Diverse host-associated fungal systems as a dynamic source of novel bioactive anthraquinones in drug discovery: Current status and future perspectives

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Highlights
- Diversity and distribution of host-living fungi producing AQs in the terrestrial ecosystem are assembled.
- AQs biosynthesis and their SAR are elucidated to guide the approaches in novel drugs design and development.
- Several examples of true endophytic fungi producing AQs like their different host plants have been reported as interesting alternative sources of drugs.
- The review recapitulates the novel AQs with rare chemical skeleton that could open future venues for investigation of their biological activities.
- Lichens are assembled as unique source of several bioactive classes of AQs.

Abstract
Background: Despite, a large number of bioactive anthraquinones (AQs) isolated from host-living fungi, only plant-derived AQs were introduced in the global consumer markets. Host-living fungi represents renewable and extendible resources of diversified metabolites to be exploited for bioactives production. Unique classes of AQs from fungi include halogenated and steroidal AQs, and absent from planta are of potential to explore for biological activity against urging diseases such as cancer and multidrug-resistant pathogens. The structural diversity of fungal AQs, monomers, dimers, trimers, halogenated, etc. results in a vast range of pharmacological activities.

Aim of review: The current study capitalizes on uncovering the diversity and distribution of host-living fungal systems producing AQs in different terrestrial ecosystems ranging from plant endophytes, lichens, animals and insects. Furthermore, the potential bioactivities of fungal derived AQs i.e., antibacterial, antifungal, antiviral (anti-HIV), anticancer, antioxidants, diuretic and laxative activities are assembled in relation to their structure activity relationship (SAR). Analyzing for structure–activity relationship among fungal AQs may facilitate bioengineering of more potential analogues. Withal, elucidation of AQs biosynthetic pathways in fungi is discussed from different fungal hosts to open up new possibilities for potential biotechnological applications. This comprehensive review unravels terrestrial host-living fungal systems...
as a treasure trove in drug discovery, in addition to future perspectives and trends for their exploitation in pharmaceutical industries.

Key Scientific Concepts of Review: Such comprehensive review unravels terrestrial-host-living fungal systems as a treasure trove in drug discovery, in addition to future perspectives and trends for their exploitation in pharmaceutical industries.

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Introduction

An increasing interest is found towards drug discovery from natural products to treat global health demand of incurable diseases [1]. Additionally, search for novel natural compounds has become an escalating promise for the treatment of recent prevalence diseases i.e., HIV/AIDS, global COVID-19 pandemic, cancer and other diseases of increasing morbidity. Historically, plants were the major source of natural drugs discovery, however, structures complexity and multi-target effects of plant preparations drove the development of synthetic drugs. Until penicillin, the first antibiotic has been discovered from a *Penicillium* fungus [2], a new era of natural bioactives discovery from microorganisms has emerged. In terrestrial system, hundreds of fungi inhabit the inner tissues of living organisms that could range from being either beneficial to the host or rather pathogenic causing disease [3]. Fungal endophytes and pathogens have been increasingly recognized as source of a myriad of biologically active secondary metabolites [4]. Three groups of fungi may comprise AQs distribution in terrestrial host-living fungi that are endophytes, pathogens and lichens, with each system to have evolved its distinct divergence of AQ classes (Fig. 1).

Up till now, hundreds of plants have been investigated for their endophytic fungi, with many novel and bioactive secondary metabolites identified [5]. Currently, endophytes producing secondary metabolites have several potential applications in medicine ever since taxol-producing endophytic fungi has been identified [6], and has yet to be exploited for the commercial production of other drugs. Lichens represent another example of host-living fungi that is also under recognized, especially that lichen-forming fungi represent nearly one-fifth of all known fungal species [7]. Up to 1800 species of fungi are identified in lichens producing a range of potentially secondary metabolites, most of which are uniquely present in lichens [7]. Although pathogenic fungi cause severe diseases in plants, insects and human, many of which produce bioactive molecules that can be exploited for the discovery of novel drugs [8,9].

AQs constitute an important class of natural compounds with a wide range of applications, with ca. 700 compounds identified till now from nature [10]. They exhibit a myriad of effects to include colorants, laxatives, antioxidant, hypotensive and analgesic, anti-inflammatory, anti-malarial and antimicrobial agents [11]. Several important drugs are AQs derivatives that are indicated for several therapeutic indications including constipation, arthritis, multiple sclerosis, and cancer [10]. Nowadays, natural AQ derivatives represent an exceptionally valuable class in cancer therapy e.g. doxorubicin, mitoxantrone and epirubicin, quite effective against several cancer types though with some toxic effects e.g., leukopenia, thrombocytopenia and cardiotoxic effects [12]. It is thus worth pursuing research on identifying new AQs with lower toxicity and improved anticancer effect. AQs exhibit their cytotoxic activity mainly through intercalation through their aromatic planar ring and trapping of DNA topoisomerase II complexes on cellular DNA [13]. Most of AQs, are well characterized as laxative drugs i.e., rhein, physcion, chrysophanol, emodin, etc... classified as irritant stimulant leading to the induction of large intestine motility [14]. Recently, a number of AQs like hypericin, aloe-emodin, quinalizarin, protohypericin, alizarin and emodin anthrone have been reported to possess antiviral activities against several animal viruses, including influenza virus-A, Coxsackie virus B3, human cytomegalovirus, Coxsackie and HIV-I virus [15]. These results suggest that AQs may be further evaluated against the newly evolved coronaviruses, i.e. COVID-19.

