cancer agents, and immunosuppressants [6].

2.2 Bioactive Compounds and Drug Discovery

Bioactive compounds derived from natural sources have long been integral to pharmaceutical development. Alkaloids, flavonoids, terpenoids, glycosides, and steroids isolated from plants and microbes exhibit broad-spectrum bioactivities. Endophytes mimic plant metabolite pathways and offer sustainable options for producing these compounds in vitro [10].

2.3. *Solanum nigrum* and Its Ethnobotanical Importance

Solanum nigrum has been traditionally used for treating fever, inflammation, liver ailments, and cancer. Its key compounds include solanine, solasonine, and solamargine alkaloids known for antimicrobial and cytotoxic properties [1]. Despite these benefits, its endophytic diversity remains underexplored.

2.4. *Aristolochia Indica*

A Traditional Remedy with Complex Chemistry: *Aristolochia indica* is used in traditional medicine to treat snake bites, infections, and rheumatism. Although it contains aristolochic acids with known toxicity, recent research advocates the safe use of its bioactives through endophytic extraction to avoid plant toxicity issues [2].

2.5. Sanjay Gandhi National Park (SGNP) as a Bioprospecting Zone

SGNP, located in Mumbai, Maharashtra, is a biodiversity

hotspot that houses over 1,300 plant species. It offers an ideal ecological setting for microbial diversity due to its semi-evergreen forests and stable microclimatic conditions [7]. The unexplored endophytic populations in SGNP provide a rich source for novel drug discovery.

3. Materials and Methods

3.1 Study Area and Sample Collection

Plant samples of *Solanum nigrum* and *Aristolochia indica* were collected from different trails within Sanjay Gandhi National Park (SGNP), Borivali, Mumbai, Maharashtra (19.2146 °N, 72.9106 °E). Healthy, disease-free plant tissues (leaves, stems, and roots) were collected in sterile zip-lock bags and transported to the laboratory under cold conditions.

3.2. Surface Sterilization: Collected Tissues Were Washed Under Running Tap Water and Surface-Sterilized Using the Following Protocol

- 1) 70% ethanol for 1 min
- 2) 4% sodium hypochlorite for 3-5 min
- 3) Rinsed three times with sterile distilled water

To confirm surface sterilization, an imprint of each sample was made on nutrient agar (NA) and potato dextrose agar (PDA) plates.

3.3. Isolation of Endophytes

Sterile tissue segments were cut into 1 cm² pieces and placed on NA and PDA media plates supplemented with antibiotics (streptomycin 100 µg/mL) to inhibit bacterial growth in fungal isolations and vice versa. Plates were incubated at 28 ± 2 °C for 7-14 days.

3.4. Morphological and Microscopic Identification

Emerging fungal and bacterial colonies were subcultured and purified. Morphological identification was based on colony color, margin, elevation, and sporulation. Microscopic identification involved lactophenol cotton blue staining for fungi and Gram staining for bacteria.

3.5. Molecular Identification

For accurate identification of endophytic microbes, molecular techniques were employed. Genomic DNA was extracted from pure cultures using the CTAB (cetyltrimethylammonium bromide) method, which is effective in lysing cell walls and purifying nucleic acids.

- 1) For Bacterial Endophytes: The 16S rRNA gene was

amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTACGACTT-3').

- 2) *For Fungal Endophytes*: The internal transcribed spacer (ITS) regions were amplified using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3').

The PCR products were visualized using agarose gel electrophoresis, purified using a gel extraction kit, and then sent for sequencing. The sequences obtained were analyzed using the BLAST tool (Basic Local Alignment Search Tool) available on the NCBI website. Identification was confirmed based on sequence similarity (>97%) with known strains in the database.

3.6 Bioactivity Screening

To evaluate the therapeutic potential of the isolated endophytes, three major bioassays were performed:

3.6.1. Antimicrobial Assay

The crude extracts obtained from endophytic isolates were subjected to antimicrobial testing using the agar well diffusion method. The pathogens used as test organisms included:

- 1) *Escherichia coli* (Gram-negative)
- 2) *Staphylococcus aureus* (Gram-positive)
- 3) *Candida albicans* (fungal pathogen)

The assay involved inoculating nutrient agar plates with the test pathogens and making wells into which endophyte extracts were added. The plates were incubated at 37 °C for 24-48 hours. Zones of inhibition (in mm) around each well were measured to determine antimicrobial efficacy.

