Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 768-772 | ISSN:2251-6727

Antimicrobial Activity of Compounds Produced by Endophytic Fungi Isolated from *Aloe Vera* Roots

Smrati Shukla [A]*, Ankit Agrawal

[A] Department of life science, Rabindranath Tagore University, Raisen Madhya Pradesh India

(Received: 10 January 2026 Revised: 19 February 2026 Accepted: 10 March 2026)

KEYWORDS

Aloe vera, endophytic fungi, antimicrobial activity, crude extracts, natural products.

ABSTRACT:

Endophytic fungi are an important source of biologically active secondary metabolites, including novel antimicrobial agents. This study aimed to isolate endophytic fungi from the roots of Aloe vera, characterize isolates morphologically, obtain fungal crude extracts and partially purified fractions, and evaluate their antibacterial and antifungal activity against a panel of wound attacking pathogens (Escherichia coli, Klebsiella, S. aureus, and E faecalis). using solid-medium agar diffusion, broth Mueller Hinton. These results confirm that Aloe vera root endophytes are a promising source of antimicrobial natural products and warrant further chemical purification and structure elucidation.

Introduction

The rise of antimicrobial resistance (AMR) is a major global health threat, creating an urgent need for novel antibiotics and antifungals (Walsh, T. R *et al*., 2023). Natural products from microbial and plant sources remain a rich reservoir for new drugs; Endophytic fungi—microorganisms that live within plant tissues without causing visible disease—have been recognized as prolific producers of structurally diverse secondary metabolites with antimicrobial, anticancer, and other bioactivities (Verma, S *et al*., 2021; Abdel-Razek *et al*., 2020). These metabolites often mirror or complement the host plant's chemistry and represent an attractive route to new bioactive compounds.

Aloe vera (Asphodelaceae) is a medicinal plant widely used for wound healing, antimicrobial, and anti-inflammatory applications; recent studies have also isolated bacterial and fungal endophytes from Aloe species that produce bioactive metabolites with antibacterial and antifungal activities. Several reports describe isolation of multiple endophytic fungal genera from Aloe tissues and demonstration of their antagonistic or antimicrobial properties (Liang, J, 2021; Lanka, S. 2018). These findings suggest the Aloe microbiome is a promising reservoir of novel antimicrobials (Rajeswari, R *et al*., 2012).

Despite these encouraging reports, several gaps remain, in this work we focused on root-associated fungal endophytes because root microenvironments often contain distinct endophyte communities and can yield unique metabolites. The objectives were: **(1)** isolate and identify root endophytic fungi from Aloe vera, **(2)** prepare crude extracts and fractions from cultures, **(3)** screen extracts for selected bacteria, antibacterial activity.

Materials and Methods

Sample collection

Healthy *Aloe vera* plants were sampled from botanical garden from Bhopal during summer season,2025. Whole root systems from 10–15 mature plants were collected and transported to the laboratory in sterile bags at 4 °C for processing within 24 hours.

Surface sterilization and isolation of endophytes

Endophytic fungus was isolated by using root fragment. After removal of the soil the sample taken into the shaken in water with detergent tween 80 at 70 rpm for 10 minutes to remove the density of the Exophytic fungus. The surface of the fragment was disinfected by successive process sequentially ethanol (70%), 2.5% sodium hypochlorite (active chlorine), and ethanol (70%) for 1min, 5min, and 30s. At the end four washes performed using autoclaved distilled water. At last, the sample were air-dried, the extern al part was removed, and the inner region of the plant segment were collected. After that the segment were placed on potato dextrose agar plate and

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incubated at 28^0 C. The incubated plates were examined for endophytic fungi and fungal hyphae were visible on the sample after 3 to 4 days. Later on, the hyphal tips of morphologically distinct endophytic fungi were carefully collected and transferred to newly prepared potato dextrose agar.

Morphological and molecular identification

Morphological assessment is usually considered as the first step of identification. The sample of isolated fungus were mounted on sterile slides after that it was stained by lacto phenol cotton blue and investigated in 40X light microscopy. The fungal culture was identified on the basis of spore shape, phenotypic characteristics, spore type, growth colour, growth rate using standard manual. Representative isolates were subjected to genomic DNA extraction and ITS rDNA amplification (primers ITS1/ITS4). PCR products were sequenced and compared to GenBank/UNITE databases for taxonomic assignment. ITS-based identification is standard for fungal endophyte studies.

Antimicrobial Screening of Endophytic Fungal Extracts

In Vitro Assays: solid-medium agar diffusion

Following the procedure outlined by Ichikawa *et al*. (1971), a solid-medium agar diffusion assay was used to assess the antibacterial activity of the isolated endophytic and exophytic fungal strains. This method allows for the quick and qualitative screening of bioactive fungal isolates. To promote the best mycelial growth and secondary metabolite diffusion into the agar medium, each fungal isolate was first grown on Potato Dextrose Agar (PDA) plates and incubated at 30 °C for seven days. Agar discs (6 mm in diameter) were aseptically cut from the fungal cultures' actively developing edges following incubation. After that, the agar discs were placed on Mueller–Hinton Agar (MHA) plates that had already been infected with pathogenic microorganisms linked to wounds. *Escherichia coli spp.*, *Klebsiella sp*p. and *Enterococcus faecalis spp.* were among the test bacterial strains that were evenly distributed around the agar surface to guarantee confluent growth. While plates containing fungal test organisms were incubated at 30 °C for 48 hours, plates inoculated with bacterial pathogens were incubated at 37 °C for 24 hours. The inhibitory diameter zones around the fungal agar discs were measured to assess antimicrobial activity.

