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Isolation and Characterization of Endophytic Fungi from Medicinal Plants in Calotropis procera

Khuman[1], zaheen e fatema[2], renuka sahu[3], khushi sharma[4]

[1,2,3,4]Kalinga university
[1]khumanbarley@gmail.com, [2]fzaheen072@gmail.com, [3]renukasahu633@gmail.com,
[4]Scarlettbuds2002@gmail.com

Abstract

Calotropis procera is a medicinal plant and endophytic fungi in Calotropis procera are widely found in arid and semi-arid regions and provide improvement in the pharmacological properties of Calotropis procera. The summary of present knowledge on isolation, identification and characterisation of endophytic fungi from C. procera were studied in this study. The approach methodological approaches, biodiversity, synthesis of bioactive chemicals and potential applications in pharmaceutical and agriculture sectors are highlighted.

Keywords: Bioremediation, Pharmacological, Antimicrobial, Endophytes

1. Introduction

Endophytic fungi from plants offer many bioactive compounds along with great potential for pharmacological molecule synthesis (Liu et al., 2023; Ogbiko et al., 2021). Endophytic bacteria inside plant tissues have become highly studied for their capacity to produce various secondary metabolites Momin and Tripathi (2018). The research team focuses on endophytes from medicinal plants because their therapeutic significance has already been proven (Makuwa & Serepa-Dlamini, 2021). The bioactivity capabilities of endophytes include two functions: either they boost preexisting biological properties or they synthesize new substances with distinct bioactivity features (Mahamed & Chenia, 2025). The symbiotic relationship between endophytes and plants results in substance production which provides plants with defense support as well as nutrition uptake and stress resistance (Omomowo & Babalola, 2019). The investigation of endophytic fungi has gained significant importance when searching for new drugs and agrarian products (Gouda et al., 2016). Scientists study medicinal plants with the purpose of detecting new substances among endophytic communities because these compounds show promise for industrial applications and medical and agricultural uses (Gouda et al., 2016; Rani, 2016). The colonization process triggers various cell wall modifications through which endophytes produce callose and cellulose and pectin structures together with phenolic compounds and biologically active substances. At sites where phytopathogens may strike the implemented structures produce a physical blockage to protect plants (Chlebek et al., 2020). The survival and fitness of plants depend on plant-microbe

interactions with endophytic mutualism being particularly important because endophytes reside entirely or partially within plant tissues to affect host cells (Jain et al., 2021). Endophytes support the mutualism with their host plant species by producing defensive chemicals that help plants grow and defend against external threats in unpredictable environmental conditions and pathogen attacks.

The relationship between plants and their microbial groups demonstrates a complex pattern according to endophyte research findings. The close physical relationship between endophytes and their host plants allows them to efficiently produce secondary metabolites (Abbas et al., 2024). Endophytes infect all plant species and proceed immediately to boost health and plant growth from seed to seed (White et al., 2019). The plants obtain benefits from several endophytic species regardless of their origin in terrestrial environments (White et al., 2019). Following plant tissue settlement endophytes form a symbiotic relation that provides multiple advantages to the hosting plant. Studied benefits from endophytes encompass two areas: they enhance plant growth while simultaneously bolstering disease and pest resistance and improving plant tolerance against environmental stress (Kumar et al. 2020). Most of an endophyte's lifespan occurs in the inside parts of a plant while the endophyte avoids damaging its host plant (Kandel et al., 2017). Anand et al. (2023) explain that this symbiotic relation produces beneficial outcomes which support both endophyte and host plant. Exhaustive mechanisms involving multiple metabolic pathways as well as molecular signaling mechanisms sustain these interactions according to Khare et al. (2018). For endophytic colonisation to succeed bacteria need multiple molecules and various activities which include chemotaxis and motility and adhesion together with bacterial cell wall characteristics and secretion and transcription regulation and substrate utilization

(Piński et al., 2019).