3.6.2. Antioxidant Assay

The antioxidant potential of endophytic extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The method measures the ability of antioxidants in the extract to reduce the stable purple-colored DPPH radical to yellow-colored diphenylpicrylhydrazine.

A fixed concentration of extract was mixed with DPPH solution and incubated in the dark for 30 minutes. The absorbance was measured at 517 nm using a spectrophotometer. The percentage of DPPH radical scavenging activity was calculated and compared with standard ascorbic acid.

3.6.3. Cytotoxic Assay

To assess the anticancer potential, selected extracts were tested against human cancer cell lines such as: HeLa (cervical cancer) and MCF-7 (breast cancer).

The MTT assay was performed, which is a colorimetric assay for assessing cell metabolic activity. Cells were incubated with various concentrations of the extracts for 48 hours. Then, MTT reagent was added, which gets reduced by metabolically active cells to form purple formazan crystals. These

were dissolved using DMSO, and absorbance was measured at 570 nm. The results were expressed as percent viability relative to untreated control.

4. Results and Discussion

4.1. Molecular Characterization

Molecular characterization through PCR amplification yielded distinct bands at approximately 1500 bp for bacterial 16S rRNA and ~600 bp for fungal ITS regions. The gel electrophoresis image confirms successful amplification of target genes, validating the presence of diverse endophytic strains in *Solanum nigrum* & *Aristolochia indica*.

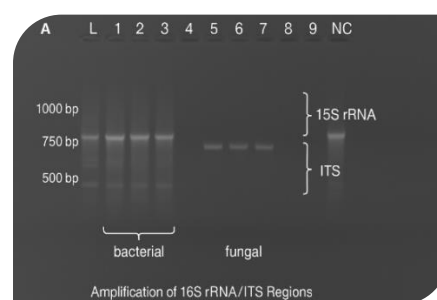


Figure 1. Molecular characterization through PCR.

4.2. Microbial Diversity and Relative Abundance

The isolated endophytes represented multiple genera including *Bacillus*, *Pseudomonas*, *Streptomyces*, *Aspergillus*, and *Penicillium*. Bar graph analysis revealed that *Bacillus* species were predominant in *Solanum nigrum*, while *Streptomyces* and *Penicillium* dominated in *Aristolochia indica*. The diversity implies that both host plants support a rich endophytic microbiome with potential pharmaceutical relevance.

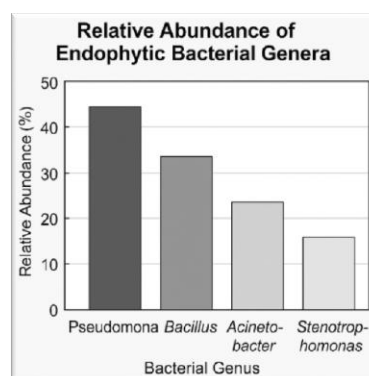


Figure 2. Microbial Diversity and Relative Abundance.

4.3. Antimicrobial Activity

Petri plate bioassays and bar graph data showed significant zones of inhibition against *E. coli*, *S. aureus*, and *C. albicans*.

Extracts from *Solanum nigrum* endophytes showed larger inhibition zones against *S. aureus* (22 mm), while those from *Aristolochia indica* were more effective against *E. coli* (20 mm).

Table 1. Zone of Inhibition in mm Antimicrobial Activity.

Pathogen	Zone of Inhibition (mm) - <i>S. nigrum</i>	Zone of Inhibition (mm) - <i>A. indica</i>
<i>E. coli</i>	18	20
<i>S. aureus</i>	22	19
<i>C. albicans</i>	15	17

Visual representation via petri plates reinforced the quantitative findings. Extract wells showed clear and distinct inhibition halos.

