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Isolation and Chemical Structural Elucidation of Antibacterial Bioactive Compounds from Endophytic Fungal Strain *Phoma* sp. D1

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Abstract

The present investigation has been performed for the isolation, optimization, and chemical characterization of antibacterial compounds from endophytic fungi that survive inside the internal tissues of plants without causing any symptoms. Endophytic fungi were isolated from four medicinal plants and screened for their antibacterial activity. The fungal strain *Phoma* sp. D1 showed maximum antibacterial activity at 18.25 ± 0.58, 22.75 ± 0.85, 19.30 ± 0.76, and 16.30 ± 0.65 against *Klebsiella pneumoniae*, *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*, respectively. For maximum production of antibacterial compounds, *Phoma* sp. D1 was further optimized on different sources such as culture media, pH, temperature, carbon and nitrogen sources, and incubation period. The optimum culture media, pH, temperature, carbon and nitrogen, and incubation period for bioactive metabolite production of the strain were recorded in SDB, dextrose, yeast extract, 25 ± 0.1, and pH 8.0. The purification and chemical characterization of the antibacterial compounds were done by using solvent–solvent extraction, thin-layer chromatography, and gas chromatography and mass spectroscopy. The chemical characterization of the extract from *Phoma* sp. D1 showed the presence of five compounds, 2-(-cyclohexenyl) ethylamine, 2-chloro-6 fluorophenol, butyl ether2-hydroxyskatole4-glycerol, p-coumarate, and bicyclo [4,2,1] nona-2,4-dien-9-one could be responsible for antibacterial activity. The computation observation of biomolecules through DFT, QSAR, EHOMO, and ELUMO was also performed.

Keywords Isolation · Chromatography · Skatole · Antibacterial activity · DFT · QSAR

Introduction

India has been considered to be a rich repository of medicinal plants which have been widely collected as raw materials for the manufacture of useful products since time immemorial. According to a recent World Health Organization (WHO) assessment, 80% of people worldwide rely on natural herbs for their basic medical needs [1, 2]. In developed nations, for example, the USA, plant based constitutes as much as 25% of the all-out medications while in fast-developing nations such as India and China, the commitment of characteristic assets for the readiness of medication is as much as 80% [3]. However, the uncontrolled exploitation of medicinal plants has resulted in a rapid decline in their populations, and some of the plants have become critically endangered. The manufacturing of plant-based medicines has increased with the population, resulting in an overuse of plants and significant loss of plant diversity. Yet symbiotic microorganisms that thrive inside plant cells also contribute to the bioactive compounds that plants manufacture. In order

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to maintain plant diversity and improve the production of bioactive substances for the treatment of various diseases, much effort is being done in the field of endophytes [4–8].

Endophytes are microorganisms that live inside the tissues of living plants without causing any overt negative effect and have been found in each plant species considered and perceived as a potential source of novel natural products for exploitation in medicine, agriculture, and industry [9]. Endophytic microorganisms are endosymbiotic groups of organisms whether bacteria or fungi that colonize intercellular or intracellular locations of plant species [10]. The endophytic parasites upgrade the development and supplement gain of host life forms and improve the host capacity to endure different sorts of abiotic and biotic stresses and develop resistance in plants against insects and pests. They additionally produce phytohormones and other few bioactive substances such as proteins and drugs of biotechnological interest [11, 12]. The endophytic fungi are known to possess biosynthetic capabilities greater than those of host plants due to their long co-evolution and genetic recombination [13, 14].

In the previous 20 years, numerous important bioactive compounds with anti-microbial, insecticidal, cytotoxic and anticancer, antioxidant, antimalarial, antiviral, immunesuppressive, anti-tuberculosis, etc. activities have been successfully isolated from the endophytic fungi [15–17]. Further, theoretical and computational approaches such as DFT, QSAR, highest occupied molecular orbitals (HOMOs), and lowest unoccupied molecular orbital's (LUMOs) help to identify the orientation of the molecules and the interaction of the molecules with active sites of the targets [18]. As a result, the primary goal of this research was to separate novel antibacterial compounds of pharmaceutical interest from endophytic fungi for use in the development of antibacterial medications to combat pathogenic and resistant bacteria.

Methods

Collection and Isolation of Endophytic Fungi

In the present study, endophytic fungi were isolated from different parts of medicinal plants, i.e., stems, leaves, and roots of *Calotropis procera*, *Ocimum basilicum*, *Bryophyllum pinnatum*, and *Colocasia esculent* collected from Jabalpur regions (M.P.). The explants were cleaned with tap water to remove the dust and debris from the outer surface of the samples and cut into 2–4-mm-sized pieces. Surface disinfection was carried out by dipping the plant parts into sterilized distilled water for up to 1–2 min and after that transferring them into 70% alcohol for 30 s, and then keeping them in 4% sodium hypochlorite (NaOCl) solution for 1–2 min [19–21]. Finally, the plant material was placed in distilled water for 1–2 min and transferred onto filter paper for removal of excess moisture from the samples. After surface disinfection, all plant parts were placed on potato dextrose agar (PDA) plates supplemented with streptomycin 500 mL/L to inhibit bacterial growth [22, 23]. All PDA plates were incubated in a fungal incubator for 3–5 days at 26 ± 1 °C, and regular growth of the hyphae was observed.

Preservation and Morphological Identification of Endophytic Fungi

All endophytic fungi isolated from different plant parts were maintained on PDA medium (potato: 200 g; dextrose: 20 g; agar: 15 g, and distilled water: 1000 mL) slants and preserved at 4–5 °C with proper labeling without antibiotics and subcultured at time to time. Morphological identification was done using the slide culture technique on the basis of various characteristics like the shape of the spores, growth and color of the cultured fungal colonies, structure of the hyphae, and size of the spore fungi [24, 25].

Production and Screening of Secondary Metabolites

For the production of secondary metabolites, the endophytic fungi were grown on potato dextrose broth (PDB) medium (potato: 200 g; dextrose: 20 g, and distilled water: 1000 mL) and incubated at 26 ± 1 °C for 7, 14, and 21 days, respectively [26, 27]. After the incubation period, the metabolites were separated by using Whatman filter paper no. 1; their antibacterial activity against test bacterial strains *Klebsiella pneumoniae* (MTCC4032), *Bacillus subtilis* (MTCC441), *Escherichia coli* (MTCC1679), and *Staphylococcus aureus* (MTCC96) was observed by using the agar well diffusion method, and the zone of inhibition was measured with the help of the Hi-Antibiotic zone scale, Hi Media Laboratories, Mumbai [28].

Optimization of Parameters for Maximum Production of Bioactive Compounds

For maximum production of bioactive compounds from potent endophytic fungi, various physical and chemical factors like incubation period, carbon and nitrogen sources, temperature, pH, and growth medium were examined.

Determination of Optimum Culture Medium

To assess optimum growth and maximum production of antibacterial compounds, the potent fungal strain *Phoma* sp. D1 was tested on different media viz. potato dextrose broth (PDB) medium (potato: 200 g, dextrose: 20 g, and distilled water: 1000 mL), Sabouraud dextrose broth (SDB) medium (dextrose: 40 g, peptone: 10 g, and distilled water:

1000 mL), maltose yeast extract broth (MYEB) medium (maltose: 4 g, yeast extract: 4 g, malt extract: 10 g, and distilled water: 1000 mL), Czapek Dox broth (CDB) medium (sodium nitrate: 2 g, dipotassium hydrogen phosphate: 1 g, magnesium sulfate: 0.5 g, potassium chloride: 0.5 g, ferrous sulfate: 0.01 g, sucrose: 30 g, and distilled water: 1000 mL), and Richard broth medium (dipotassium dihydrogen phosphate: 5 g, magnesium sulfate: 2.5 g, potassium nitrate: 10 g, ferric chloride: 0.02 g, sucrose: 50 g, and distilled water: 1000 mL). After 7, 14, and 21 days, the antibacterial activity of the selected media was observed by using the agar well diffusion method [29].