2. Literature Review

Various therapeutic compounds emerge from endophytic fungi that persist inside plant tissues causing no harm (Liu et al., 2023). Due to their medicinal compound synthesis potential biotechnologists explore these fungal species (Ogbiko et al., 2021). According to Momin and Tripathi (2018) endophytes represent microorganisms that penetrate plant tissues causing no harm to their host. Gouda et al. (2016) demonstrate their ability to generate various helpful secondary metabolites which benefit pharmaceutical practices as well as industrial manufacturing and agricultural production. Endophytic organisms obtained genetic information from their resident plant population while developing their useful compounds (Rani, 2016). Endophytic microorganisms have gain use for agricultural applications and drug development because they produce a diverse mixture of bioactive compounds (Abbas et al., 2024). The health sector in Traditional Asian and African medicine relies on medicinal plants to treat sicknesses for a long time (Makuwa & Serepa-Dlamini, 2021). Bioactive characteristics that these plants possess are correlated to their endophytic content according to Mohamed and Chenia (2025). Scientists have focused their search for medicine-based drug discovery chemicals among natural compounds within endophytic organisms according to Gouda et al. (2016). Phytopathogens are deterred by structural barriers that endophytic colonisation creates in the cell wall while accumulating phenolic substances together with callose and pectin and cellulose (Chlebek et al., 2020). Endophytes enhance plant growth while improving nitrogen absorption as they simultaneously perform biocontrol functions (Kumar et al., 2020; Omomowo & Babalola, 2019). The cell wall modifications together with phytohormone signaling controls the molecular processes that occur during colonization by managing chemotaxis and motility as well as adhesion and secretion functions (Piński et al., 2019).The production or enhancement of metabolites by endophytes

makes it possible for plants to defend themselves alongside adapting to their environment (Jain et al., 2021). The American Microbiologist Journal finds that endophytic bacteria alter ecological habitats by facilitating nutrient cycles and conducting biodegradation and bioremediation processes (Nair & Padmavathy, 2014).Endophytes, which live inside many plants, can be transferred by seeds and help plants stay healthy from the start (White et al., 2019). Various endophytes can be collected from soil and help plants similarly (White et al., 2019). Endophytes help plants survive drought, salinity, temperature stress, heavy metal stress, and nutrient scarcity (Eid et al., 2021).These bacteria help the host cope with biotic and abiotic stressors, according to Rana et al. (2020). In 1866, "endophyte" was used to describe fungus and bacteria that live on host plants without showing disease (Anand et al., 2023).

2.1. Current Research on Calotropis procera

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2.2 Gaps in Knowledge

Although a large number of endophyte and related gene candidates have been identified, diversity in life styles and functions of the endophytes remain largely unexplored (Khare et al., 2018). The production of bioactive compounds by endophytes is now proven but the mechanism of their synthesis is still not fully understood.

Table: Research Gaps in the Study of Endophytic Fungi from Calotropis procera

S. No.	Research Gap	Description	Reference
1	Limited exploration of fungal diversity	Most studies report only culturable endophytes; non-culturable fungi remain unexplored.	Kusari et al., 2012; Strobel & Daisy, 2003
2	Inadequate use of molecular tools	Few studies have used ITS or next-generation sequencing for accurate fungal identification.	White et al., 1990; Raviraja, 2005
3	Limited metabolomic profiling	Limited analysis of secondary metabolites using advanced tools like LC-MS, GC-MS, NMR.	Gunatilaka, 2006
4	Lack of bioassay-guided fractionation studies	Crude extracts are often tested, but specific active compounds are rarely isolated and characterized.	Scarcity of Schulz et al., 2002
5	Neglected root and flower-associated endophytes	Most studies focus on leaves and stems; roots and reproductive parts are underexplored.	Jalgaonwala et al., 2011
6	Minimal evaluation of host metabolism	The functional role of endophytes in endophytic interactions with modulating host plant secondary metabolism remains unclear.	Porras-Alfaro & Bayman, 2011
7	Absence of in vivo validation of bioactivity	Antimicrobial/anticancer assays are usually in vitro; efficacy and toxicity in animal models are rare.	Zhang et al., 2006

8	No standardized protocol for endophyte isolation from C. procera	Methods vary greatly across studies, affecting reproducibility and comparative analysis.	Strobel et al., 2004
9	Few reports on ecological and seasonal variation in endophyte community structure in C. procera	Lack of long-term or seasonal studies to understand population dynamics.	Kumaresan & Suryanarayanan, 2002
10	Limited commercial application of promising isolates	Biotechnological potential remains untapped due to lack of pilot-scale fermentation or field trials.	Aly et al., 2010