Results

1. **Isolation and identification:** A total of 6 fungal isolates were recovered from surface-sterilized *Aloe vera* roots. Morphology and molecular sequence identified isolates belonging to genus level of species *as Microsporum, Tricoderma, Fusarium, Candida, Mucor* and *Aspergillus.*

2. **Antimicrobial screening:** Antibacterial Activity of endophytic Fungal Extracts was evaluated against gram-positive (*S. aureus* and *E. faecalis*) and gram-negative (*E.coli* and *Klebsiella*) bacteria. The antibacterial activity of endophytic fungal extracts was evaluated against four pathogenic bacterial strains, namely *Escherichia coli*, *Klebsiella spp*., *Staphylococcus aureus spp*., and *E. faecalis spp.*, using six different samples (S1–S6) as indicated by zones of inhibition (mm). The results demonstrated variable antibacterial efficacy among the tested extracts. Against *E. coli*, the highest inhibitory activity was observed with sample S4 (22 mm), followed by S3 (18 mm), while the lowest activity was recorded with S6 (13 mm). In the case of *Klebsiella spp*., sample S6 exhibited the maximum zone of inhibition (19 mm), whereas S1 and S5 showed comparatively lower activity (13 mm). Notably, *S. aureus spp*. showed strong susceptibility to samples S2 and S4, both producing prominent zones of inhibition (20 mm and 19 mm), indicating pronounced antibacterial potential against this Gram-positive bacterium. Similarly, E. faecalis exhibited maximum sensitivity to sample S6 (19 mm), followed by moderate inhibition with S4 and S5 (16 mm each). Overall, sample S4 displayed broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria, as showing all result in table number-1. suggesting that the endophytic fungal extract possesses promising antibacterial properties and may serve as a potential source of bioactive compounds for further pharmacological investigation.

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Table:1 Antimicrobial activity against selected bacteria *spp*. to S1-S6.

Name of bacteria *spp.*	S1	S2	S3	S4	S5	S6
E. coli spp.	17mm	16mm	18mm	22mm	15mm	13mm
Klebsiella spp.	13mm	14mm	15mm	17mm	13mm	19mm
S. aureus spp.	10mm	19mm	14mm	20mm	18mm	10mm
E. faecalis spp.	11mm	11mm	14mm	16mm	16mm	19mm

