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Original article

Exploration of Endophytic Fungi and Their Bioactive Potential Isolated from the Medicinal Plant *Adhatoda vasica*

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Abstract

Background and objective: The plants with pharmacological potential host potential endophytic fungi having metabolic interaction. *Adhatoda vasica* Nees, a well-reputed medicinal plant in Asia, has very few investigations on the plant's endophytic fungi available. This study reports the isolation, identification, and bioactive potential determination of the endophytes from the leaf and stem of the plant growing in Bangladesh. **Methods:** A protocol for fungus isolation was followed, including the significant steps: sample collection, surface sterilization, cultivation, preliminary selection, and purification. The fungal species were identified by morphological and molecular features, and then, small-scale cultivation followed solvent treatment (chloroform) to extract secondary metabolites. The extract's cytotoxic, antimicrobial, and antioxidant activities were determined by brine shrimp lethality bioassay, disc diffusion method, and DPPH free radical scavenging activity, respectively. **Results:** Eight endophytic fungi were isolated and identified: four *Fusarium sp.*, two *Colletotrichum sp.*, one *Phacidiopycnis sp.*, one *Lasiodiplodia sp*. Genome sequence confirmed two novel fungi from the plant: *Fusarium solani* (OR414980) and *Colletotrichum gloeosporioides* (OR420097). In bioactivity testing, the fungi from the stem exhibited better activity than the leaf fungi. Among the eight fungi, *Lasiodiplodia sp.* showed the highest and most significant potential in each bioactivity test. Its extract (100 µg/disc) was approximately 80% susceptible against Gram-negative and Gram-positive bacteria and a fungus *A. flavus* compared to references (30 µg/disc). The fungus's LC_{50} (4 h) was 0.45 µg/mL, whereas vincristin sulfate showed nearly half. **Conclusion:** The study recorded uncommon fungi of four genera from *A. vasica*; some showed remarkable bioactivity.

Keywords: *Adhatoda vasica;* antimicrobial; cytotoxicity; DNA sequencing; endophytes

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Introduction

Endophytic fungi are taxonomically and ecologically heterogeneous groups of organisms. They reside in the tissues of living plants for at least part of their life cycle without causing apparent disease [1,2]. At the same time, the fungi evolve to the adaptive response against

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diseases, insects, pests, and herbivores that also provide their protective role for the host plants [3–6]. Their synthesis of different molecules characterizes the evolution as like the secondary metabolites of the plants. The astounding chemical variety of their secondary metabolites is consistent with entophytic fungi's tremendous diversity and ecological roles [7,8]. For this reason, endophytes have been of great interest during the last two decades to discover biologically active chemical constituents and economically synthesize these molecules [9].

Usually, medicinal plant carries the potential endophytic fungi to produce secondary metabolite due to metabolic interaction [10]. On behalf of these points, *Adhatoda vasica* Nees, a well-investigated medicinal plant with a wide range of chemical constituents, might be a good candidate for fungal isolation and getting pharmacologically active compounds from their metabolites [11]. The plant belongs to the Acanthaceae family and is a well-known medicinal plant for respiratory tract ailments in treating cough, bronchitis, asthma, and common cold [12]. However, it has more diverse therapeutic activities, including antiulcer, hepatoprotective, cardioprotective, abortifacient, antitubercular, antimutagenic, antibacterial, antiasthmatic, and antitussive properties [13].

Endophytic fungi contain biosynthetic gene clusters (BGCs) encoding the biosynthetic enzymes responsible for producing several classes of secondary metabolites, which sometimes follow the biosynthetic pathways analogous to plants [14,15]. Quinazoline alkaloids are the most important bioactive compounds isolated from *A. vasica*. Several important bioactive alkaloids of quinolone, isoquinoline, indole, ergot, pyridine and quinazoline class have already been separated from a different genus of endophytic fungi [16]. If *A. vasica* collected in Bangladesh harbor similar type fungi, they might give forth similar bioactive metabolites. Endophytic fungi belonging to several genera of *Colletotrichum*, *Diaporthe*, *Flavodon*, *Corynespora*, and *Mycoleptodiscus* were isolated from leaves of *Justicia brandegeana* plant collected in Brazil [17]. A report described the isolation of endophytic fungi of *Chaetomium* sp. from leaves of *A. vasica* collected from Islamabad, Pakistan[18] . The present study is the first attempt to isolate endophytic fungi from *A. vasica* collected in Bangladesh, along with the bioactivity determination of the fungal metabolites.

Material and Methods

Ethical considerations

This study was approved at the Faculty of Science by Noakhali Science and Technology University Ethical Committee, Bangladesh, with the ethical code: NSTU/SCI/EC/2018/148.

Chemicals

Water agar medium (HiMedia, Germany); Potato dextrose agar medium (HiMedia, Germany); 2,2-Diphenyl-1-Picrylhydrazyl (Merck, Germany); Chloroform (S D Fine Chem, India); Dimethyl sulfoxide (Rx Chemicals, India) were used in the study.

Plant material

Healthy and mature plant leaves and stems were carefully collected from the Botanical Garden of Jahangirnagar University, Savar, Dhaka, in May 2018 and identified by a taxonomist of the Bangladesh National Herbarium, Dhaka, Bangladesh (DACB 78253).