Determination of Optimum Incubation Period

Phoma sp. D1 was used in the steady incubation process for 21 days on a selected medium at 25 ± 1 °C. The cell-free culture filtrate (CFCF) of fungi was extracted from the first day of incubation, and the antibacterial activity was observed by using the agar well diffusion method [30].

Determination of Optimum Nitrogen and Carbon Sources

To determine the effect of carbon and nitrogen sources on the production of secondary metabolites, various carbon and nitrogen sources like maltose, glucose, dextrose, sucrose, mannitol, yeast extract, peptone, potassium nitrate, ammonium chloride, and ammonium nitrate with 1% concentration were amended to the selected medium [31–33]. After the addition of carbon and nitrogen sources separately in the growth medium, all flasks were incubated at 25 ± 1 °C for 12 days and the CFCF of each broth was observed for antibacterial activity.

Determination of Optimum Incubation Temperature

The variations in temperature directly impact the growth and production of metabolites from fungi [34]. Therefore, for maximum production of antibacterial compounds, *Phoma* sp. D1 was incubated at different temperatures of 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, and 40 °C for 12 days in an incubator (REMI, India) to determine antibacterial activity.

Determination of Optimum pH

The pH of the growth medium indirectly affects the metabolism of organisms and influences the enzymatic action of fungi. For the optimization of the pH of the medium, fermentation broth with eight different pH values, 3, 4, 5, 6, 7, 8, 9, and 10, was prepared and incubated for 12 days at 25 ± 1 °C. After incubation, the metabolite was separated using filter paper and antibacterial activity was observed against the test pathogen by the agar well diffusion method [35].

Purification of Bioactive Compounds

The purification of bioactive compounds from endophytic fungi is a crucial process. Therefore, the purification of antibacterial bioactive compounds from *Phoma* sp. D1 using solvent–solvent extraction and thin-layer chromatography approaches was implemented.

Solvent–Solvent Extraction of fungal metabolite

In the case of solvent–solvent extraction, antibacterial compounds were extracted with a wide range of solvents having different polarities like cyclohexane, carbon tetrachloride, ethyl acetate, toluene, benzene, and dichloromethane. Equal volumes of (1:1 v/v) of CFCF and solvents were taken in a separating funnel, shaken for 10–15 min, and kept stationary for 25–30 min. After that solvent fractions were separated successfully and evaporated solvent in sterilized condition. The remaining residue left after evaporation was mixed with dimethyl sulfoxide (DMSO) and their antibacterial activity against bacterial strain was observed. After confirmation of the solvent in which a positive result was obtained, further extraction of the compound was done in a rotary vacuum evaporator and the yielded extract was used for separation of the antibacterial compound.

Thin-Layer Chromatography (TLC)

In this method, a number of different solvent systems were tried in order to attain a good resolution for purification of the antibacterial compounds. Finally, the solvent system benzene:methanol (50:50 v/v) was further used for purification of the compound in TLC and two spots were observed on the TLC plate with different retention factor (R*f*) values.

Table 1 The endophytic fungi isolated from different plants

Name of medicinal plant	Endophytic fungi	Class
Calotropis procera	*Aspergillus flavus*	Eurotiomycetes
	Penicillium chrysogenum	Eurotiomycetes
	Aspergillus niger	Eurotiomycetes
	Monilia sp.	Leotiomycetes
Ocimum basilicum	*Alterneria alternata*	Dothideomycetes
	Nigrospora sp.	Sordariomycetes
	Fusarium oxysporum	Sordariomycetes
Bryophyllum pinnatum	*Phoma* sp. D1	Dothideomycetes
	Fusarium solani	Sordariomycetes
	Colletotrichum sp.	Sordariomycetes
Colocasia esculenta	*Fusarium moniliformi*	Sordariomycetes
	Aspergillus fumigatus	Eurotiomycetes

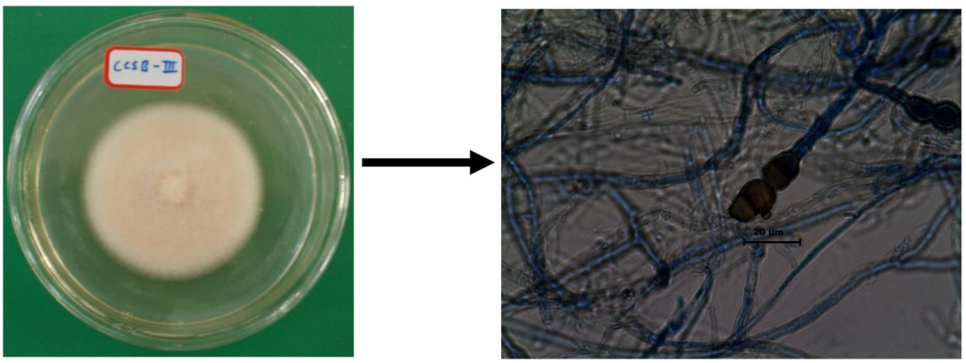


Fig. 1 Morphological identification of potent fungus *Phoma* sp. D1

The spots were scraped out separately from the TLC plate, mixed with DMSO, and centrifuged at 8000 rpm for 20 min. The collected fractions were tested for antibacterial activity by agar well diffusion.

R*f* = migration distance of compound/migration distance of solvent front.

Chemical Characterization of Antibacterial Compound

In order to characterize antibacterial compounds, active fractions collected after TLC were subjected to gas chromatography–mass spectrometry (GC–MS) analysis. The sample was analyzed in an Agilent 19091 s-431u1 GCMS/MS 700 1C analyzer and introduced via an all-glass injector working in the split-less mode, with helium as the carrier gas with a linear velocity of 25.779 cm/s. An HP-5 MS UI fused silica capillary column (length: 15 m, film thickness: 250 µm, I.D.: 0.25 mm) was used. The temperature program was as follows: 60–300 °C at 40 °C/min, 170–310 °C at 10 °C/min, and a 5-min hold at 310 °C. The identification of bioactive compounds was performed by mass spectra with data from the library.

Computational Observation (DFT Evaluation)

In the present scenario, the computation observation such as DFT evaluation plays a significant role in gaining experimental date and predicts the different properties of the bioactive compounds. Therefore, in the present study, initially, all the characterized bioactive compounds were optimized and analyzed using the Gauss View 6.0.16 program, GAUSSIAN 09 suite of programs, and the most reliable theoretical method and density functional computations (DFT) were performed [36, 37]. Carbon, nitrogen, oxygen, and hydrogen atoms were primarily calculated with the help of B3LYP and exchange correlation functional with 6–31 G (d, p) basic sets.

Table 2 Screening of endophytic fungi for antibacterial activity by the agar well diffusion method

Endophytic fungal strain	Zone of inhibition (mm)			
	K. pneumoniae	*B. subtilis*	*E. coli*	*S. aureus*
Aspergillus flavus	00.00 ± 0.00	07.35 ± 0.90	00.00 ± 0.00	00.00 ± 0.00
Penicillium chrysogenum	10.40 ± 0.75	12.45 ± 0.96	00.00 ± 0.00	00.00 ± 0.00
Aspergillus niger	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
Monilia sp.	08.40 ± 0.76	10.45 ± 0.84	07.30 ± 0.62	00.00 ± 0.00
Alterneria alternata	00.00 ± 0.00	12.45 ± 0.65	00.00 ± 0.00	00.00 ± 0.00
Nigrospora sp.	10.75 ± 0.70	15.60 ± 0.65	13.25 ± 1.20	00.00 ± 0.00
Fusarium oxysporum	14.00 ± 0.60	16.35 ± 0.96	18.00 ± 0.68	16.50 ± 0.70
Phoma sp. D1	18.25 ± 0.58	22.75 ± 0.85	19.30 ± 0.76	16.30 ± 0.65
Fusarium solani	00.00 ± 0.00	16.45 ± 0.45	09.80 ± 0.74	10.40 ± 0.82
Colletotrichum sp.	00.00 ± 0.00	00.00 ± 0.00	11.45 ± 0.80	00.00 ± 0.00
Fusarium moniliformi	11.75 ± 0.60	11.45 ± 0.70	12.30 ± 0.85	00.00 ± 0.00
Aspergillus fumigatus	00.00 ± 0.00	12.80 ± 0.70	00.00 ± 0.00	00.00 ± 0.00

