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Antimicrobial activity of culturable endobionic fungi residing lichens and lower plants

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ABSTRACT

We urgently need new antimicrobials to treat systemic fungal infections, food and plant pathogens, and bacterial drug resistance. Many bacterial and fungal diseases remain uncontrolled despite research on antibacterial and antifungal agents. Many regions have long used plant and microbial metabolites as therapeutic drugs. Plants contribute 50–60% of these compounds, including alkaloids, flavonoids, steroids, terpenoids, and polysaccharides, while microbial metabolites contribute 5%. The vast unexplored diversity of microorganisms may yield novel and bioactive metabolites. Thus, research is increasingly focused on rare and understudied microorganisms from diverse ecological habitats. Endolichenic fungi live in lichen thalli with algae. They are similar to plant endophytes, which live inside the cells of their host plants. Several antibiotics and natural bioactive compounds with multiple uses have developed them. According to the WHO, a large percentage of developing countries' populations rely on local medicinal products for primary health care, driving up demand for medicinal plants worldwide. Plants' ability to synthesize antimicrobial potency compounds (secondary metabolites) helps fight antibiotic resistance. We reviewed these organisms' potential as sources of novel antimicrobial compounds. These fungi may produce bioactive substances that fight harmful microorganisms. Understanding their antimicrobial properties could lead to new antibiotics that address antibiotic resistance. This research is essential for finding alternative treatments and understanding natural antimicrobials.

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Introduction

Over two decades after Sir Alexander Fleming's discovery of penicillin, the widespread use of antibiotics for agricultural and medical purposes leads to the unfavorable outcome known as multidrug resistance (MDR). Antibiotics were initially regarded as "magic bullets," assumed to be universally safe and effective, with little consideration given to their distinct properties or the potential for developing resistance due to insufficient understanding. (Lax minarayan et al. 2013). These factors have resulted in multiple global epidemics caused by drug-resistant pathogens, including multidrug and extensively drug-resistant tuberculosis (M/XDR-TB), Drug-resistant pathogens such as *Vibrio cholerae*, vancomycin-resistant enterococci (VRE), and methicillin-resistant *Staphylococcus aureus* (MRSA) were historically restricted to hospitals and other healthcare environments (Monowar et al., 2018).Remarkably, MRSA, VRE, and MDR-TB have resistance rates of 97%, 59%, and 52%, respectively (CDC, 2019). Additionally, in some countries, resistance to ciprofloxacin, a commonly used treatment for urinary tract infections, has escalated to as high as 92.9%.

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These microorganisms have developed strategies to become resistant to and reduce the effectiveness of conventional antibiotics. These strategies include compromising the efflux mechanisms that allow the drugs to exit the cell, enzymatic modifications that alter the antibiotics, mutating drug targets, decreasing the permeability of cell membranes, modifying lipopolysaccharides, and biofilm formation (Blair et al. 2015, Redgrave et al. 2014, Olaitan et al. 2014, Li & Webster 2018). Due to the current lack of efficient therapies approaches for multidrug-resistant (MDR) infections, the worldwide disease burden continues to rise, resulting in greater death rates, longer illness durations, and dramatically higher treatment costs. Additionally, MDR has increased the virulence of resistant strains, and this heightened virulence can be readily transmitted to other strains via horizontal gene transfer. (Davies & Davies 2010). The necessity of finding innovative therapeutic compounds that may serve as "game changers" in the battle against multidrug resistance has been highlighted by the rise in resistance and the possibility of treatment failure.

The fungal kingdom is becoming recognized as a major source of pharmacologically useful chemicals as scientists turn their attention to natural products as substitute sources of antimicrobial agents (Radic & Strukelj 2012, Abo Nahas et al. 2023). medication development and discovery from fungal sources has increased since the endophytic fungus *Taxomyces andreanae* was found to produce the anticancer medication Paclitaxel (Taxol). As a result, both endophytic and endolichenic fungi are now given the greatest attention. Endophytes have been shown to have great potential as antimicrobial agents in this regard, making them viable options for effective biocontrol of infections that are resistant to drugs.