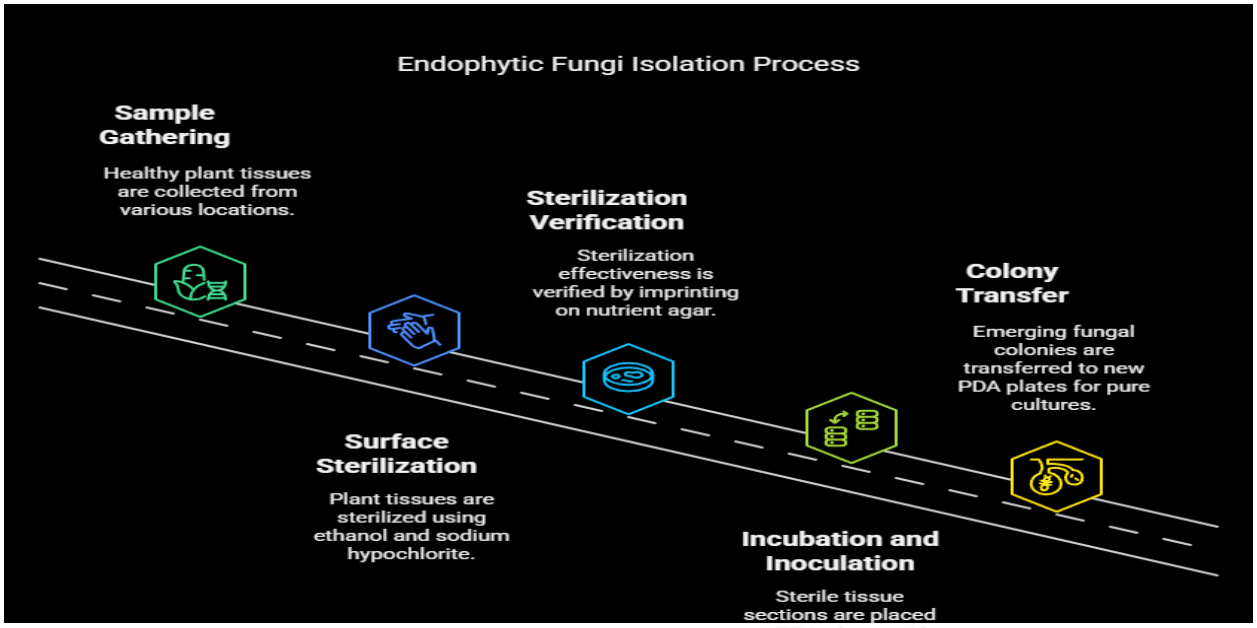
3. Diversity of Endophytic Fungi in Calotropis procera

Research shows that C. procera is home to a variety of fungal species, including:

Aspergillus,Penicillium,The Fusarium,Alternaria,Cladosporium,Colletotrichum

The plant component, time of year, and geographic location all affect the diversity and frequency of colonisation. Compared to stems and roots, leaves frequently show higher colonisation rates (Hashem et al., 2023).

4. Methodology for Isolation of Endophytic Fungi



5. Bioactive Potential of C. procera Endophytic Fungi

5.1. Antimicrobial Performance

Many of the fungal isolates of C. procera are strong antibacterial and antifungal. Extracts free of fatty acids and other chemical compounds are able to stop pathogens such as E. coli, S. aureus, Candida albicans, and Aspergillus niger from growing (Jain et al., 2021).

5.2. Action of Antioxidants

Endophytic fungus makes molecules that neutralises free radicals using DPPH and ABTS tests (Abbas et al., 2024; Hashem et al., 2023).