K. pneumoniae (*Klebsiella pneumoniae*), *B. subtilis* (*Bacillus subtilis*), *E. coli* (*Escherichia coli*), *S. aureus* (*Staphylococcus aureus*)

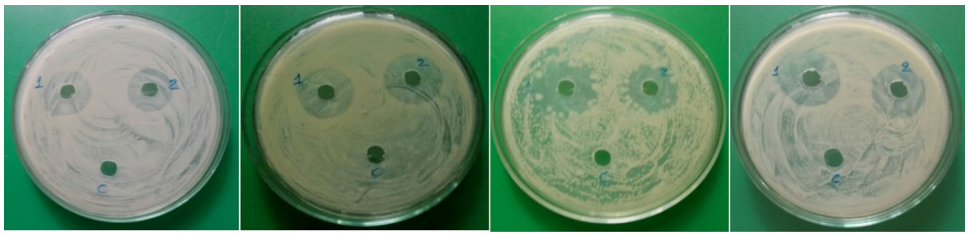


Fig. 2 Antibacterial activity by the agar well diffusion method. 1, Antibacterial activity of *Phoma* sp. D1; 2, antibacterial activity of *Fusarium oxysporum*; C, control

Results

Isolation and Identification of Fungi

In the present study, a total 12 endophytic fungi were isolated from different parts of four medicinal plants collected from different sites of Jabalpur (M.P.), India. All isolated fungal strains were identified by using the slide culture technique on the basis of their morphological (Fig. 1) and microscopic characteristics. The endophytic fungi belong to different classes in the fungal family as depicted in Table 1.

Screening of Endophytic Fungi for Antibacterial Activity

In the antibacterial screening of endophytic fungi, all fungal strains were grown on PDB medium for 7, 14, and 21 days and incubated in a fungal incubator at 25 ± 1 °C. Out of 12 endophytic fungi, only two displayed maximum zones of inhibition against the test bacterial strain after 14 days of incubation period (Table 2).

The fungal strain *Phoma* sp. D1 isolated from *Bryophyllum pinnatum* (Lam.) Oken, was observed for the highest zone of inhibition against *Klebsiella pneumoniae* (18.25 ± 0.58 mm), *Bacillus subtilis* (22.75 ± 0.85 mm), *Escherichia coli* (19.30 ± 0.76 mm), and *Staphylococcus aureus* (16.30 ± 0.65 mm). Similarly, *Fusarium oxysporum* showed antibacterial activity against *Klebsiella pneumoniae* (14.00 ± 0.60 mm), *Bacillus subtilis* (16.35 ± 0.96 mm), *Escherichia coli* (18.00 ± 0.68 mm), and *Staphylococcus aureus* (16.50 ± 0.70 mm) respectively. The other endophytic fungi like *Nigrospora* sp., *Fusarium solani*, *Aspergillus fumigatus*, *Monilia* sp., and *Fusarium moniliformi* were also observed for their antibacterial activity against the pathogenic bacteria (Fig. 2).

Determination of the Optimum Medium

For the evaluation of the suitable medium for growth and production of antibacterial secondary compounds, five different media, potato dextrose (PDB) medium, Sabouraud dextrose (SDB) medium, maltose yeast extract broth (MYEB), Czapek Dox broth (CDB), and Richard broth (RB) medium (Fig. 3), were used for potent *Phoma* sp. D1 fungi. The SDB medium was observed for maximum production of antibacterial activity after 14 days of incubation against *Klebsiella pneumoniae* (19.40 ± 0.60 mm), *Bacillus subtilis* (23.80 ± 0.90 mm), *Escherichia coli* (21.40 ± 0.75 mm), and *Staphylococcus aureus* (18.90 ± 0.80 mm).

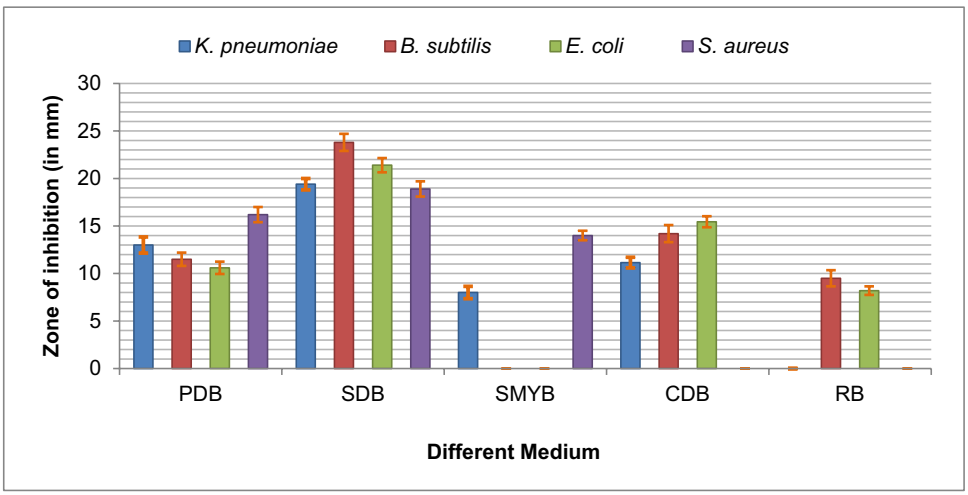


Fig. 3 Effect of various growth media on bioactive metabolite production of *Phoma* sp. D1

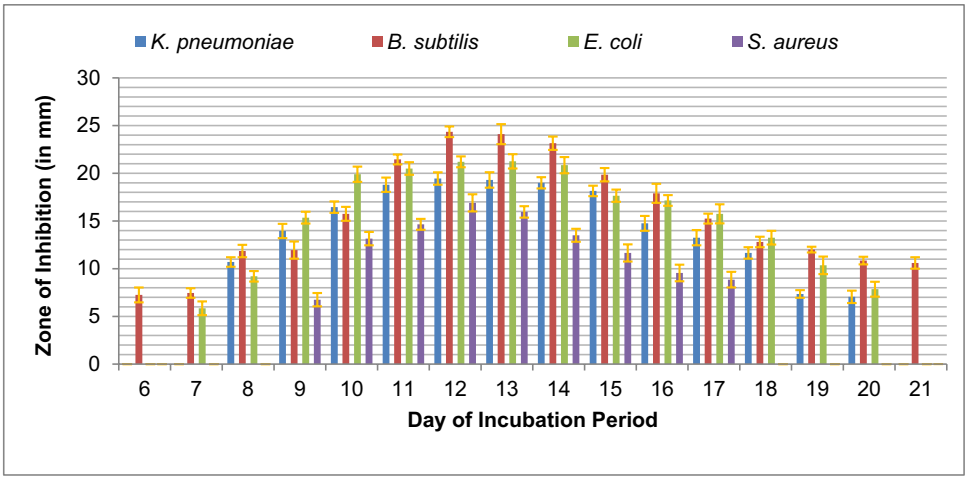


Fig. 4 Effect of various incubation periods on bioactive metabolite production of *Phoma* sp. D1

Determination of the Optimum Incubation Period

To find out the appropriate incubation period, *Phoma* sp. D1 was inoculated on SDB medium for 21 days and it showed minor antibacterial activity from the 6th day of its incubation and maximum antibacterial activity was observed on the 12th day of its incubation period against *Klebsiella pneumoniae* (19.45 ± 0.65 mm), *Bacillus subtilis* (24.35 ± 0.56 mm), *Escherichia coli* (21.20 ± 0.57 mm), and *Staphylococcus aureus* (16.90 ± 0.90 mm). After the 12th day of incubation, the activity declined continuously and stopped in the 20th day of incubation (Fig. 4).