Endophytes are endosymbiotic microorganisms that live inside the tissues of different plant parts, including the roots, leaves, stems, flowers, fruits, and seeds. They work in mutually beneficial relationships and symbiosis with their hosts. The properties they possess include being antimicrobial, immunomodulatory, insecticidal, antioxidant, and growth-promoting. They are thought to be an excellent source of several biologically active compounds (Strobel 2018). Similarly, a parallel group of fungi, termed endolichenic fungi, inhabit lichen thalli in a similar asymptomatic manner. Lichen thalli are the result of a symbiotic relationship between a fungal organism (mycobiont) and at least one photosynthetic organism (photobiont), which contains chlorophyll. The photobiont can be a microalga, cyanobacterium, or a combination of both (Lutzoni & Miadlikowska 2009), also harbor numerous asymptomatic microfungi closely associated with the photobiont. These diverse fungi groups residing within the interior of lichen thalli are referred to as 'endolichenic fungi' (Arnold et al. 2009, Miadlikowska et al. 2004).

Endolichenic fungi exhibit similar behavior to endophytic Fungi that live inside plants. Lichens, which cover a minimum of 8% of the planet's surface, demonstrate a remarkable capacity to colonize a variety of surfaces, such as vascular plants, rocks, and man-made objects including concrete, glass, plastic, and metal. These lichens can host a phylogenetically and ecologically diverse array of fungi, providing a promising way for finding new natural products (Balasubramanian & Nirmala 2014).

Lichens are widely regarded as a rich source of natural antioxidants, particularly phenolic compounds, which are highly valued for their strong antioxidative and antiradical capabilities. Consequently, they are associated with numerous therapeutic benefits, including antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, and anticancer effects (Tomović et al. 2024).

Endophytes: The Undiscovered treasure

Plant physiology is significantly influenced by the plant microbiome, which enhances growth, nutrient uptake, and stress tolerance. According to Passari et al. (2015) endophytes are common microorganisms that live inside the tissues of different plant parts. They develop mutualistic interactions with their host plants without producing signs of illness (Abdel-Azeem et al. 2016). It is thought that these endophytes release secondary metabolites that increase the host plant's evolutionary fitness and survival by offering protection against pathogens. One way they achieve this is by producing powerful hydrolytic enzymes that prevent pathogen, nematode, or insect invasion by stimulating the defense mechanisms of the plant to prevent diseases (Martinez-Klimova et al. 2017; Hajji-Hedfi et al. 2025). Endophytes have also been shown to improve the therapeutic qualities of plants having ethnobotanical roots. Endophytes are attractive targets for medication research because their secondary metabolites might occasionally resemble those of the host plant (Mohamed & Abdel-Azeem 2024). In the Taxaceae family, for instance, the endophyte *Pestalotiopsis microspore* produces torreyanic acid, a potent anticancer compound originally found in its plant host (Ludwig-Müller 2015). Similarly, the endophytic fungus *Eupenicillium parvum*, found in *Azadirachta indica* (neem tree), It was previously thought that the neem tree was the only source of the insecticides azadirachtin A and B (Kusari et al. 2012). Another instance is the fungus strain QJ18, which generates the chemical gentiopicrin, which has antifungal, antihepatotoxic, chlorotic, and anti-inflammatory qualities. Additionally, demonstrating the

potential of endophytes in creating innovative therapeutic approaches, this strain was originally isolated from the medicinal plant *Gentiana macrophylla* pall , further supporting the potential of endophytes in developing novel therapeutic strategies (Kumar et al. 2014). Endophytic fungi have been extensively researched across different climates and geographical areas, and may be found in a broad range of plant tissues, displaying a rich diversity of species (Ghimire et al. 2011, Vieira et al. 2014). Numerous studies have shown that endophytes significantly impact plant evolution, fitness, and ecology, which affect the variety and structure of plant communities (Arnold & Lutzoni, 2007, Yuan et al. 2011).