Isolation of endophytic fungi

The respective plant parts were washed with tap water, followed by washing with distilled water. The leaves and stems were then cut into small pieces. Then, the smaller plant parts were surface-sterilized by sequential immersion in 70% ethanol, 1.3 M sodium hypochlorite, 70% ethanol, and sterile distilled water. Surface-sterilized leaf and stem fragments spaced in Petri dishes containing water agar medium amended with streptomycin (100 mg/L) and incubated at 28± 2 °C until fungal growth started. Unsterilized stem segments were set under the same conditions in parallel to isolate the surface-contaminating fungi as control. The hyphal tips, which grew from sample segments over 4-6 weeks, were separated and purified by sub-culturing onto potato dextrose agar (PDA) medium [19].

Identification of the fungi

Morphology and DNA sequence was used to identify the fungus. Conidial size, shape, structure, color, and growth pattern on PDA media were observed at different intervals. Furthermore, microscopic observation of

endophytic fungal spores confirmed the genus of the fungi. For microscopic identification, the slide specimen was stained with lactophenol cotton blue reagent and examined with a bright field and phase-contrast microscope. The characteristic arrangement of spores under 10x, 40x, and 100x objective lenses of a compound microscope was observed (Krüss, Germany). Finally, fungal taxonomical positions were determined by described methods by Humber, 1997 [20]. To get the conformation up to species, we amplified and sequenced the intervening 5.8S rRNA and the Internal Transcript Spacer regions (ITS4 and ITS5) in an electrophoretic sequencing DNA analyzer (Applied Biosystems, USA) applying Big Dye Terminator v 3.1 cycle sequencing kit for molecular identification. The universal ITS primers were 5"-TCC GTA GGT GAA CCT GCC G-3" for ITS4 region and 5"-GGA AGT AAA AGT CGT AAC AAG G-3" for ITS5 region. Hot Start Green Master Mix (Cat. M7432, Promega, USA) was used to purify and desalting of the PCR products [21]. The sequences were aligned using Chromas software (V 2.6.2) and matched against the nucleotide-nucleotide database BLASTn of NCBI (National Center for Biotechnology Information) for the final determination of the endophytic isolates [22].

Extraction of fungal secondary metabolites

The isolated fungal strains were cultivated on a small scale for 21 days on PDA medium. Then the cultures were kept in a freezer (-4 to -10 °C), and after 24 h, these were transferred to a fume hood at average temperature. The resulting water portions from the media were filtrated, and the remaining solid cultures were cut into small fragments and soaked into ethyl acetate. After the 7^{th} and 14^{th} day, liquid ethyl acetate portions were filtrated and evaporated using a rotary evaporator to get dry and solid crude extracts.

Bioactivity determination

The study evaluated the biological potential of the fungi through antioxidant properties, antimicrobial activity, and brine shrimp lethality assyas. Additinally, the leaf and stem extracts of *A. vasica* were undertaken in the study to compare the biochemicals of fungi to their host.

Antioxidant activity

The free radical scavenging activity of the plant and endophyte extracts on the stable radical DPPH were estimated by modification of the method described by Brand –Williams et al. [23]. The amount of 1.6 mg samples and standards (ascorbic acid and tert- butyl-1-hydroxytoluene) were dissolved in 4 mL methanol to make a stock solution. Then, 2 mL was transferred to ten test tubes with consecutive addition of 2 mL methanol into the solution. Afterwards, 2 mL DPPH methanol solution of 20 µg/mL was added to each test tube to maintain a serial concentration of 200.0 to 0.78125 µg/mL of sample. After 30 minutes of reaction period at room temperature in a dark place, the absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer (SPECORD 250 PLUS: Analytik Jena double-beam spectrometer, Japan).

Inhibition of free radical DPPH in percent (I%) was calculated as follows:

$$I\% = (1 - A_{sample}/A_{control}) \times 100$$

Then, 50% inhibitory concentration (IC_{50}) was determined by linear regression analysis.

Antimicrobial activity

The disc diffusion method of antimicrobial activity determination was followed as suggested by Toma & Barriault against two Gram-positive bacteria, two Gram-negative bacteria, and two fungi [24]. The bacterial and fungal strains were *Bacillus megaterium* (BTCC 18), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 28739), *Pseudomonas aeruginosa* (ATCC 27833), *Aspergillus niger*, and *Aspergillus flavus.* Kanamycin (antibacterial agent) and ketoconazole (antifungal agent) standard discs were used to compare the sample activity. The entity concentrations per disc were 30 µg for standards, 100 µg for fungal extracts, and 500 mg for plant extracts.

Brine shrimp lethality assay

The test was followed as suggested by Meyer et al. with simplification [25]. As the standard, vincristine sulfate was used. Every ten living brine shrimp nauplii were transferred into ten test tubes containing 5 mL salt water (salt concentration maintained like seawater). Then samples were added, dissolving in dimethylsufoxide (DMSO) by serial dilution to maintain concentration from 100 to 0.19531

µg/mL. In the control group, only 30 µL DMSO was added to 5 mL saltwater. After 4 h, the test tubes were observed using a magnifying glass, and the number of survived nauplii in each vial was counted. From this data, the percent (%) of the lethality of the brine shrimp nauplii was calculated for each concentration.