Determination of Optimum Carbon Source

To observe the effect of carbon sources on the production of antibacterial compounds of *Phoma* sp. D1, the SDB medium was replaced with different carbon sources such as glucose, galactose, dextrose, maltose, and sucrose and the maximum activity was observed in the case of dextrose-supplemented medium against test bacterial strains *K. pneumoniae* (21.05 ± 0.68 mm), *Bacillus subtilis* (24.88 ± 0.70 mm), *Escherichia coli* (22.25 ± 0.94 mm), and *Staphylococcus aureus* (17.75 ± 0.55) as shown in Fig. 5.

Determination of Optimum Nitrogen Source

Similarly, to examine the effect of nitrogen sources on the production of antibacterial compounds from *Phoma* sp. D1, different nitrogen sources such as peptone, yeast extract, ammonium chloride, ammonium nitrate, and potassium nitrate were added in the medium and maximum antibacterial activity was observed in the case of yeast extract against bacterial strains *K. pneumoniae* (21.20 ± 0.75 mm), *Bacillus subtilis* (24.50 ± 0.77 mm), *Escherichia coli* (22.70 ± 0.95 mm), and *Staphylococcus aureus* (16.85 ± 0.80 mm) as displayed in Fig. 6.

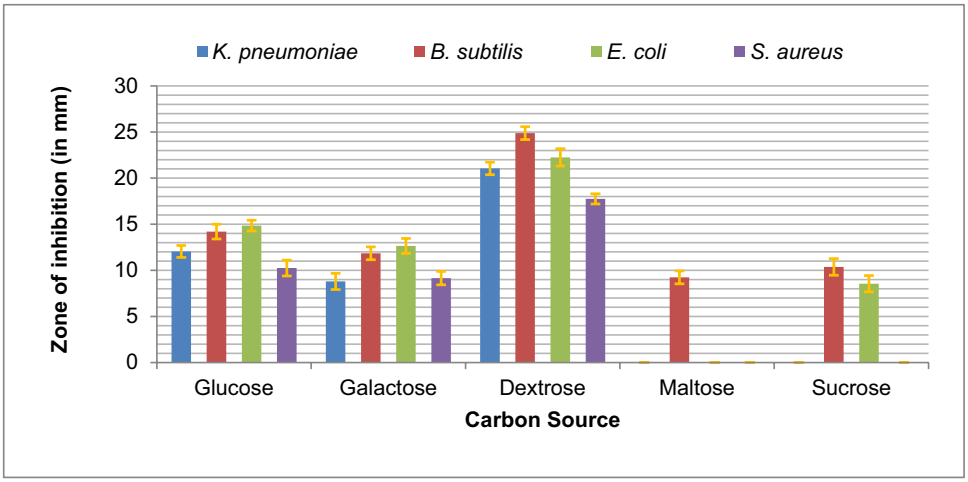


Fig. 5 Effect of various carbon sources on bioactive metabolite production of *Phoma* sp. D1

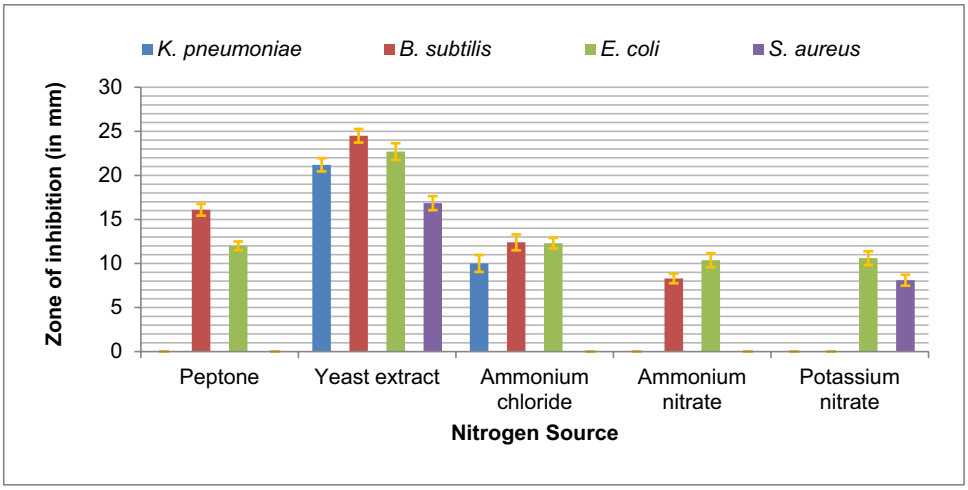


Fig. 6 Effect of various nitrogen sources on bioactive metabolite production of *Phoma* sp. D1

Determination of Optimum Incubation Temperature

The fungal strain *Phoma* sp. D1 was incubated at different temperatures for maximum growth and antibacterial metabolite production followed by 15, 20, 25, 30, 35, and 40 °C. The maximum activity was observed at 25 ± 1 °C against *K. pneumoniae* (20.95 ± 0.90 mm), *Bacillus subtilis* (24.10 ± 0.71 mm), *Escherichia coli*, (23.03 ± 0.60 mm), and *Staphylococcus aureus* (17.06 ± 0.83 mm) as depicted in Fig. 7.

Determination of Optimum pH

The pH of the culture medium is one of the very important determining parameters for the biosynthesis of secondary metabolites, so *Phoma* sp. D1 was grown on pH levels 4, 5, 6, 7, 8, and 9. The maximum antibacterial activity was observed at pH 8 against *Bacillus subtilis* (24.50 ± 0.94 mm), *Escherichia coli* (22.60 ± 0.82 mm), *K. pneumoniae* (21.96 ± 0.64 mm), and *Staphylococcus aureus* (17.44 ± 0.56 mm) as displayed in Fig. 8.

Purification of Bioactive Compound

Solvent–Solvent Extraction

In the present study, the CFCF of potent endophytic fungi *Phoma* sp. D1 was extracted with different polarity solvents like cyclohexane, carbon tetrachloride, ethyl acetate, toluene, benzene, and dichloromethane (Fig. 9) in 1:1 (v/v) for the purification of antibacterial bioactive compounds. The entire fractions extracted with the solvents were tested for their antibacterial activity by the agar well diffusion method, and it was observed that ethyl acetate extract exhibited the maximum zone of inhibition against the bacteria *Bacillus subtilis* (25.10 ± 0.64 mm), *Escherichia coli* (22.30 ± 0.70 mm), *K. pneumoniae* (21.40 ± 0.77 mm), and *Staphylococcus aureus* (16.45 ± 0.76 mm).

Thin-Layer Chromatography (TLC)

In thin-layer chromatography, ethyl acetate extract was further purified by using the benzene:methanol solvent

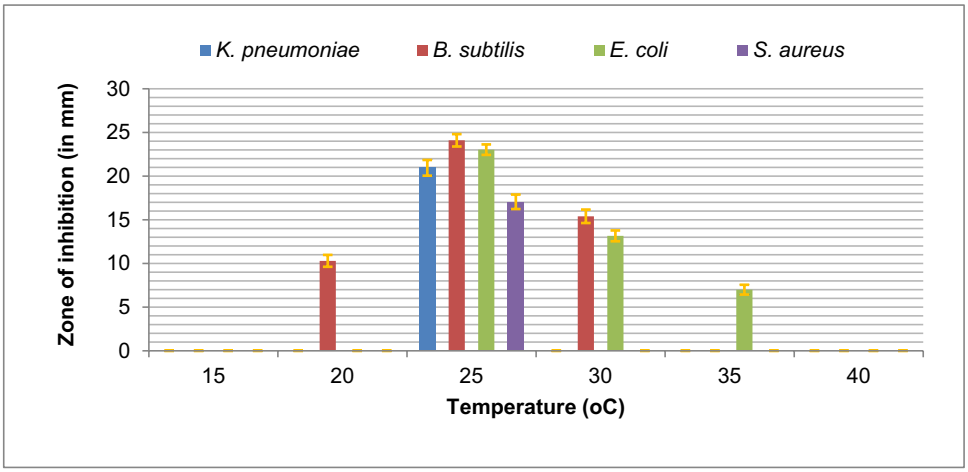


Fig. 7 Effect of various temperatures on bioactive metabolite production of *Phoma* sp. D1