Endophytes have even been identified in plant fossils dating back 400 million years (Krings et al. 2007). Consequently, Endophyte-host plant interactions, particularly their possible contribution to plant evolution, have drawn a lot of attention. Researching the variety and composition of endophytic fungus (EF) linked to plants at different phases of evolution is essential. In this context, "evolutionary stages" refers to several taxonomic levels found in phylogenetic categorization schemes, where classification is based on evolutionary relationships among organisms. Most botanists regard bryophytes and pteridophytes as the oldest surviving eukaryotic plants that colonized land (Renzaglia et al. 2000).

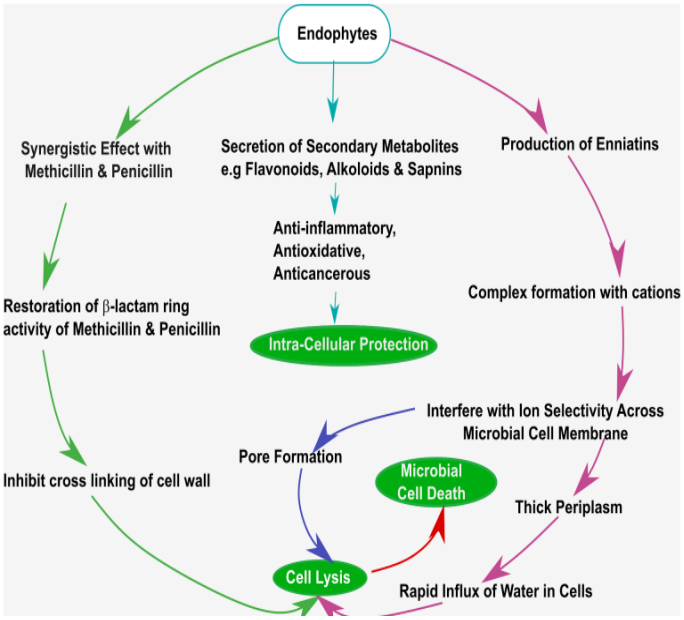

Fig. 1. A flow diagram illustrating how endophytes work to combat drug-resistant microorganisms (Pasrija et al.2022).

Endophytic fungi as sustainable source of secondary metabolites with antimicrobial properties

As new infectious diseases and multidrug-resistant bacteria increase, there is an increasing demand for new antimicrobial agents. Scientific efforts are focused on discovering antimicrobial metabolites from endophytes. A wide variety of metabolites from endophytes have demonstrated antimicrobial properties against various pathogenic microorganisms, making them valuable for use in medicine, pharmaceuticals, and agriculture (Gunatilaka et al. 2006). One of the largest groups of compounds made by endophytic fungus that have antibacterial properties are terpenes. Notable examples include the diterpenoids periconicin A, periconicin B, guanancastepene A, and guanacastepene, which are generated by an unnamed endophytic fungus from *Daphnopsis americana*. Additionally, Colletotric acid from *Artemisia annua's Colletotrichum sp*. has demonstrated antifungal and antibacterial activities (Vigneshwari 2020).

The medicinal plant *Erythrina crista* contains an endophytic fungus (*Phomopsis sp*.) from which phomol, a new antibacterial chemical, has also been extracted. Using spectroscopic techniques, this molecule was shown to be a polyketide lactone (Guo et al. 2000). Common secondary metabolites found in endophytes include alkaloids, some of which have antibacterial properties. While 3-O-methylalaternin and altersolanol, which are derived from the endophyte *Ampelomyces sp*. showed antibacterial activity against gram-positive pathogens including *Staphylococcus aureus* and *S. epidermidis*, derived from the medicinal plant *Urospermum picroides*, and *Enterococcus faecalis*, chaetoglobosins A and C were isolated from an endophytic *C. globosum* culture obtained from *Ginkgo biloba* leaves (Aly et al. 2008).