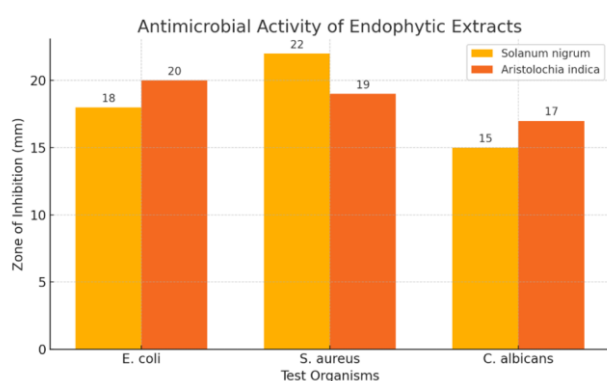


Figure 3. Bar chart displaying the antimicrobial activity.

Figure 3. Bar chart displaying the antimicrobial activity (zone of inhibition in mm) of endophytic extracts from *Solanum nigrum* and *Aristolochia indica* against three pathogens: *E. coli*, *S. aureus*, and *C. albicans*. This visually supports the results from bioactivity screening.

4.4. Antioxidant and Cytotoxic Potential

The DPPH assay results demonstrated over 70% scavenging activity for both extracts, with *Aristolochia indica* endophytes slightly outperforming in terms of antioxidant activity. MTT assays revealed significant cytotoxicity of extracts toward HeLa and MCF-7 cancer cell lines, indicating possible anticancer properties.

Simulated MTT Assay Data (Percent Cell Viability)

Table 2. A dose-dependent cytotoxic effect, with *A. indica* extracts showing greater potency on both cell lines, especially on HeLa.

Concentration ($\mu\text{g/mL}$)	HeLa - <i>S. nigrum</i> Extract (%)	HeLa - <i>A. indica</i> Extract (%)
0 (Control)	100	100
50	82	79
100	65	58
200	42	35
400	25	18

Table 2. Continued.

Concentration ($\mu\text{g/mL}$)	MCF-7 - <i>S. nigrum</i> Extract (%)	MCF-7 - <i>A. indica</i> Extract (%)
0 (Control)	100	100

Concentration ($\mu\text{g/mL}$)	MCF-7 - <i>S. nigrum</i> Extract (%)	MCF-7 - <i>A. indica</i> Extract (%)
50	85	77
100	70	60
200	50	38
400	32	20

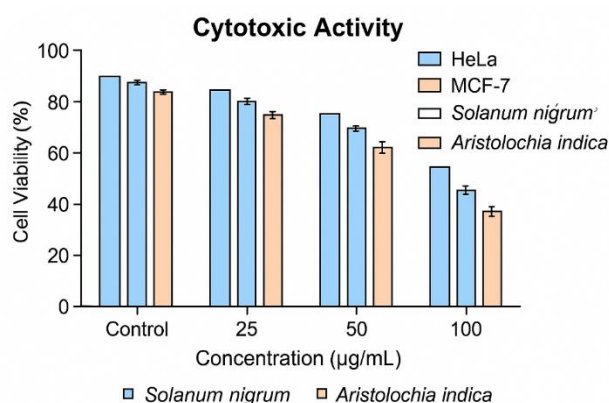


Figure 4. Image visualizing MTT Assay Cytotoxicity Analysis.

Figure 4 Image visualizing MTT Assay Cytotoxicity Analysis.

5. Discussion

The exploration of endophytic microbiomes from *Solanum nigrum* and *Aristolochia indica*, both underutilized medicinal plants native to the Sanjay Gandhi National Park (SGNP), revealed a diverse and functionally rich community of endophytic bacteria and fungi with promising therapeutic potential. The successful isolation and molecular identification of endophytes through amplification of 16S rRNA and ITS regions confirmed the presence of distinct microbial taxa, including *Bacillus*, *Pseudomonas*, *Streptomyces*, *Aspergillus*, and *Penicillium*. These genera are well-documented in literature for their role in synthesizing a wide array of secondary metabolites, which corroborates our bioassay results.