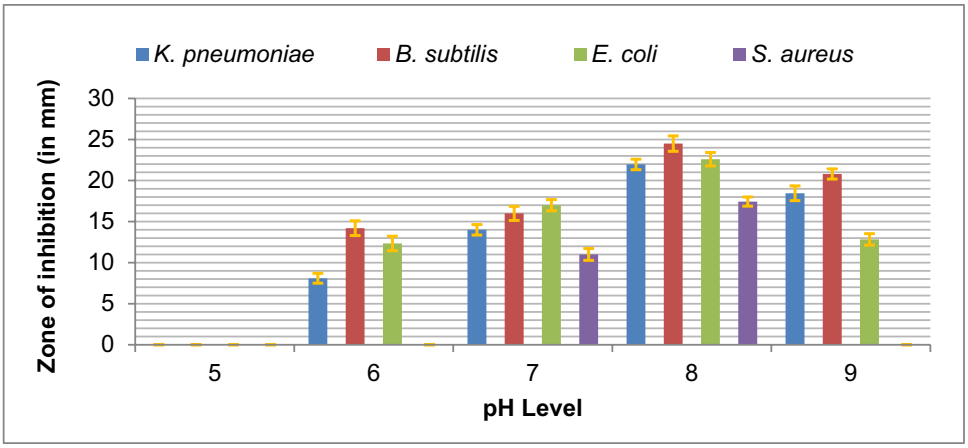


Fig. 8 Effect of various pH levels on bioactive metabolite production of *Phoma* sp. D1

system. In TLC, two fractions with different R*f* values of 0.25 and 0.96 were observed (Fig. 10). The fractions were tested for antibacterial activity, and the fraction with an R*f* value of 0.25 exhibited antibacterial activity against *K. pneumoniae* (20.20 ± 0.60 mm), *Bacillus subtilis* (22.12 ± 0.82 mm), *Escherichia coli* (21.70 ± 0.90 mm), and *Staphylococcus aureus* (14.85 ± 0.76 mm) (Fig. 11).

Structure Elucidation of Bioactive Compounds by GC–MS/MS

The TLC fraction that exhibited antibacterial activity was subjected to GC–MS/MS for structure analysis. The sample was run counts vs. acquisition time (in min), and a number of peaks were observed with different molecular weights. The chromatogram shows the presence of one major peak in a TLC extracted spot, and some other small peaks were also found as shown in Fig. 12. A total of five compounds, 2-(-cyclohexenyl) ethylamine, 2-chloro-6 fluorophenol, butyl ether2-hydroxyskatole4-glycerol, p-coumarate, and bicyclo [1, 2, 4] nona-2,4-dien-9-one, were identified on different RT and with different % area. But the intense peak of the ESI/MS spectra was observed at RT 11.085 with transition at 147 and was identified as 2-hydroxy skatole (C_9H_9N) as displayed in Table 3.

Investigative Computation (DFT Studies)

The essential method for interpreting the atomic arrangements of various prepared compounds for DFT research was conducted on a GAUSSIAN platform. To theoretically establish the geometry of all compounds (Fig. 13), the B3LYP/ 6–31 + G (d, p) basic set was used to optimize the geometry of each compound. By applying the DFT/ B3LYP 6–31 + G (d,p) fundamental sets, the single point energy and dipole moment (*D*) value of all compounds are evaluated (Table 4). It is evident from the energy values that compound 3 has a greater single point energy than the other compounds [38, 39]. Compound 5 assumes that less energy equals greater stability, based on the total energy of all the compounds. The dipole moment of compound 3 is greater than that of the other compounds. From these

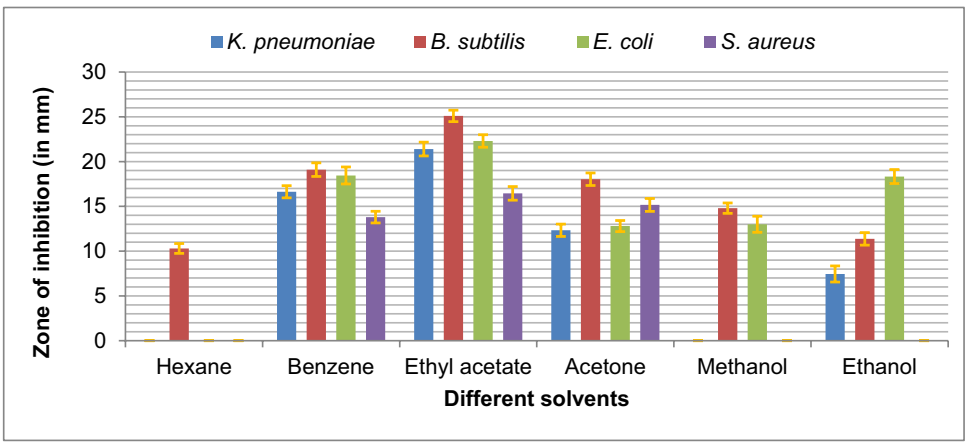


Fig. 9 Antibacterial activity of different solvent-extracted fractions by *Phoma* sp. D1

comparison values, it is evident that all compounds possess dipole–dipole interaction.

Frontier Molecular Orbital (FMO) Studies

The FMO studies make it easy to comprehend the reaction of the compound and predict the active site in a conjugated system. The energies of the highest occupied molecular orbitals (EHOMO) and the energies of the lowest unoccupied molecular orbitals (ELUMO) of all compounds explain the global reactivity descriptors such as chemical hardness, chemical potential, and electrophilicity. Negative EHOMO and ELUMO values for all compounds confirm their stability (Fig. 14). The electron cloud of compound 1 is localized on N26 for the active atomic sites in the position of neucleophilic attack. From the difference in bond energy, the chemical reactivity and chemical stability of the active molecule are explicable. The difference energy [EHOMO – ELUMO] for compound 5 is established to be less than the energy gap of the other compounds based on the energy difference values, which are the strongest evidence for the greater reactivity of compound 5. In contrast, compound 2 is more stable.

The FMO's research explains the chemical reactivity of the compound and the molecular selection of its active sites. From the FMO band energy values and the energy difference between HOMO and LUMO, the charge transfer (CT) interaction is described. Few significant chemical reactivity parameters including electronegativity (χ), chemical potential (μ), global hardness (η), global softness (S), and global electrophilicity index (ω) are listed in Table 5 [40].

$$\chi = -\frac{(E_{\mathrm{LUMO}} + E_{\mathrm{HOMO}})}{2}$$

$$\mu = -\chi = \frac{(E_{\mathrm{LUMO}} + E_{\mathrm{HOMO}})}{2}$$

$$\eta = \frac{(E_{\mathrm{LUMO}} - E_{\mathrm{HOMO}})}{2}$$

$$S = \frac{1}{2\eta}$$

$$\omega = \frac{\mu^2}{2\eta}$$

$$\sigma = \frac{1}{\eta}$$

The EHOMO and ELUMO plots (Fig. 6) of the compounds determine the exact orbital energy gap within the mapped molecular orbitals. Few essential quantum chemical parameters, such as chemical softness (S), are lower for compound 2 than for the other compounds; consequently, the stability of compound 2 is greater than that of the other compounds. Some additional crucial parameters, such as the electrophilicity (ω) value, are assigned as positive quantities that evaluate the tendency of the system to accept electrons from its environment. Comparing all compounds, compound 2 is the most stable because its electrophilicity value is less than those of the other compounds (Table 5).