Cryptocandin A is a special peptide that has been studied to cure fungal infections in humans. It was isolated from the endophyte *Cryptosporiopsis quercina* of the medicinal plant *Tripterigeum wilfordii*, native to Eurasia (Strobel et al. 2003). Furthermore, antibacterial activity has been shown for both jesterone and hydroxyjesterone from *Pestalotiopsis sp*. (Li et al. 2001).

Mechanism of Action

Endophytic compounds have been shown to be highly effective against various bacterial and fungal pathogens responsible for human diseases. However, the exact mechanism behind this biocontrol activity remains unclear. Several in vitro studies on different pathogens have been conducted, and Scanning Electron Microscopy (SEM) images from these studies have provided some clues about how these compounds work. In a study by Yenn et al. (2017), extracts from the endophytic fungus *Penicillium purpurogenum* ED76, isolated from *Swietenia macrophylla* King, exhibited strong bioactivity against pathogens such as *Staphylococcus aureus*,

Acinetobacter anitratus, and *Candida albicans*. SEM images of *Staphylococcus aureus* treated with the sterol-containing extract showed significant changes in cell shape, including invaginated cell walls and leakage of internal components. Similarly, a study by Chatterjee et al. (2019) used extracts from *Alternaria alternata* AE1, isolated from the medicinal tree *Azadirachta indica* A.Juss. The SEM micrographs of the treated bacteria revealed severe damage to the cell structure, resulting in the release of DNA and proteins from the bacterial cells. These consistent findings suggest that bioactive metabolites extracted from endophytes disrupt both cell wall synthesis and membrane permeability, causing cell rupture and the leakage of intracellular contents (Figure 1). The damaged cells also lose their metabolic functions and become irreparable (Taufiq and Darah 2019). Additionally, studies have shown that endophytic metabolites have significant catalase-inhibiting effects, which reduce the pathogen's resistance to oxidative stress and increase its vulnerability to macrophage attacks (Mbekou et al. 2021).

Endolichenic fungi

The unique class of symbiotic biota known as lichens grow when a photosynthetic partner (photobiont) and a fungal partner (mycobiont) associate. While the photobiont is frequently a cyanobacterium or green alga, the mycobiont is usually a member of the Ascomycota phylum (Nash 2008). Recent research has proposed the inclusion of yeast in lichen thalli as a third symbiotic partner (Spribille et al. 2016). The discovery of endolichenic fungi, a group related to Pezizomycotina within the Ascomycota phylum, further confirmed the existence of a microbial consortium within lichen thalli. These fungi form intimate associations with the green algal photobiont (Arnold et al. 2009). This observation was made unexpectedly during the isolation of the lichen mycobiont into pure cultures, leading to the fungi's outgrowth from the sample of thallus (Kellogg & Raja 2017). Endolichenic fungi reside solely into the lichen's interior tissues, causing no visible harm to the thallus (Tripathi & Joshi 2015, Wang et al. 2016), Similar to plant endophytes, which inhabit host plant tissues without causing damage (U'ren et al. 2010, Kellogg & Raja 2017), these fungi are non-damaging. However, endolichenic fungal communities are distinct from endophytes found in vascular plants, except for those residing in mosses (U'ren et al. 2012). The identification of endolichenic fungi and their interactions through endolichenism is considered a significant milestone in understanding microbial interactions and the ecological roles of Ascomycetes (Arnold et al. 2009, U'ren et al. 2012). Arnold et al. (2009) suggested that endolichenism played a pivotal role in the evolution of endophytism within Ascomycota, proposing that endolichenic fungi are the ancestors of numerous endophytic fungi found in nature. This idea implies that lichens serve as "cradles" for fungal diversification, potentially containing a variety of missing fungi species yet to be discovered among the estimated 5.1 million fungal species on Earth (Blackwell 2011), particularly in tropical forests and other unexplored habitats (Hawksworth & Rossman 1997). A variety of endolichenic fungal species have been identified, including *Aspergillus*, *Phaeosphaeria*, *Ulocladium*, *Penicillium*, *Nodulisporium*, *Neurospora*, *Chaetothyriales*, and *Xylaria*. Researchers are looking at these fungi's potential bioactive properties, such as antimicrobial, antifungal, antioxidant, and cytotoxic effects (Aziz & Abdel-Azeem 2024). The interest in their bioactive compounds is due to their distinct chemical structures compared to the ones made by lichen (Kellogg & Raja 2017, Singh et al. 2017).