Results and Discussion

Eight endophytic fungi were isolated from *A. vasica* where four were from leaves, code AVLE-1, AVLE-2, AVLE-3, and AVLE-4, and four were from stems, code AVSE-1, AVSE-2, AVSE-3, and AVSE-4. They exhibited characteristic colonies and microscopic morphology that assisted in differentiating them. According to macroscopic and microscopic view, AVLE-1, AVLE-2, AVLE-4, and AVSE-4 had white, salmon, or gray-colored colonies with velvety to cottony surfaces and microscopically, the filaments were hyaline, and some septate (Figures 1 and 2). AVLE-3 showed few unique criteria such as gray myceliam with rapid growth, curved created circular patterns on top and bottom; chonidiospores simple; conidia hyaline, one-celled, and ovoid. AVSE-1 and AVSE-3 showed wooly and gray tops with the brown bottom view; conidiophores simple, elongated; conidia hyaline. AVSE-2 exhibited wooly and raised top with an entire margin and circular form, dark bottom, and coenocytic hyphae, obovate and dark spore (Figure 2).

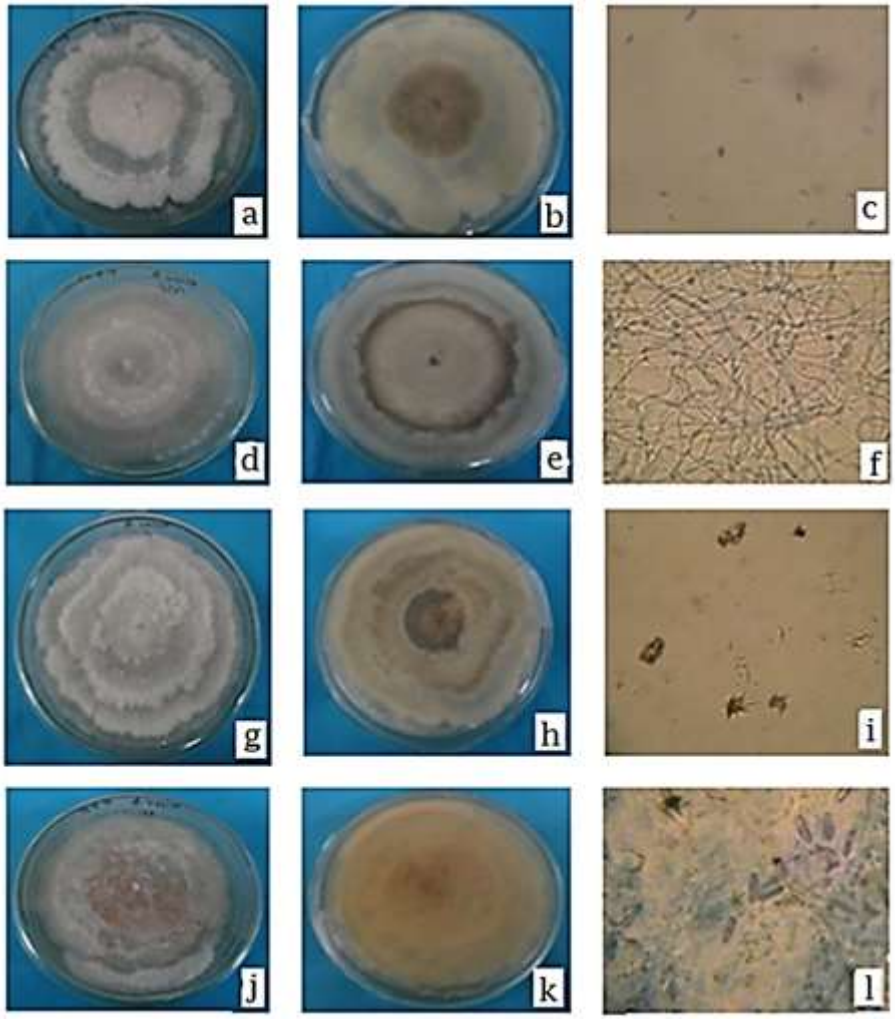

Figure 1. The pictures represent a macroscopic and microscopic view of fungus from **leaves** of *Adhatoda vasica* after 11 days of cultivation and a microscopic view of hyphae and spores (40X); a, b, c indicate the top, bottom, and microscopic views of AVLE-1, respectively. In the same way, (d, e, f), (g, h, i), (j, k, l) are the top, bottom, and microscopic view of AVLE-2, AVLE-3, AVLE-4, respectively.