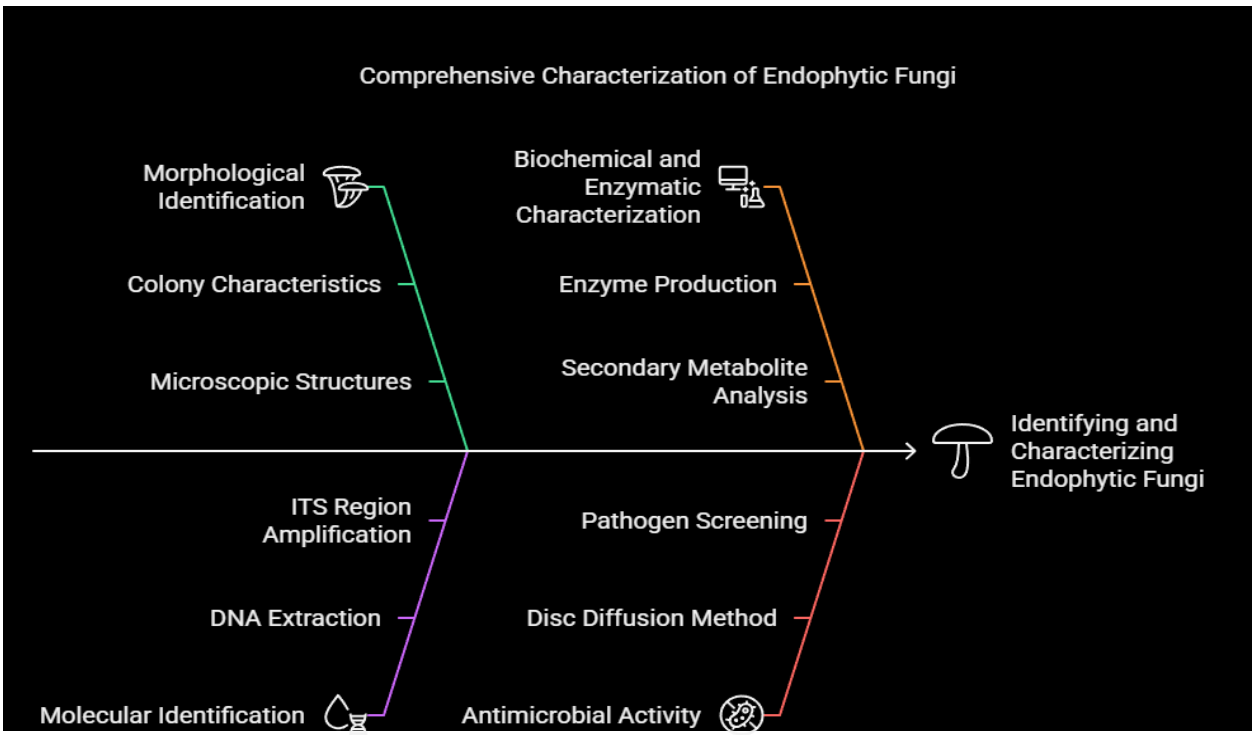
5.3. Cytotoxic and Anticancer Characteristics

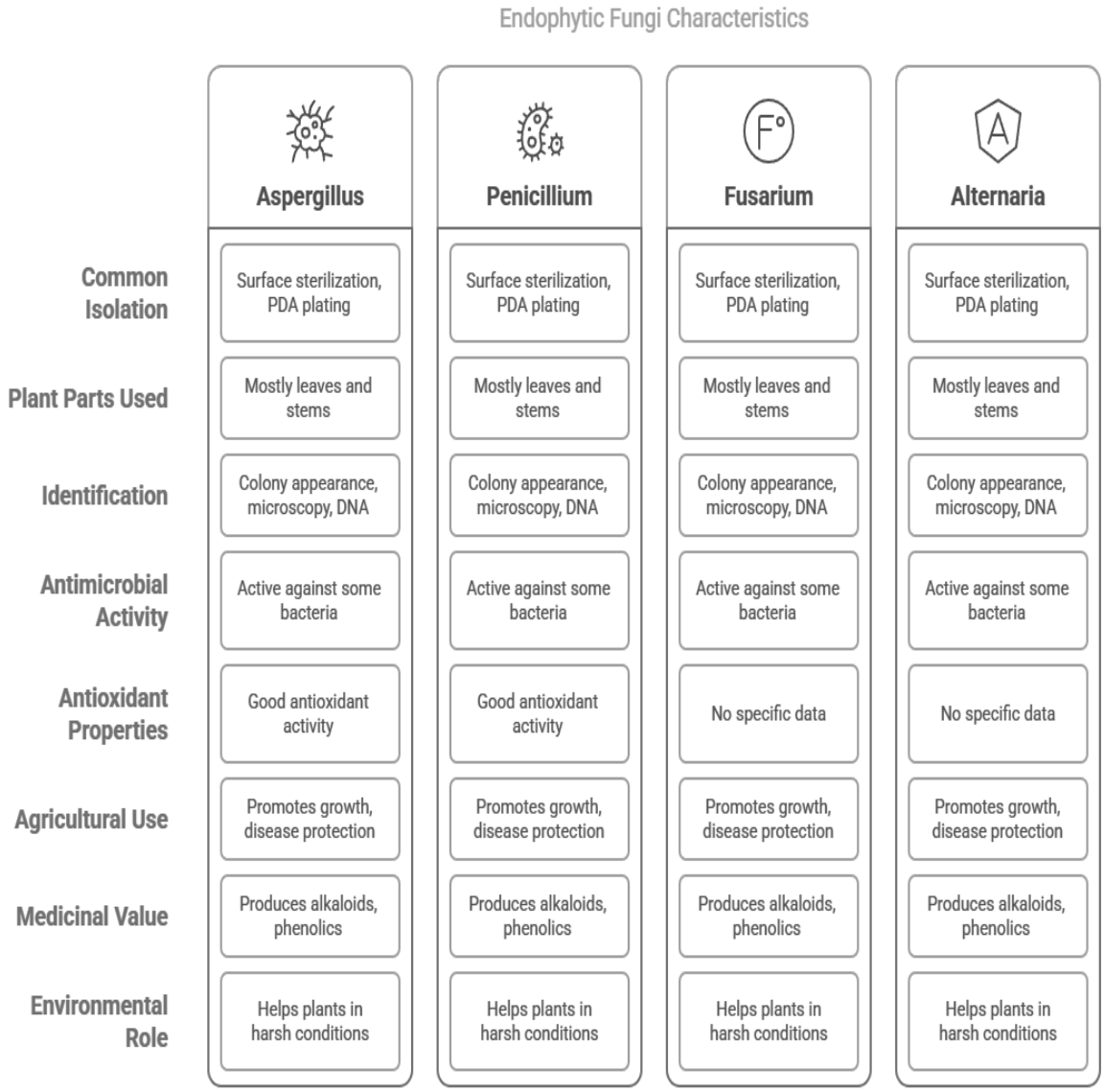
Some isolates have indications of their use as anticancer drugs, as they are cytotoxic to cancer cell lines such as colon, breast, and liver malignancies (Khare et al., 2018).