Lichens are at risk of overexploitation and collecting due to the growing demand for endolichenic fungus. Therefore, conservation is crucial to ensure sustainable exploration of their bioactive compounds, especially since lichens grow slowly (Shukla et al. 2014). Strategies for conservation and sustainable forest management are crucial in mitigating risks to lichen biodiversity (Shukla et al. 2014). Key actions consist of compiling detailed inventories of lichen species, establishing uniform sampling protocols, and reducing environmental disturbances. These measures are aimed at ensuring the protection of lichen species and the preservation of their habitats (Scheidegger & Worth 2009). The biological activities attributed to endolichenic fungi (ELF) are akin to those observed in endophytic fungi residing within plant tissues. ELF inhabit healthy lichen tissues, utilizing the photobiont for nutrition and shelter. In return, they contribute various active compounds that aid in protecting the host lichen from both biotic and abiotic stresses. Biochemical analyses have highlighted that ELF produce a diverse array of bioactive substances with significant ecological functions, including photoprotection, allelopathy, responses to pollution, and activities against viruses, herbivores, and insects. This potential makes endolichenic microorganisms a promising source for discovering novel natural products with potent pharmacological properties, emphasizing their importance as bioresources in biotechnology and pharmaceutical research (Figure 2). (Nguyen et al. 2013, He et al. 2012, Li et al. 2015, Kellogg & Raja 2017, Suryanarayanan & Thirunavukkarasu 2017).

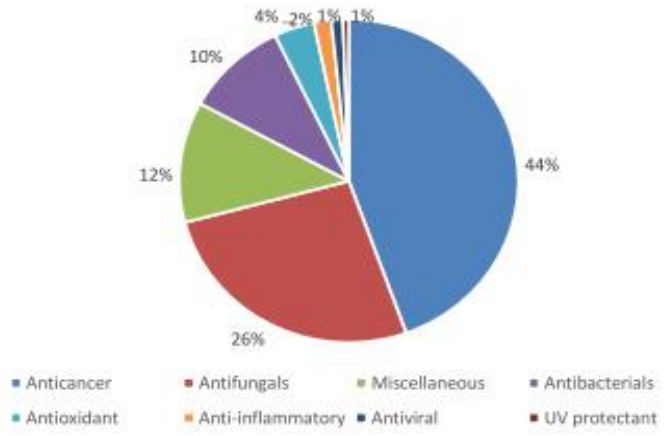

Fig 2. Percentage of endolichenic fungal bioactive metabolites reported for a variety of activities between 2008 and 2019 (Agrawal et al. 2020).

Antibacterial activities

The discovery of fungi as a source of antibiotics dates back to Sir Alexander Fleming's pioneering work in 1928, which led to the identification of penicillin, and later, Brotzu's discovery of cephalosporin C. These breakthroughs marked significant milestones in the field of antibacterial drug discovery. However, in recent decades, there has been a notable increase in antibiotic-resistant pathogens such as *Methicillin-resistant Staphylococcus aureus* (MRSA), *Penicillin-resistant Streptococcus pneumoniae* (PRSP), and *Vancomycin-resistant Enterococcus faecium* (VRE). This trend has rendered newer drugs like linezolid and daptomycin ineffective due to acquired resistance. Moreover, multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) have emerged as global threats, posing challenges in diagnosis and treatment efficacy (Agrawal et al. 2020). In response to these ongoing developments, there is a critical need to search for novel drug scaffolds. Endolichenic fungi (ELF) have emerged as a ubiquitous source of antibacterial compounds. For instance, compounds such as 8-methoxy-1-naphthyl-b-glucopyranoside and phomol were separated from *Hypoxylon fuscum* obtained from spruce bark in the Lilong Snow Mountain region of Lijiang, Yunnan, China, and that are associated with *Usnea sp*. (Agrawal et al. 2020).