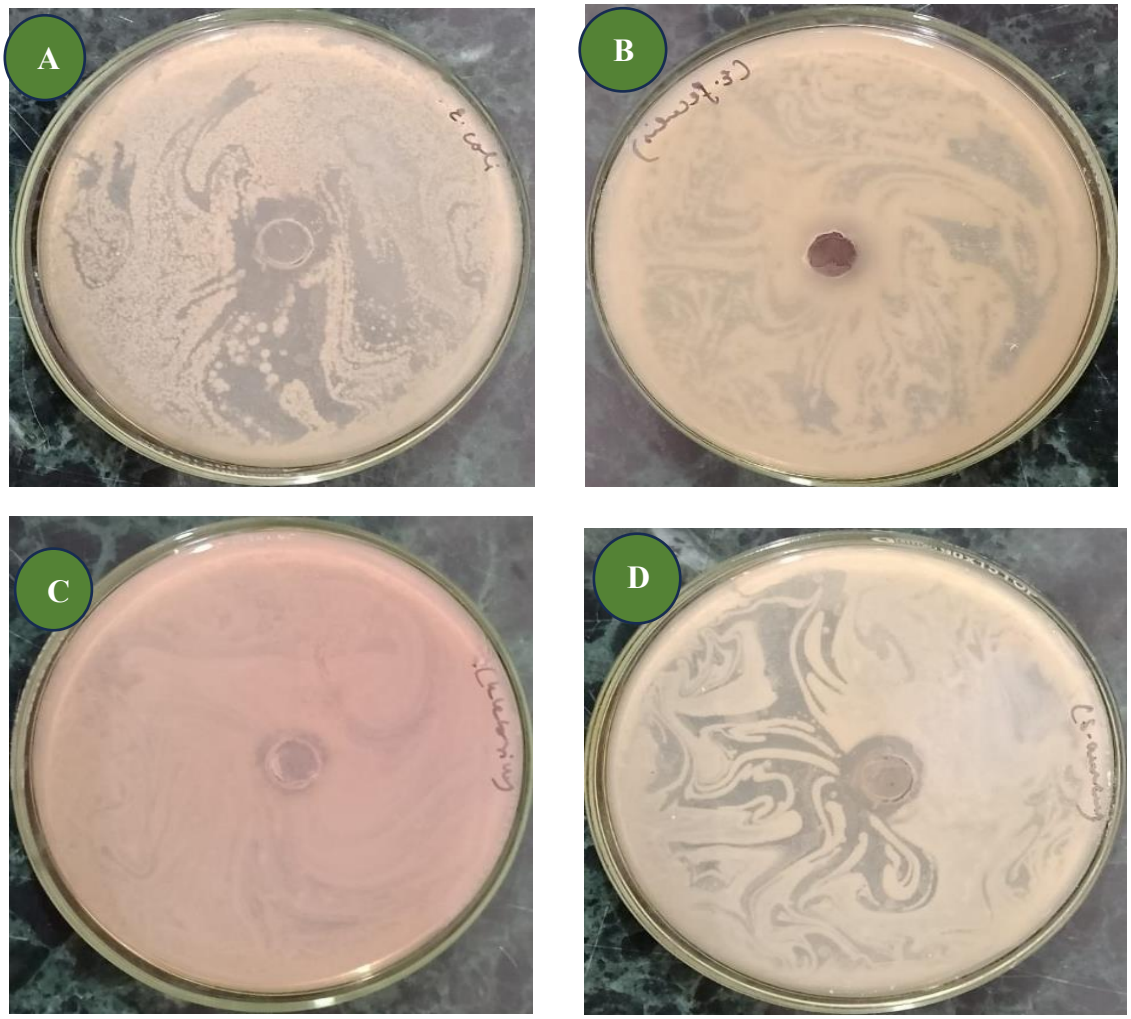

Fig.: 1 Antimicrobial assay of endophytic Fungal Extracts extract against selected pathogens where; A- *E. coli*, B- *E. faecalis,* C- *Klebsiella* and D- *S. aureus*

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Discussion

This present study is reporting the successful isolation of six endophytic fungal genera from surface-sterilized roots of Aloe vera, which include *Microsporum*, *Trichoderma*, *Fusarium*, *Candida*, *Mucor*, and *Aspergillus*. In the process, it provides a main insight into the diversity of the internal fungal community associated with this medicinal plant. The occurring genera agree with previous studies indicating that medicinal plants harbor a very taxonomically diverse group of endophytic fungi with the capacity to biosynthesize biologically active secondary metabolites.

In general, endophytic fungi are well-known as rich sources of antimicrobial compounds, taking into consideration that they help in the protection of their host plants from the incursion of pathogenic agents. In fact, the antibacterial activity disclosed by the fungal extracts against Gram-positive bacteria like *S. aurenus* and *E. faecalis*, and Gram-negative bacteria like *E. coli spp.* and *Klebsiella spp*., is consistent with this. The differences in antimicrobial efficiency between S1-S6 samples clearly explain the variabilities in metabolite profiles, extraction efficiency, or species-specific biosynthetic pathways.

Sample S4 exhibited the widest of antibacterial activity, forming the largest inhibition zones against E. coli and S. aureus at 22 and 20 mm each. This is supported by previous literature that reports endophytic fungi, particularly species of the genera *Aspergillus*, *Trichoderma*, and *Fusarium*, producing bioactive antimicrobial polyketides, alkaloids, and terpenoids. Stronger inhibitory action against *S. aureus spp*. and *E. faecalis spp*. indicated that these extracts might interfere with the synthesis of cell walls or permeabilize cellular membranes, as is common for most metabolites produced by fungi.

The moderate activity Gram-negative bacteria exhibited may be attributed to its lipopolysaccharide-rich outer membrane, which can inhibit the entry of the antimicrobial agent. On the other hand, the appreciable activity against *E. coli spp.* and *Klebsiella spp*. suggests that some metabolites can permeate these barriers or act through alternative mechanisms.

Overall, the antibacterial potential presented here adds to the growing evidence that endophytic fungi associated with medicinal plants, such as Aloe vera, represent a highly useful, yet underexplored reservoir of antimicrobial agents. This is much more significant considering the rise of antimicrobial resistance, and the need for discovering new bioactive compounds from natural sources is therefore urgent.

Conclusion

Endophytic fungal extracts from Aloe vera species have wide applications in topical therapies, cosmetics, and health beverages, among many other medical uses. Based on a wide range of studies in various countries involving different species of Aloe, and focusing on the antimicrobial properties of the plant, commercially prepared products claim uniform medicinal value.

The results of this study indicate that a variety of endophytic fungi from Aloe vera roots have significant antibacterial activity. In the six fungal extracts tested, Sample S4 exhibited potent antimicrobial activity against both Gram-positive and Gram-negative pathogenic bacteria. These findings indicate that endophytic fungi associated with Aloe vera could be a promising source for bioactive secondary metabolites of potential therapeutic interest. Further studies are necessary to establish their pharmacological relevance and possible development as novel antimicrobial compounds, by means of purification, structural characterization, and investigation of their mechanisms of action.

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JCHR (2026) 16(2), 768-772 | ISSN:2251-6727

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