![Fig. 1. Distribution of host-living fungal AQs producers in terrestrial ecosystem; endophytic fungi, pathogenic fungi and lichens-associated fungi, with representative example of main AQs.](image-url)
Moreover, emodin was found to exhibit a potent antibacterial action against a number of bacterial pathogens including *Hae-mophilus parasuis*, *Bacillus cereus* and *Staphylococcus aureus* [16, 17].

Currently, host-living fungi have been explored extensively for the discovery of novel drugs. Drugs discovery from fungi hold some promises compared to plants in term of their high availability, stability, and low residues. To overcome though endophytes limitations of high-yield bioactives production at a commercial scale, there are recent approaches designed to enhance the *in vitro* production of active metabolites using isolated endophytic fungi [18]. Currently, over 1000 secondary metabolites are already reported from lichens, several of which are specific for lichens [19]. It has been reported that all of these secondary metabolites isolated from lichens are of fungal origin [20].

Recent reviews highlighted potential bioactive AQ metabolites isolated from marine fungal endophytes as targets for discovery and development of new drugs, especially anticancer, antibacterial, antifungal, and anti-parasitic among others [21]. Several AQSs are produced by marine-derived endophytes such as catenarin, emodin, erythroglaucin, anthraquinone 5,5’-biphyscion, physcion, questin, and rubrocristin or physcion anthrone. *Phomopsis*, *Aspergillus*, *Nigrospora*, *Halorosellinia*, *Eurotium*, *Alternaria*, *Microsphaeropsis*, and *Fusarium* were as marine-derived anthraquinones producers [22].

The high rate of publications on AQ chemistry and biological activity from host-living fungi reflects their potential in drug discovery. Consequently, the current review comprehensively covers the diversity and distribution of host-living fungi in context to AQ production in terrestrial ecosystems classified into endophytes, pathogenic fungi and lichens. Withal, the potential results on the different biological effects i.e., antibacterial, antifungal, antiviral, anticancer, antioxidant, diuretic and laxative of isolated AQSs will be assembled and discussed in relation to their chemistry backbone and biosynthetic origin (Fig. 1).

### AQs formation via the octaketide biosynthetic pathway in fungi

AQs belong to polyketides constructed of a tricyclic anthracene ring harbouring at least one aromatic ring with carbonyl groups at positions 9 and 10 [23]. They are produced by two main pathways including, the polyketide pathway in fungi in addition to shikimate pathway in *plant*. Even though, the widespread distribution and broad benefits of AQSs, their biosynthesis and more over metabolic regulation have yet to be fully elucidated especially in case of fungal associated systems [24].

In the fungal polyketide pathway, large multi-domain proteins named polyketide synthases (PKSs) are responsible for polyketide biosynthesis using acetyl-CoA (CoA) and malonyl-CoA (M-CoA) as substrates. Generally, three fundamental domains are found in all PKSs that are acyltransferase (AT), β-ketosynthase (KS), and the acyl carrier protein (ACP). Altogether, these domains represent the minimal modular components indispensable for polyketide chain elongation [25]. AT domain is accountable for picking and transporting of the major building block, a malonyl–CoA, in each chain elongation cycle to an ACP domain that covalently tether the growing polyketide chain by its phosphopantetheinyli arm. While the KS domain spurs the chain elongation between the ACP-bound extender unit and the growing polyketide chain through the repetition of decarboxylation condensation reactions [23] (Fig. 2).