QSAR Studies

QSAR is useful for predicting the activity, reactivity, and properties of reported compounds. All computations were performed using the HyperChem Professional 8.0.3 program. The compounds' structures were optimized using the (MM+) force field, with semi-empirical PM3 methods, and the Fletcher–Reeves conjugate gradient algorithm to

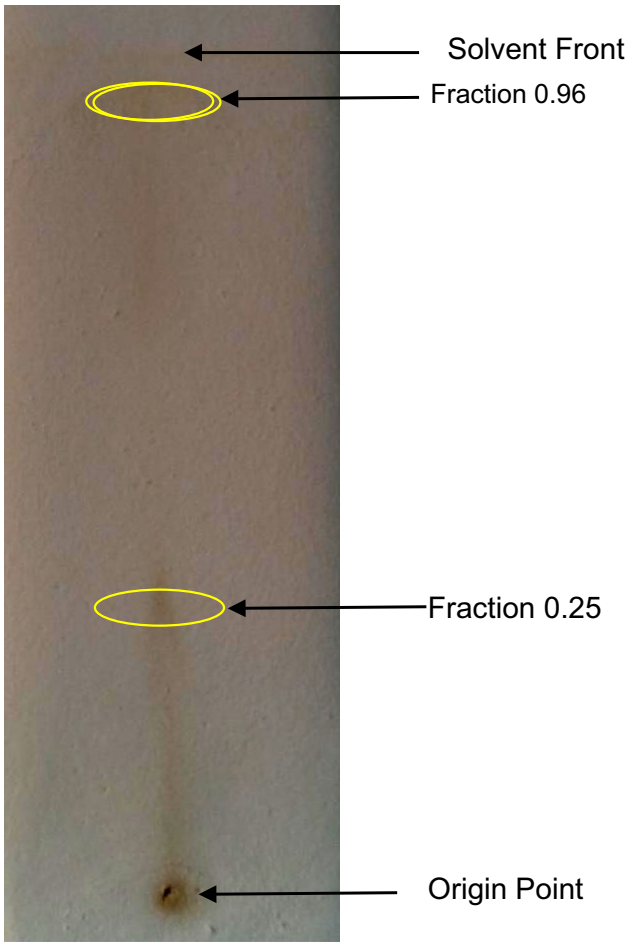


Fig. 10 TLC chromatogram of purified bioactive metabolite of *Phoma* sp. D1

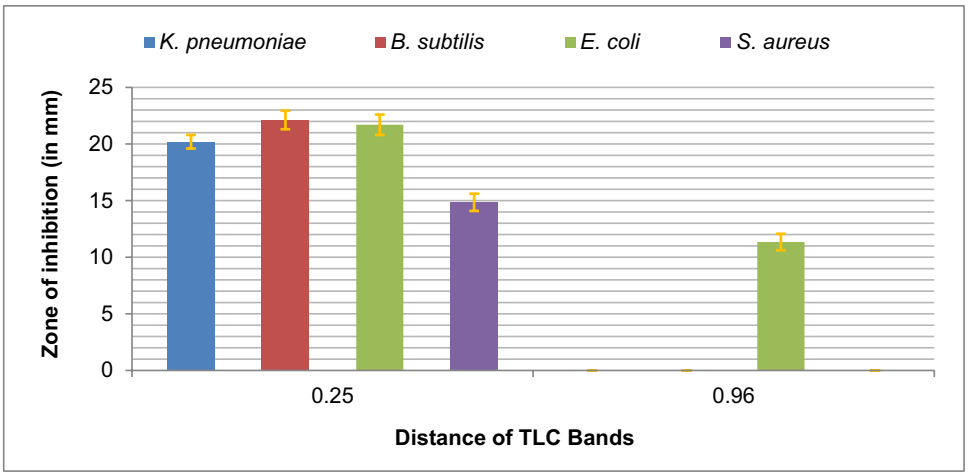


Fig. 11 Antibacterial activity of thin-layer chromatography (TLC) purified fractions

minimize energy. The evaluated log *P* values of compound 5 are higher than those of all other compounds. The partition coefficient (log *P*) values play a crucial role in explaining the biological activity of synthesized compounds. Specifically, the log *P* is essential for measuring the permeability of the employed compound into the cell membrane. Other essential physical parameters, such as surface area, volume, hydration energy, refractivity, polarizability, mass, total energy, free energy, and RMS gradient, are also calculated to suggest the action of the compounds listed in Table 6.

Discussion

The exploration of endophytic fungi is continuously being conducted in order to isolate and characterize novel bioactive compounds against a number of ailments. Because of this, huge studies were conducted on the endophytic fungi especially associated with medicinal plants and observed that these myco-organisms present a great source of bioactive compounds. In one of the studies, the endophytic fungi *Aspergillus* sp., *Alternaria alternata*, *Cladosporium* sp., *Diaporthe* sp., *Curvularia* sp., *Fusarium* sp., *Macrophomina*

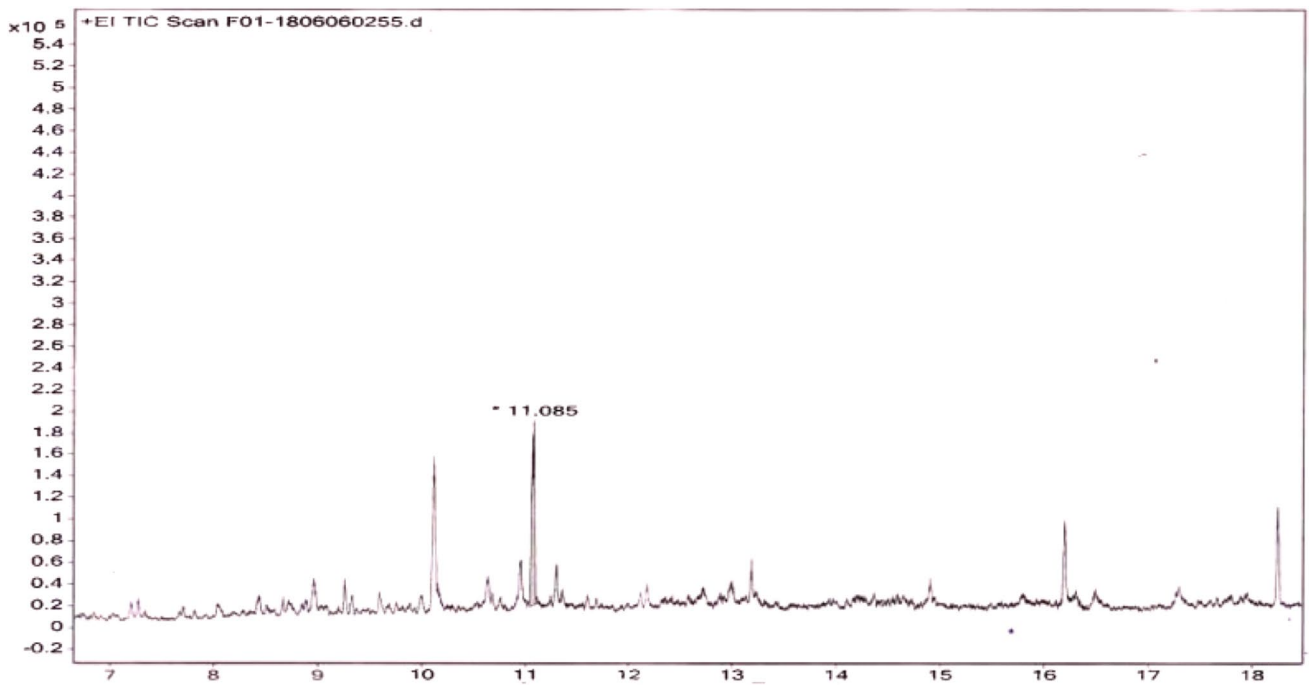
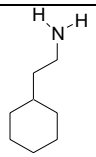
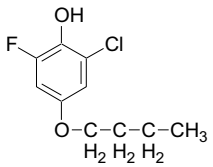
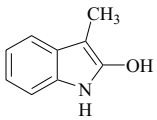
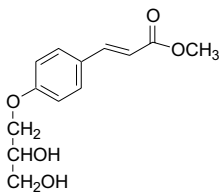
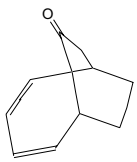