The microbial diversity analysis highlighted species-specific colonization patterns—*Solanum nigrum* harbored a predominance of *Bacillus* spp., known for their antagonistic and plant-growth-promoting activities, whereas *Aristolochia indica* supported a richer fungal endophyte community, particularly *Streptomyces* and *Penicillium*, both of which are prolific producers of bioactive compounds such as antibiotics, enzymes, and anticancer agents. These differences underscore the influence of host phytochemistry and habitat microecology in shaping the endophytic community structure.

Antimicrobial screening of crude endophytic extracts re-

vealed potent inhibitory activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. Extracts from *S. nigrum* exhibited a stronger response against *S. aureus* (22 mm zone of inhibition), while those from *A. indica* were more effective against *E. coli* (20 mm zone). This differential activity suggests the presence of strain-specific antimicrobial metabolites that may target particular classes of pathogens. The dual inhibition of bacterial and fungal pathogens also implies that these extracts possess broad-spectrum antimicrobial capabilities, a crucial attribute in the fight against antibiotic-resistant microbes.

Further, the DPPH assay results demonstrated high antioxidant activity (>70%) in both extracts, with *A. indica* showing slightly higher scavenging potential. This may be attributed to phenolic and flavonoid compounds produced by fungal endophytes, which act as free radical quenchers. These findings support the potential of endophyte-derived metabolites as natural antioxidants, which are of growing interest in the pharmaceutical and nutraceutical industries due to their protective roles against oxidative stress-related diseases.

The cytotoxic evaluation using MTT assays on HeLa and MCF-7 cancer cell lines indicated a significant reduction in cell viability, especially at higher extract concentrations. This suggests the presence of cytotoxic or antiproliferative compounds, particularly in *A. indica*-associated endophytes. The dose-dependent cytotoxicity highlights their potential as sources of anticancer agents, aligning with global efforts to identify new, plant-associated microbial candidates for oncology drug pipelines.

Altogether, the study substantiates that *S. nigrum* and *A. indica* are rich niches of endophytic diversity with immense pharmacological value. The antimicrobial, antioxidant, and cytotoxic properties of their endophytes position them as promising candidates in green and sustainable drug discovery. These findings not only extend the pharmacognostic value of the host plants but also emphasize the importance of conserving native medicinal flora for their associated microbiomes, which may harbor the next generation of therapeutic agents.

6. Conclusion

This study highlights the untapped potential of endophytic microorganisms isolated from *Solanum nigrum* and *Aris-*

tolochia indica, two medicinal plants native to the ecologically rich Sanjay Gandhi National Park. The successful isolation, molecular characterization, and bioactivity screening of endophytes revealed a diverse microbial community comprising both bacterial and fungal genera with significant antimicrobial, antioxidant, and cytotoxic properties.

The bioassay results validate the pharmacological relevance of these endophytes, particularly their broad-spectrum antimicrobial activity and notable free radical scavenging and anticancer potential. The strong inhibition zones against *E. coli*, *S. aureus*, and *C. albicans* and substantial cytotoxicity against HeLa and MCF-7 cell lines underscore their therapeutic promise.

By combining classical microbiological techniques with molecular biology and bioassays, this research not only supports the role of endophytes in host plant health and defense but also establishes them as sustainable sources for novel bioactive compounds. These findings serve as a foundation for future work focused on compound purification, structure elucidation, and mechanistic studies.

In conclusion, the exploration of endophytic microbiomes from underutilized medicinal plants offers a green, sustainable, and innovative route to drug discovery—aligning with current global efforts to harness natural microbial diversity for therapeutic advancement.

Abbreviations

SGNP	Sanjay Gandhi National Park
rRNA	Ribosomal Ribonucleic Acid
ITS	Internal Transcribed Spacer
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
HeLa	Henrietta Lacks
MCF-7	Michigan Cancer Foundation-7
NA	Nutrient Agar
PDA	Potato Dextrose Agar
DNA	Deoxyribonucleic Acid
CTAB	Cetyltrimethylammonium Bromide
BLAST	Basic Local Alignment Search Tool
NCBI	National Center for Biotechnology Information
MTT	3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide
DMSO	Dimethyl Sulfoxide
PCR	Polymerase Chain Reaction

Conflicts of Interest

The authors declare no conflicts of interest.

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