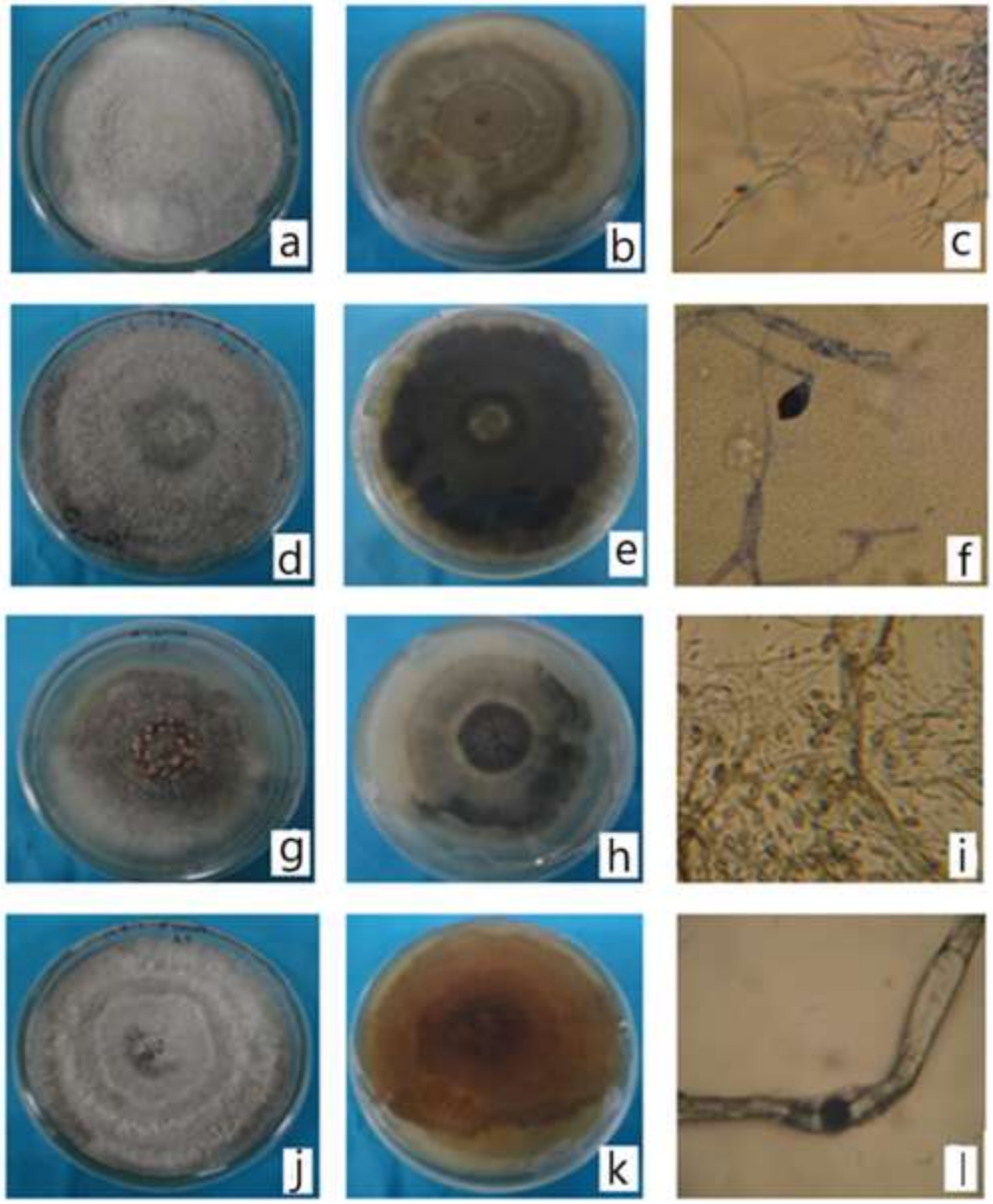

Figure 2. The macroscopic and microscopic view of fungus isolated from stems of *Adhatoda vasica* after 11 days of cultivation and a microscopic view of hyphae and spores (40X); a, b, c indicates the top, bottom, and microscopic views of AVSE-1, respectively. In the same way, (d, e, f), (g, h, i), (j, k, l) are the top, bottom, and microscopic view of AVSE-2, AVSE-3, AVSE-4, respectively.

The eight fungi were taxonomically identified based on morphological features and DNA sequencing. Based on microscopic and morphological characters, the fungi exhibited the probability of the following fungi: AVLE-1, AVLE-2, AVLE-4, and AVSE-4 were *Fusarium sp.*; AVLE-3 was *Phacidiopycnis sp.*; AVSE-1 and AVSE-3 were *Colletotrichum sp.*; AVSE-2 was *Lasiodiplodia sp.* Gene sequences of AVLE-4 and AVSE-3 based on ITS-5.8S rRNA showed 99% similarity to *Fusarium solani* and *Colletotrichum gloeosporioides*; National Center for Biotechnology Information (NCBI) accession numbers are OR414980 and OR420097, respectively.

Evaluating the antioxidant activity, the stem extract of *A. vasica* showed thirteen times more antioxidant activity than the leaf extract, with IC_{50} of 31.69 µg/mL. The endophytic fungi from the stem had relatively more activity than the fungi from the leaf. Fungal extract of AVSE-2 (*Lasiodiplodia sp.*) exhibited the highest free

radical scavenging property among all endophytes with IC_{50} value of 100.8 µg/mL (Figure 3). On the other hand, AVSE-4 (one *Fusarium sp.*) showed the least activity.