These compounds exhibited varying degrees of antibacterial activity against *Staphylococcus aureus*, albeit weaker than the positive control vancomycin hydrochloride (Basnet et al. 2019). Specifically, phomol, demonstrated MICs of 51.2 mM, , likewise, in contrast to the MIC of 2.1 mM for vancomycin hydrochloride. These findings underscore the potential of ELF as a valuable source for discovering new antibacterial agents, which is crucial in addressing the escalating challenge of antibiotic resistance in clinical environments. *Aspergillus niger* was extracted from the lichen thallus of *Parmotrema ravum*, which is found in India, to produce a variety of compounds.

These compounds include Carbonarone A, fonsecinone A, asperpyrone A, aurasperones A, and pyrophen. Aurasperone A demonstrated antibacterial effects versus *Staphylococcus aureus* and *Pseudomonas aeruginosa*, having IC50 values of 135 and 160 mg/mL, respectively. With an IC50 of 112 mg/mL, asperpyrone A demonstrated specific activity against *Escherichia coli*. Fonsecinone A shown antibacterial effects against *E. coli* (IC50 47 mg/mL) and *S. aureus* (IC50 120 mg/mL), as well as *Pseudomonas syringae pv. maculicola* (IC50 154 mg/mL) efficacy against the plant pathogen. With an IC50 of 88 mg/mL, carbonarone A demonstrated efficacy against the plant disease *Dickeya solani*. A*eromonas hydrophila* (IC50 78 mg/mL), *Listeria innocua* (IC50 86 mg/mL), and *Micrococcus luteus* (IC50 63 mg/mL) were all susceptible to Pyrophen's antibacterial action (Padhi et al. 2019). *Talaromyces funiculosus*, an endolichenic fungus symbiotically linked with the lichen thallus of *Diorygma hieroglyphicum* found in India, yielded funiculosone and its analogues mangrovamide J and ravenelin. The antibacterial activity of these compounds was evaluated against *Escherichia coli* and *Staphylococcus aureus*, yielding IC50 values in the range of 23 to 104 mg/mL, indicating their potential as bioactive agents with broad-spectrum antibacterial properties (Padhi et al. 2019a).

Additionally, compounds griseoxanthone C, norlichexanthone, and 6-O-methylnorlichexanthone were isolated from *Ulocladium sp*., a fungus linked to the lichen *Everniastrum sp*. that was gathered in China's Yunnan region. These compounds have shown strong antibiotic properties. They displayed a remarkable IC50 of 15 µM against *Bacillus subtilis*. Furthermore, norlichexanthone, with an IC50 of 20.95 µM, was especially successful in preventing the growth of *Methicillin-resistant Staphylococcus aureus* (MRSA). According to Wang et al. (2012) this shows that these substances may play a significant part in combating bacterial strains that are resistant.

A newly discovered naphthalenone, (R)-4,6,8-trihydroxy-3,4-dihydro-1(2H)-naphthalenone, along with a novel isocoumarin, 6,8-dihydroxy-(3R)-(2-oxopropyl)-3,4-dihydroisocoumarin, was isolated from the endolichenic fungus CR1546C, found in association with the Costa Rican lichen *Sticta fuliginosa*. These compounds demonstrated promising antibacterial properties against *Bacillus subtilis*, showing MIC values of 100 mg/mL and 150 mg/mL (Kim et al. 2014). Ophiobolane sesterterpenes, ophiobolin P and ophiobolin T, which extracted from *Ulocladium sp.*, a fungus that was collected in the Yunnan