Fig. 12 Chromatogram of the TLC-purified fraction obtained from GC–MS/MS

spp., and *Trichoderma* spp. were isolated from various parts of *Cupressus torulosa* D. Don., *Alstonia boonei-ahun*, *Enantia chlorantha-Awopa*, and *Kigelia africana* plants [41]. The findings of this study are in agreement with a previous report in which endophytic fungi were isolated from *Sceletium tortuosum* L., *Moringa oleifera*, and *Withania somnifera* and their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* was observed [42, 43]. In many of the studies, it was found that the in vitro optimization of endophytic fungi on different media, temperatures, carbon and nitrogen sources, and pH levels supports the maximum production of novel bioactive compounds [44, 45]. Therefore, Deka et al. [46] observed the effect of optimization of the culture medium for maximum production of bioactive compounds on Czapeck–Dox broth (CDB), potato–dextrose broth (PDB), malt extract broth (MEB), Sabouraud dextrose broth (SDB), and V8 juice broth, and the highest fungal growth and bacterial zone of inhibition was found in potato–dextrose broth. Similarly, maximum growth and antibacterial metabolite production from *Nigrospora* sp. ML#3 were observed in Sabouraud dextrose broth [30]. In another research work, endophytic fungi *Aspergillus* sp. CPR5 and *Cymbidium aloifolium* were also optimized on various carbon and nitrogen for maximum production of secondary metabolites and the fungal strain [31, 47]. For biosynthesis of natural bioactive compound naphthoquinones, the endophytic fungi *Fusarium solani* was tested on different carbon sources, glucose, fructose, xylose, sucrose, sodium acetate, and glycerol. In the case of Altenuene synthesis from *Alternaria alternata* isolated from *Catharanthus roseus*, the fungal media were replaced with a number of nitrogen sources such as soybean meal, yeast extract, tryptone, beef extract, peptone, casein, and urea, and the best production was observed in the case of soybean meal and glycerol [48]. In the case of temperature, the fungal strain *Aspergillus fumigatus* strain KARVS04 was optimized in a range of 25–30 °C and a better result was obtained at 29 °C [49]. In another research work, the endophytic fungus *Fusarium solani* showed maximum growth and antibacterial activity at an optimized temperature of 25 °C [50]. Endophytic fungus *Athelia rolfsi* strain displays maximum growth of biomass at pH 5 and maximum production of antibacterial bioactive compounds from potent endophytic fungal strain *Fusarium* sp. DF2 was obtained at pH 6 that showed maximum zone of inhibition against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* [51].

For purification and characterization of bioactive compounds from the endophytic fungi, there are many

Table 3 Chemical composition of the TLC-purified fraction of *Phoma* sp. D1 (GC–MS/MS analysis)

S. No.	Compound	RT	Response	Transition	Structure
1.	2-(-Cyclohexenyl) ethylamine	10.055	3526	125	
2.	2-Chloro-6 fluorophenol, butyl ether	11.055	1055	148	
3.	2-Hydroxyskatole	11.085	117689	147	
4.	4-Glycerol p-coumarate	16.197	7771	148	
5.	Bicyclo [4,2,1] nona-2,4-dien-9-one	18.261	16380	79	

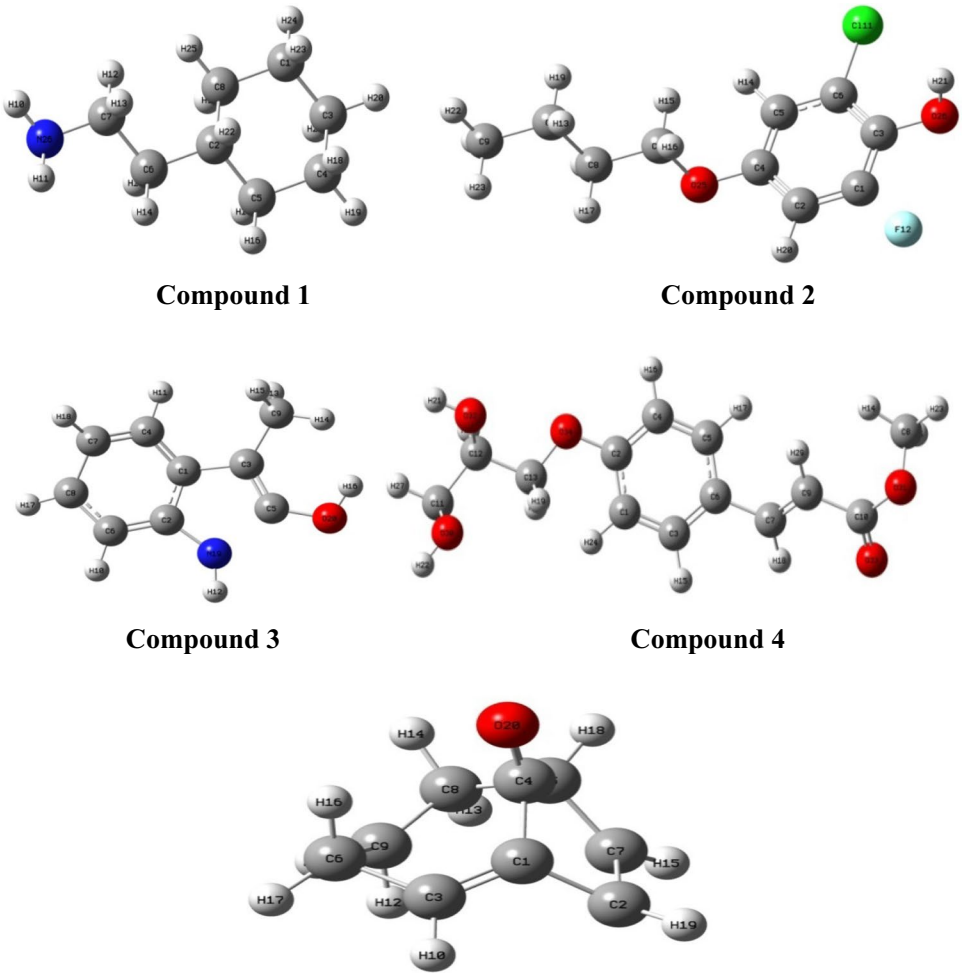


Fig. 13 Optimized geometry of compound 1, compound 2, compound 3, compound 4, and compound 5

techniques being carried out like solvent–solvent extraction, thin-layer chromatography column chromatography, gas chromatography, etc. In the case of endophytic fungus *Thielaviopsis basicola*, the bioactive compounds were extracted through an aqueous solvent system and further purification was carried out in thin-layer chromatography by using chloroform and methanol solvent system [52]. In a previous finding, the antibacterial compounds extracted from different types of 40 endophytic fungi isolated from 10 medicinal plants were separated by using thin-layer chromatography and the activity bacterial strain [53]. In the case of endophytic fungi *Colletotrichum* sp. and *Fusarium* sp., the antibacterial compounds were also separated by thin-layer chromatography by using a mixture of hexane, ethyl acetate/hexane, ethyl acetate, and acetate/methanol [54].