Leaf extract of the plant (AVL) exhibited moderate activity against *Bacillus megaterium*, *Pseudomonus aeruginosa*, and suiatbel activity against *E. coli*. The stem extract of the plant (AVS) did not show activity against tested bacteria at a concentration of 500 µg/disc. AVSE-2 (*Lasiodiplodia sp*.) exhibited the highest activity against *E. coli*, *Staphylococcus aureus*, *P. aeruginosa*, *B. megaterium and Aspergillus flavus* at 100 µg/disc was roughly 80% of references at 30 µg/disc (Table 1). Two *Fusarium sp*. AVLE-2 and AVLE-4 exhibited activity against *P. aeruginosa* with 10 mm and 7 mm inhibition zone, respectively. AVSE-3 (*Colletotrichum gloeosporioides*) showed activity against both Gram-positive and Gram-negative bacteria. But, no one except AVSE-2 successfully exhibited antifungal activity with a zone inhibition of 25 mm. The *Lasiodiplodia sp.* showed the most prominent antimicrobial activity compared to *Fusarium sp*. and *Colletotrichum sp.* In the cytotoxicity test, leaf fungal extracts of *A. vasica* showed no substantive cytotoxicity, whereas the values for stem fungi were fungi were comparable with reference vincristine sulphate. The significant results are shown in Figure 4.

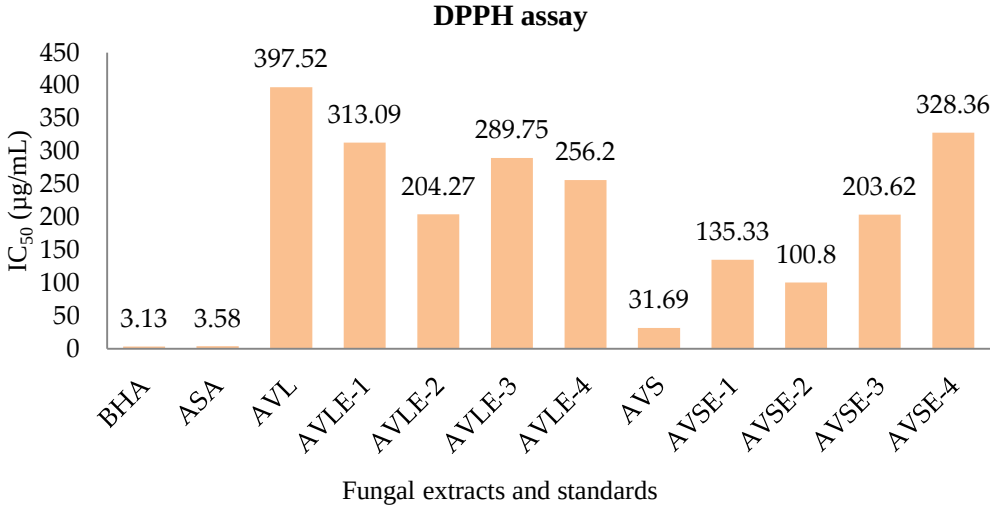

Figure 3. IC_{50} values of test samples and standard molecules; BHA, butylated hydroxyanisole; ASA: ascorbic acid; AVL: *Adhatoda vasica* leaf; AVLE: *A. vasica* leaf endophyte; AVS: *A. vasica* stem; AVSE: *A. vasica* stem endophyte

Table 1. Antimicrobial activity of plant extracts and endophytic fungal strains

Tests	**Antibacterial activity Zone of inhibition (mm)**				**Antifungal activity Zone of inhibition (mm)**	
Samples	***Escherichia coli***	***Pseudomonus aurigenosa***	***Bacillus megaterium***	***Staphylococcus aureus***	***Aspergillus niger***	***Aspergillus flavus***
Standards	25	25	27	23	35	45
AVL	13	12	-	12	-	-
AVLE-1	-	-	-	-	-	-
AVLE-2	-	10	-	-	-	-
AVLE-3	-	-	-	-	-	-
AVLE-4	-	7	-	-	-	-
AVS	8	-	-	-	-	-
AVSE-1	7	-	-	-	-	-
AVSE-2	19	20	15	20	-	25
AVSE-3	11	9	-	7	-	-
AVSE-4	-	-	-	-	-	-

-: no activity; AVL: *Adhatoda vasica* leaf; AVLE-1: *A. vasica* leaf endophyte 1; AVLE-2: *A. vasica* leaf endophyte 2; AVLE-3: *A. vasica* leaf endophyte 3; AVLE-4: *A. vasica* leaf endophyte 4; AVS: *A. vasica* stem; AVSE-1: *A. vasica* stem endophyte 1; AVSE-2: *A. vasica* stem endophyte 2; AVSE-3: *A. vasica* stem endophyte 3; AVSE-4: *A. vasica* stem endophyte 4

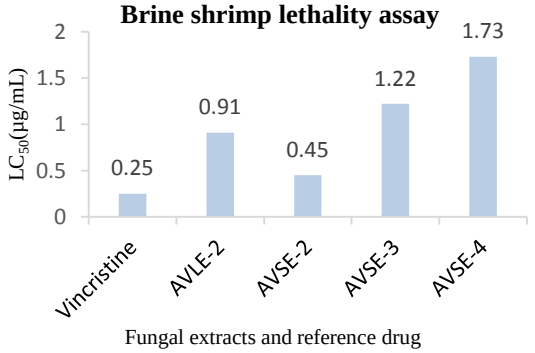

Figure 4. LC_{50} values of test samples and standard drug; AVLE-2: *Adhatoda vasica* leaf endophyte 2; AVSE-2: *A. vasica* stem endophyte 2; AVSE-3: *A. vasica* stem endophyte 3; AVSE-4: *A. vasica* stem endophyte 4

The lowest LC_{50} (4 h) value among the fungi strains was for the AVSE-2 (*Lasiodiplodia sp.)* extract with 0.45 µg/mL, whereas vincristine sulfate had nearly a half of AVSE-2 logarithmic LC_{50} (4 h) values of AVLE-2, AVSE-3, and AVSE-4 were considerable, and these were 0.91, 1.22, and 1.73 µg/mL, respectively (Figure 4).