5.4. Plant Development Enhancement

Endophytes are also able to produce phytohormones such siderophore and indole-3-acetic acid (IAA) under stress to enhance growth and nutrient absorption (Kumar et al., 2020; Rani, 2016).

6. Characterization of Endophytic Fungi





7. Discussion

Living in plant tissues without inflicting any obvious damage, endophytic fungus are a mostly unexplored source of bioactive secondary metabolites with great therapeutic potential (Nazir & Rahman, 2018). These substances show potential for treating diseases and battling pathogens as well as for addressing other human health issues (Gouda et al., 2016). Due to their possible to generate chemicals with increased bioactivity or unique structures compared to those found in other environments, the investigation of endophytic fungus from medicinal plants is of especially interest (Liu et al., 2023; Rana et al., 2020). The meeting of conventional medicine with contemporary biotechnology emphasises the need of knowing the interactions of plants and their related microbes (Gouda et al., 2016). Almost every plant species studied possesses endophytic fungus, implying a substantial and mostly unknown reservoir of these microorganisms in the natural world (Nahas, 2019). On continents like Asia and Africa, plants with medicinal qualities have been used to treat disorders including diarrhoea, headaches, and fever (Makuwa & SerepaDlamini, 2020). Ayurveda has long used these endophytes, which are crucial in the synthesis of

medicinal drugs (Deshmukh et al., 2015). Recent research have underlined the biotechnological curiosity in secondary metabolites originating from endophytes, showing their capacity to generate drugs of therapeutic relevance (Ogbiko et al., 2021).

The bioactive features of the host can be much influenced by the tight interaction between medicinal plants and endophytic bacteria (Mohamed & Chenia, 2025). Microorganisms known as endophytes colonise plant tissues within of themselves without producing clear disease signs (Momin & Tripathi, 2018). Usually producing bioactive secondary metabolites with a spectrum of biological activity, these microbial symbionts interact intricately with their host plants (Midhun & Mathew, 2021).

8. Conclusion

Fungi endophytiques are a good source of bioactive compounds that could be used in many others fields (medecine, agriculture, etc.). The studies indicate that only approximately a small fraction of identified plant species have been looked upon for possible endophytic associates, which implies that there should be more study. Interaction between the plant microbiome and microbes are vital for plant survival and overall fitness. The endophytes enhance the host plant capabilities including tolerance, growth. Endophytic fungi from the medicinal plants including

Calotropis procera could be used to identify new drug candidates and sustainable bioproducts. Endophytic fungi isolated from medicinal plant such as Calotropis procera is a promising opportunity for the discovery of novel bioactive compounds as concluded in this work.

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