region of China and found to be associated with *Everniastrum sp*. With MIC values of 62.5 µM for ophiobolin P and 31.3 µM for ophiobolin T, both substances produce antibacterial action toward *Methicillin-resistant Staphylococcus aureus* (MRSA). Furthermore, ophiobolin T exhibited significant efficacy against the *Bacillus subtilis* and *Bacille Calmette-Guerin* strains, with MIC values of 15.6 µM and 31.3 µM, as well. The MIC values for positive control vancomycin, gentamicin, and hygromycin against *MRSA*, *B. subtilis*, and BCG, respectively, were 0.70 µM, 0.10 µM, and 0.68 µM (Wang et al. 2013b). Tricycloalternarenes (TCA 1b), which were linked to the lichen *Everniastrum sp*. and isolated from the endophytic fungus *Ulocladium sp*., shown a moderate degree of antibacterial activity against the BCG strain, with a minimum inhibitory concentration (MIC) of 125 mg/mL (Wang et al. 2013). Two sulfur-containing xanthone derivatives that were isolated from *Coniochaeta sp.*, coniothiepinol A and coniothienol A, had antibacterial activity against *Enterococcus faecium* and *E. faecalis*. Coniothienol A demonstrated IC50 values of 2.00 mg/mL and 4.89 mg/mL, whereas coniothiepinol A demonstrated IC50 values of 3.93 mg/mL and 11.51 mg/mL, respectively. With IC50 values of 0.51 mg/mL and 2.61 mg/mL against these strains, the antibiotic ampicillin, in contrast, showed more potent antibacterial activity (Wang et al. 2010). Ambuic acid and its derivative originated from *Pestalotiopsis sp*., which is isolated from the lichen *Clavaroids sp*. that grows in the Hainan area of China. The bactericidal activity of these compounds against *S. aureus* (ATCC 6538) was demonstrated by their respective IC50 values of 43.9 µM and 27.8 µM. Additionally, these compounds' antimicrobial peptides (AMP) demonstrated bactericidal effects against *S. aureus*, with an IC50 value of 1.40 µM (Ding et al. 2009).

Antifungal activities

The increasing prevalence of fungal infections in scenarios such as allogeneic bone marrow transplants, cancer therapies, and organ transplants has underscored the need for effective antifungal compounds that are both potent and compatible (Lockhart & Guarner 2019). Currently, For the treatment of different systemic and topical fungal infections, there is a restricted selection of antifungal medications. Products made naturally from endolichenic fungus (ELF) represent a significant reservoir of novel metabolites with potential applications in medicine and agriculture. *Aspergillus niger* was obtained from the lichen thallus *Parmotrema ravum* gathered in India, along with the known compounds aurasperone A, carbonarone A, and pyrophen, as well as a novel 6-benzyl-γ-pyrone named aspergyllone. Aspergyllone demonstrated antifungal efficacy against *Candida parapsilosis* with an IC50 value of 52 µg/mL. Aurasperone A exhibited anti-candidal effect toward *Candida krusei* with an IC50 of 373 µg/mL. In the same way, carbonarone A displayed antifungal activity against *C. krusei* and *Candida albicans*, with corresponding IC50 values of 31 µg/mL and 103 µg/mL. With an IC50 of 35 µg/mL, Pyrophen demonstrated antifungal efficacy against *Candida parapsilosis*, then *Candida utilis* (IC50 of 62 µg/mL) and *Candida albicans* (IC50 of 74 µg/mL) (Padhi et al. 2019).

The lichen *Cetraria islandica*, collected from Yunnan, China, was found in association with *Pestalotiopsis sp*., from which two ambuic acid derivatives, along with a known derivative, were extracted. The ambuic acid derivatives showed strong antifungal activity that inhibits action that inhibits *Fusarium oxysporum* with MIC values of 8 µg/mL, similar to ketoconazole, and significant activity toward *Fusarium gramineum* with MIC values of 8 µg/mL (Yuan et al. 2017). Through extensive screening of the endolichenic fungus *Phialocephala fortinii*, palmarumycin P3 and phialocephalarin B were extracted. These compounds specifically regulate MDR1 expression in *Candida albicans*, inhibiting drug efflux pump activity and showing efficacy against azole-resistant strains (Xie et al. 2016). The fungus *Floricola striata*, which inhabits the lichen *Umbilicaria sp.*, yielded three novel p-terphenyl derivatives, Floricolins A, B, and C. With MIC values of 16.0, 8.0, and 8.0 µg/mL, respectively, Floricolin A, B, and C showed fungicidal efficacy against *Candida albicans*. According to recent research, Floricolins can damage mitochondria, which causes reactive oxygen species (ROS) to accumulate. Moreover, glutathione (GSH) or thiol-group proteins may react with the α, β-unsaturated carbonyl group in Floricolins, leading to an imbalance in intracellular redox potential and the accumulation of ROS.