Endophytic fungi are abundant in the plant kingdom, including algae, ferns, lichens, mosses, and vascular plants [26]. They are of growing interest to scientists as rich sources of diversified secondary metabolites. As a result, this research aimed to investigate the diversity of fungi isolated from the medicinal plant *A. vasica*, which is predominantly grown in Bangladesh.

A total of eight endophytic fungi were isolated and purified from the plant*:* four *Phacidiopycnis sp.*, two *Colletotrichum sp.*, one *Fusarium sp.* and one *Lasiodiplodia sp*. Previous studies reported the presence of endophytic fungi of multiple genera from the plant, namely *Aspergillus sp., Emericella sp., Fusarium sp., Gliocladium sp., Penicillium sp. Colletotrichum sp. Chaetomium sp., Glomerella sp.,* and *Alternaria sp.* [18,27,28]. Therefore, *Phacidiopycnis sp.* and *Lasiodiplodia sp.* are reported for the first time from the plant in this study. Moreover, although *Fusarium oxysporum, Fusarium avenaceum,* and *Colletotrichum dermatium* were reported before, the present species *Fusarium solani* and *Colletotrichum gloeosporioides* are also novel as the plant's fungi.

Some fungal endophytes, including *Fusarium sp*., *Colletotrichum sp*., previously identified from the medicinal plants of Bangladesh exhibited varied bioactivity such as antioxidant, antimicrobial, cytotoxic, and anti-cancer properties [29,30]. Researchers from several ecoregions have uncovered specific bioactive endophytes producing essential molecules [31–33]. Therefore, the study analyzed the biological activities of the isolated fungi.

IC_{50} values of the free radical scavenging test and LC_{50} values of lethality bioassay showed that the fungi from the stem had better activity than the fungi from the leaf. Extracts of *Lasiodiplodia sp.* exhibited significant bioactivy. A study described the presence of 134 compounds of the fungi belonging to the category of secondary metabolites including cyclohexenones, indoles, jasmonates, lactones, melleins, and phenols. These compounds were prone to potential antimicrobial and cytotoxic activities [34]. In the antibacterial assay, compounds from *Lasiodiplodia theobromae* have shown significant susceptibility against *S. aureus*, with MIC levels from 1.6 to 13 µg/mL. Also, those compounds revealed cytotoxicity against human cancer cell lines, with IC_{50} values range of 2.5–9.4 µM [35]. In the present study, *Lasiodiplodia sp.* exhibited excellent antimicrobial activities against *E. coli*, *S. aureus*, *P. aeruginosa*, *B. megaterium*, and *A. flavus*. Moreover, LC_{50} of brine shrimp lethality bioassay for *Lasiodiplodia sp.* was the lowest among all samples that indicated the toxicity of its metabolites. Also, the results match the finding described by Cimmino et al. [36].

We identified *Fusarium solani* and *Colletotrichum gloeosporioides by* ITS 5.8S ribosomal gene sequencing technique. *Fusarium solani* is a plant pathogen and saprophyte in the soil, and its direct inoculation or spore inhalation causes a variety of illnesses, including keratitis, onychomycosis, eumycetoma, and skin lesions [37]. *Colletotrichum gloeosporioides* is also a plant pathogen that causes bitter rot in various crops worldwide, particularly perennials in tropical regions [38]. The fungal metabolites exhibited significant antimicrobial and cytotoxic effects in several investigations. A study isolated napthaquinone aza-anthraquinones from the *F. solani* that had antimicrobial and cytotoxic properties [39]. Another study revealed that the endophytic fungus *Colletotrichum gloeosporioides* produced novel bioactive compounds against multidrug-resistant *Staphylococcus aureus* [40]. Our study complied with the reports to some extent, except for the cytotoxic effects of AVLE-4 (*F. solani*).

Conclusion

The findings explored the significant scientific and industrial potentials of *A. vasica* and its endophytic fungi. Eight fungi were isolated, and few were first reported. The bioactivity of isolated few fungal metabolites give a clue to potentially valuable compounds. Further investigation is required to isolate and characterize the molecules from the potential fungi.

Acknowledgments

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Author contributions

Prodip Kumar Baral conducted the study in the laboratory and wrote the manuscript; the study was conceptualized and supervised by Mohammad. Hossain Sohrab and Sakina Sultana, Farhana Afroz and Nadira Begum were associated with fungus isolation and identification; Satyajit Roy Rony and Suriya Sharmin contributed to manuscript preparation and proofreading; Fatema Moni and Shammi Akhter contributed to the bioactivity testing of the fungi.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

References

[1] Gokhale M, Gupta D, Gupta U, Faraz R, Sandhu SS. Patents on endophytic fungi. *Recent Pat Biotechnol*. 2017; 11(2): 120–140.