The bioactive compounds isolated from the endophytic fungus *Arthrinium* sp. MFLUCC16-1053 were characterized by using GC–MS, and the mass spectra of the molecules were compared with library data and verified through relative peak area percentage that were related to C8–C23 n-alkanes [55]. In another study, the bioactive metabolites extracted from entophytic fungi *Phoma* sp. isolated from *Tectona grandis* L. were analyzed by GC–MS/MS and a total of eleven compounds were characterized [56]. Further, the atomic arrangements of antibacterial compounds were studied through computational analysis such as DFT and FMO which

Table 4 Energetic properties of the evaluated compounds

Compound (no.)	Single point energy (kcal/mol) DFT/B3LYP 6.31G + (d, P)	Dipole moment (*D*) DFT/B3LYP 6.31G + (d, P)
$C_8H_{17}N$ (1)	-3.3209×10^5	0.227
$C_{10}H_{12}ClFO_2$ (2)	-6.8947×10^5	0.466
C_9H_9NO (3)	-3.0007×10^5	0.394
$C_{13}H_{16}O_5$ (4)	-5.5287×10^5	0.628
$C_9H_{10}O$ (5)	-2.6595×10^5	−0.610

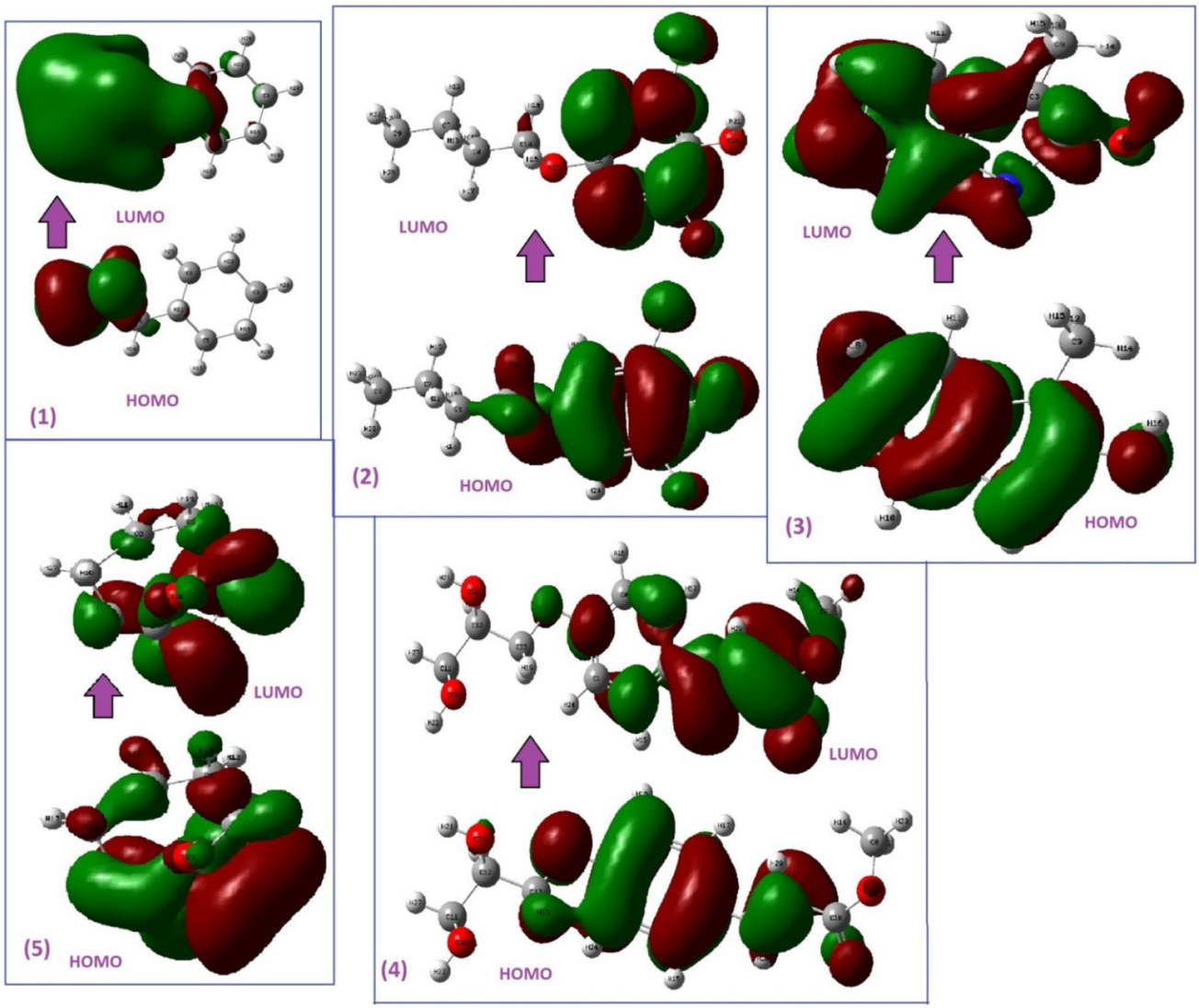


Fig. 14 HOMO–LUMO energy comparison of **(1)** compound 1, **(2)** compound 2, **(3)** compound 3, **(4)** compound 4, and **(5)** compound 5

helps to find out and predict the active site in a conjugated system. Due to the advancement in the sciences and technology, various software programs like QSAR are widely used for predicting the activity, reactivity, and properties of reported compounds isolated from the endophytic fungi [40, 57]. Hence, the endophytic fungi widely exploited from plants act as a replenished source of novel bioactive compounds and have a wide range of activities. The results described in this study definitely help to scale up for bioactive molecule production and formulation of pharmaceutical products in future from the endophytic microbes.

Table 5 Computed quantum chemical parameters of few compounds

Compound (no.)	HOMO in eV	LUMO in eV	ΔE in eV	χ Pauling	η in eV	σ	μ in eV	S	ω in eV
$C_8H_{17}N$ (1)	−0.2202	−0.0827	0.1375	−0.1514	0.0687	14.55	0.1514	7.2780	0.1666
$C_{10}H_{12}ClFO_2$ (2)	−0.2448	−0.0274	0.2174	−0.1361	0.1087	9.19	0.1361	4.5998	0.0851
C_9H_9NO (3)	−0.1853	−0.0196	0.1657	−0.1024	0.0828	12.07	0.1024	6.0386	0.0632
$C_{13}H_{16}O_5$ (4)	−0.2243	−0.0539	0.1704	−0.1391	0.0852	11.73	0.1391	5.8685	0.1134
$C_9H_{10}O$ (5)	−0.1762	−0.0946	0.0816	−0.1354	0.0408	24.50	0.1354	12.2549	0.2246

Table 6 QSAR evaluation for optimized compounds

Function	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5
Surface area (approx) (Å^2)	296.33	381.14	268.07	505.05	218.22
Surface area (grid) (Å^2)	335.44	420.02	328.71	512.30	302.22
Volume (Å^3)	511.15	651.51	499.32	805.28	459.40
Hydration energy (kcal/mol)	−2.60	−6.57	−10.61	−16.19	−1.28
Log *P*	1.61	2.71	2.10	2.96	3.78
Refractivity (Å^3)	40.34	26.05	14.66	34.19	22.15
Polarizability (Å^3)	16.03	18.57	16.96	25.72	15.44
Mass (amu)	127.23	195.34	147.18	252.27	134.18
Total energy (kcal/mol)	−31,681.3	−4.74616	−17.0852	−8.30409	26.4735
Dipole moment (Debye)	1.296	1.336	0.8822	2.323	0
Free energy (kcal/mol)	−31,681.3	−4.74616	−17.0852	−8.30409	26.4735
RMS gradient (kcal/Å mol)	0.09873	0.0952	0.09812	0.09708	0.09855

Conclusions

The endophytic fungi are unique microorganisms for producing antibacterial secondary metabolic compounds and also have gained the attention of the scientific community because of their potential applications in the fields of agriculture and pharmaceutical industries. The result of this study substantiates the presence of antibacterial compound from the endophytic *Phoma* sp. D1 fungal strain isolated from the *Bryophyllum pinnatum.* This is easily cultured and optimized in in vitro conditions for maximum production of the antibacterial bioactive compound. From the present observation, it can be concluded that *Phoma* sp. D1 has potential to produce an antibacterial bioactive compound which may be used in the field of pharmacology and also as a prospective source of valuable drugs. However, further work will need to be undertaken to determine its toxicity profile, reliability, and economical value.

Author Contribution We declare that this work was done by the authors named in this article and the authors will bear all liabilities about claims relating to the content of this article.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Conflict of Interest No conflict of interest associated with this work.

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