These accumulating ROS cause apoptosis, nuclear disintegration, and mitochondrial malfunction (Li et al. 2016, Zhang et al. 2018). *Biatriospora sp.* (8331C), which was isolated from the lichen *Pseudosyphellaria sp.*, was collected from Changbai Mountain in China's Jilin area. From this species, biatriosporin D and K were isolated, along with previously identified compounds 6-deoxy-7-O-demethyl-3,4-anhydrofusarubin and 2-acetonyl-3-methyl-5-hydroxy-7-methazarin. Compared to fluconazole (2 µg/mL) and terbinafine (16 µg/mL), biatriosporins D and K exhibited efficacy against *fluconazole-resistant Candida albicans*, with MIC80 values of 16.0 and 64.0 µg/mL, respectively. These compounds are known to inhibit efflux pumps and reduce the transcription of efflux-pump-related genes *CDR1* and *CDR2* (Agrawal et al. 2020). Additionally, biatriosporins D and K displayed antivirulence effects by preventing biofilm formation and filamentation in *C. albicans*. Notably, biatriosporin D

increased the expression of *Dpp3* and inhibited filamentation at a concentration below its MIC value, leading to the increased production of farnesol. Consequently, the pathogenicity of *Candida albicans* was reduced by inhibiting Cdc35 activity, lowering intracellular cAMP levels, and interfering with morphological transitions (Zhang et al. 2017). With a MIC value of 31 mg/mL, pericoterpenoid A, a new cadinane-type sesquiterpene, demonstrated considerable fungicidal activity against *Aspergillus niger* after being extracted from *Periconia sp*. associated with *Parmelia sp*. (Wu et al. 2015). The fungus *Nodulisporium sp*., which is linked to *Everniastrum sp.,* a lichen found in the Yunnan area of China, was also the source of Nodulisporipyrones AD, a class of α-pyrone derivatives. With a MIC of 31 mg/mL, these substances likewise demonstrated antifungal efficacy on *A. niger* (Zhao et al. 2015).

A. versicolor, an endolichenic fungus, disrupts Candida cell membranes, altering their permeability and causing intracellular glycerol accumulation. This compound also down-regulates genes involved in cell membrane synthesis and increases reactive oxygen species (ROS) levels, ultimately damaging mitochondria. According to Li et al. (2015), diorcinol D act as fungicidal agent by inducing ROS production and destroys cytoplasmic membranes. In addition to Pericocins C-D, one new dihydroisocoumarin, 3-(2-oxo-2H-pyran6-yl) propanoic acid, two new a-pyrone derivatives, pericocin A, and one new chromone, pericocin B were isolated from *Periconia sp*., which is found inside the lichen *Parmelia sp*., which was collected from the Jilin region of China. The antifungal efficacy of pericocins A , B , CD , and 3-(2-oxo-2H-pyran-6-yl) propanoic acid towars *A. niger* was moderate, with MIC of 31 mg/mL, whereas cycloheximide had a MIC of less than 0.3125 (Wu et al. 2011).

Conclusions

In conclusion, the study of endophytic and endolichenic fungi represents a nexus of ecological understanding and biotechnological innovation, offering insights into microbial interactions and promising avenues for the development of sustainable antimicrobial solutions. Continued interdisciplinary research is essential to harnessing the full potential of these fungi for future biomedical and environmental applications.

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