[2] Khan N, Afroz F, Begum MN, Rony SR, Sharmin S, Moni F, Hasan CM, Shaha K, Sohrab MH. Endophytic *Fusarium solani*: a rich source of cytotoxic and antimicrobial napthaquinone and aza-anthraquinone derivatives. *Toxicol Rep*. 2018; 5: 970–976.

[3] Chowdhury NS, Sohrab MH, Rana MS, Hasan CM, Jamshidi S, Rahman KM. Cytotoxic naphthoquinone and azaanthraquinone derivatives from an endophytic *Fusarium solani*. *J Nat Prod*. 2017; 80(4): 1173–1177.

[4] Knop M, Pacyna S, Voloshchuk N, Kant S, Müllenborn C, Steiner U, Kirchmair M, Scherer HW, Schulz M. *Zea mays*: Benzoxazolinone detoxification under sulfur deficiency conditions- a complex allelopathic alliance including endophytic *Fusarium verticillioides*. *J Chem Ecol*. 2007; 33: 225–237.

[5] Clay K, Fuqua C, Lively C, Wade MJ. Microbial community ecology of tick-borne human pathogens. In: Collinge SK, Ray C, Eds. Disease ecology: Community structure and pathogen dynamics. Oxford: Oxford Academic, 2006.

[6] Clay K, Schardl C. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *Am Nat.* 2002; 160(S4): 99–127.

[7] Khan MI, Sohrab MH, Rony SR, Tareq FS, Hasan CM, Mazid MA. Cytotoxic and antibacterial naphthoquinones from an endophytic fungus, *Cladosporium* sp. *Toxicol Rep.* 2016; 3: 861–865.

[8] Strobel G, Daisy B, Castillo U, Harper J. Natural products from endophytic microorganisms. *J Nat Prod*. 2004; 67(2): 257–268.

[9] Abdel-Azeem AM, Abdel-Azeem MA, Khalil WF. Endophytic fungi as a new source of antirheumatoid metabolites. In: Watson RR, Preedy VR, Eds. Bioactive food as dietary interventions for arthritis and related inflammatory diseases. Academic Press (Online edition), 2019.

[10] Kogel KH, Franken P, Hückelhoven R. Endophyte or parasite–what decides? *Curr Opin Plant Biol*. 2006; 9(4): 358–363.

[11] Baral PK, Roy S, Sultana S. A review article on *Adhatoda vasicanees*: a potential source of bioactive compounds. *Int J Dev Res*. 2018; 8(11): 23874–23882.

[12] Karthikeyan A, Shanthi V, Nagasathaya A. Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatoda vasica* L. *Int J Green Pharm.* 2009; 3(1): 78–80.

[13] Singh TP, Singh OM, Singh HB. *Adhatoda vasica* Nees: phytochemical and pharmacological profile. *Nat Prod J*. 2011; 1(1): 29–39.

[14] Rokas A, Mead ME, Steenwyk JL, Raja HA, Oberlies NH. Biosynthetic gene clusters and the evolution of fungal chemodiversity. *Nat Prod Rep*. 2020; 37(7): 868–878.

[15] Deepika VB, Murali TS, Satyamoorthy K. Modulation of genetic clusters for synthesis

of bioactive molecules in fungal endophytes: a review. *Microbiol Res*. 2016; 182: 125–140.
[16] Zhang Y, Han T, Ming Q, Wu L, Rahman K, Qin L. Alkaloids produced by endophytic fungi: a review. *Nat Prod Commun*. 2012; 7(7): 963–968.
[17] Silva AA, Polonio JC, de Oliveira JA, Bulla AM, Golias HC, Polli AD, Soares LC, Vicentini VE, de Oliveira AJ, Gonçalves JE, Gonçalves RA. Multilocus sequence analysis of endophytic fungi from *Justicia brandegeana* with the culture-dependent method and their bioprospection for health field. *S Afr J Bot.* 2020; 134(1): 359–368.
[18] Fatima N, Mukhtar U, Qazi MA, Jadoon M, Ahmed S. Biological evaluation of endophytic fungus *Chaetomium* sp. NF15 of *Justicia adhatoda* L.: a potential candidate for drug discovery. *Jundishapur J Microbiol*. 2016; 9(6):1–12.
[19] Kusari S, Lamshöft M, Zühlke S, Spiteller M. An endophytic fungus from *Hypericum perforatum* that produces hypericin. *J Nat Prod.* 2008; 71(2): 159–162.
[20] Humber RA, Lacey LA. Fungi: identification. Manual of techniques in insect pathology. Academic Press, 1997.
[21] Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibillo A. Real-time DNA sequencing from single polymerase molecules. *Science*. 2009; 323(5910): 133–138.
[22] Abarenkov K, Nilsson RH, Larsson KH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, Sen R. The UNITE database for molecular identification of fungi–recent updates and future perspectives. *New Phytol*. 2010; 186(2): 281–285.
[23] Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci Technol.* 1995; 28(1): 25–30.
[24] Toma E, Barriault D. Antimicrobial activity of fusidic acid and disk diffusion susceptibility testing criteria for Gram-positive cocci. *J Clin Microbiol*. 1995; 33(7): 1712–1715.
[25] Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med*. 1982; 45(5): 31–34.
[26] Huang WY, Cai Y, Surveswaran S, Hyde KD, Corke H, Sun M. Molecular phylogenetic identification of endophytic fungi isolated from three *Artemisia* species. [Accessed 2023]. Available form: https://www.fungaldiversity.org/fdp/sfdp/FD36-5.pdf.
[27] Barman S, Ghosh R, Mandal N. Assessment of antimicrobial activity of endophytic fungi isolated from *Justicia adhatoda* L. *UGB J Plant Biol Biotech*. 2016; 1(1): 19–23
[28] Mishra Y, Sharma L, Dhiman M, Sharma MM. Endophytic fungal diversity of selected medicinal plants and their bio-potential applications. In: Sharma VK, Shah MP, Parmar S, Kumar A, Eds. Fungi bio-prospects in sustainable agriculture, environment and nano-technology. Academic Press (Online edition), 2021.
[29] Chowdhury NS, Sohrab MH, Rony SR, Suriya S, Begum MN, Rana MS, Hasan CM. Identification and bioactive potential of endophytic fungi from *Monochoria hastata* (L.) Solms. *Bangladesh J Bot.* 2016; 45(1): 187–193.
[30] Mahmud SN, Sohrab MH, Begum MN, Rony SR, Sharmin S, Moni F, Akhter S, Mohiuddin AK, Afroz F. Cytotoxicity, antioxidant, antimicrobial studies and phytochemical screening of endophytic fungi isolated from *Justicia gendarussa*. *Ann Agric Sci*. 2020; 65(2): 225–232.
[31] Kaul S, Gupta S, Ahmed M, Dhar MK. Endophytic fungi from medicinal plants: a treasure hunt for bioactive metabolites. *Phytochem Rev*. 2012; 11: 487–505.
[32] Hazalin NAMN, Ramasamy K, Lim SSM, Wahab IA, Cole ALJ, Abdul Majeed AB. Cytotoxic and antibacterial activities of endophytic fungi isolated from plants at the National Park, Pahang, Malaysia. *BMC Complement Altern Med*. 2009; 9: 1–5.
[33] Shah A, Rather MA, Hassan QP, Aga MA, Mushtaq S, Shah AM, Hussain A, Baba SA, Ahmad Z. Discovery of anti-microbial and anti-tubercular molecules from *Fusarium solani*: an endophyte of *Glycyrrhiza glabra*. *J Appl Microbiol*. 2017; 122(5): 1168–1176.
[34] Salvatore MM, Alves A, Andolfi A. Secondary metabolites of *Lasiodiplodia theobromae*: distribution, chemical diversity, bioactivity, and implications of their occurrence. *Toxins.* 2020; 12: 1–29.
[35] Chen S, Chen D, Cai R, Cui H, Long Y, Lu Y, Li C, She Z. Cytotoxic and antibacterial

preussomerins from the mangrove endophytic fungus *Lasiodiplodia theobromae* ZJ-HQ1. *J Nat Prod.* 2016; 79(9): 2397–2402.

[36] Cimmino A, Cinelli T, Masi M, Reveglia P, da Silva MA, Mugnai L, Michereff SJ, Surico G, Evidente A. Phytotoxic lipophilic metabolites produced by grapevine strains of *Lasiodiplodia* species in Brazil. *J Agric Food Chem.* 2017; 65(6): 1102–1107.

[37] Kuruvilla TS, Dias M. *Fusarium Solani*: a causative agent of skin and nail infections. *Indian J Dermatol*. 2012; 57(4): 308–309.

[38] Alam MW, Malik A, Rehman A, Hameed A, Tahir U, Sarwar M, Hussain M, Hussain D, Shafeeq T. First record of *Colletotrichum gloeosporioides* causing anthracnose of banana in Pakistan. *Plant Dis*. 2021; 105(7): 2013.

[39] Khan N, Afroz F, Begum MN, Rony SR, Sharmin S, Moni F, Hasan CM, Shaha K, Sohrab MH. Endophytic *Fusarium solani*: a rich source of cytotoxic and antimicrobial napthaquinone and aza-anthraquinone derivatives. *Toxicol Rep*. 2018; 5: 970–976.

[40] Arivudainambi USE, Anand TD, Shanmugaiah V, Karunakaran C, Rajendran A. Novel bioactive metabolites producing endophytic fungus *Colletotrichum gloeosporioides* against multidrug-resistant Staphylococcus aureus. *FEMS Immunol Med Microbiol*. 2011; 61(3): 340–345

Abbreviations

AVL: *Adhatoda vasica* leaf; AVS: *Adhatoda vasica* stem; AVLE: *Adhatoda vasica* leaf endophyte; AVSE: *Adhatoda vasica* stem endophyte; DCM: dichloromethane; IC_{50}: inhibitory concentration 50%; LC_{50}: lethal concentration 50%; DMSO; dimethyl sulfoxide; PDA: potato dextrose agar; TLC: thin layer chromatography; DPPH: 2,2-diphenyl-1-picrylhydrazyl