| Three major types of PKSs, i.e., type I, II and III PKSs were reported. Type I are large, multifunctional and highly modular proteins. In contrast, Type II are monofunctional proteins with free-standing catalytic components. Whereas Type III comprise multifunctional enzymes that use malonyl–CoA (M-CoA) as substrate instead of ACP domains [26]. Further Type I PKSs can be classified into iterative or noniterative according to the number of rounds in chain elongation. Non-iterative (Modular) Type I PKSs encompass a characteristic sequence of modules, each one in this sequence proceeds only one cycle of polyketide chain elongation. While each domain in iterative Type I PKSs can repeatedly modify and activate the same monomer [26]. Generally, most fungal PKSs are multifunctional type I PKSs with an iterative strategy (iPKSs) [27]. Based on their ability to reduce the β-keto carbon, type I iterative PKSs could be classified as non-reducing (NR-iPKSs), partially reducing (PR-iPKSs), or highly reducing (HR-iPKSs). HR-iPKSs contain a fully reducing modifying region. On the contrary, NR-iPKSs lack all the β-keto modifying domains and use the polyketide chains for polycyclic aromatic cyclizations. HR-iPKSs reduce the β-keto to a hydroxy group, dehydrate the hydroxyl to an enol group, which can be further reduced to an alkyl group to produce a huge diversity of PK compounds (alcohols, ketones, alkenes) [28]. In contrast, these reduction steps are omitted in polyketide synthesis by NR PKSs, resulting in a highly diverse polyketide chain with respect to the occurrence of β-ketone, β-hydroxyl and alkyl groups [27]. Indeed, NR-iPKSs initiate the biosynthesis of aromatic polyketide compounds including AQSs in fungi [21]. NR-iPKSs comprise three additional domains including starter-unit ACP transacylase (SAT), product template (PT), and thioesterase/Claisen cyclase (TE/CLC) domains. The domains organization inside a NR-PKS is the starter unit: ACP transacylase (SAT), KS, AT, PT, ACP; in addition to the region that may variably contain thioesterase/Claisen cyclase (TE/CLC), or other chain-modification domains [29]. The role of SAT domain lies in the selection of the starting unit, while PT domain in NR-iPKSs selectively modulates the cyclization of the polyketide chains to construct the final structure of the final PK compounds. Finally, the C–C bond closure reaction of the last ring is catalyzed by TE/CLC domain [29].

Generally, the regioselective cyclization or folding of the polyketide chain in eukaryotes introduces two intact acetate units in the first aromatic ring of the final product (designated folding mode F), while the first aromatic ring in prokaryotes incorporates three such acetate building units (designated folding mode S) [30]. In F-mode, there are three different forms of first-ring cyclization patterns for polyketide chain: C2 – C7, C4 – C9, and C6 – C11. For AQ formation in fungi, one acetate and seven malonate units are involved in the biosynthesis of the unstable β-polyketide chain. The acetyl-CoA molecule acts as a primer to which malonyl-CoA molecules are continually attached via a condensation process. In this process, the C–C bond is formed by the release of a free carboxyl group, and generating an intermediate chain of octa-β-ketoacyl-CoA [22].

PT determines the regioselective cyclization of the polyketide backbone, and ultimately the final structure of products. For the first cyclization, two common cyclic patterns (C4-C9, C6-C11) showed an aldol cyclization involving the synthesis of AQSs. Further, the cyclization process results in three rings formation, which are precursors of various stable AQSs. This pathway generates several well-known AQSs such as emodin, aloe-emodin, chrysophanol and physcion. Additionally, these later AQSs could also act as precursors for several other AQSs [22].
Anthraquinones producing-endophytic fungi

Host-living fungi are distributed widely in terrestrial ecosystems as pathogens or as endophytes of living organisms, i.e., planta or in lichens. Sometimes it would be difficult to discriminate between endophytic and pathogenic microorganisms, especially when both share similar genetic signatures [32]. Strikingly, several fungal species combine subspecies that have both endophytic and pathogenic properties [33], which complicates the clear dissection of a pathogen versus mutualistic fungi. While, most pathogenic microorganisms generate toxins harmful to the host plant, secondary metabolites produced by endophytic fungal strains may protect their host plants against pathogenic microorganisms [34]. Endophytic fungi are microorganisms that inhabit the internal tissues of the plants without causing apparent disease symptoms [35]. The endophyte-host interaction and communications have been described as a balanced symbiotic relationship, with endophytic fungi to generate metabolites that are sometimes characteristic to their host plants due to endophytes plant interaction as explained in the next section [6]. On the other hand, selected examples of endophytic microorganisms can be cultured and grown after isolation from their host on their own to yield novel pharmacologically active and structurally diverse secondary metabolites that are not present in their host’s tissues (Fig. 1).

Host-AQs producing endophytic fungi

Such host-endophyte interactions, genetic recombination of the endophyte may occur during the long period of co-evolutionary process with their hosts. This may explicate the ability of several endophytic species to produce some phytochemicals originally characteristic of their hosts. Endophytic fungi could insert their DNA segments into the host genomes or take some plant DNA segments within their own genomes [6]. However, the biosynthetic pathways harboured inside the endophytic fungi might be different and/or governed by a different molecular mechanism than the host plant. The ability of endophytic fungi to produce the same active constituents of their hosts represents a very interesting area in the exploration of alternative routes for the production of these metabolites [36], especially in case of endangered plants. Wherever explored, these fungi showed significant scientific and industrial potential to produce natural products in cost-effective and reproducible manner. Compared to fungal culture, several factors may affect the production of bioactive metabolites in planta e.g. the plant health status, climate fluctuations, soil differences, genotypic changes, etc. Further, the extraction of medicinal plants may contain a combination of constituents [37], and might be complicated due to chlorophyll rich matrix in case of stems and leaves. It is captivating to analyse reported examples of true endophytic fungi producing AQs like their different host plants as illustrated in the following subsections (Fig. 3).

Hypericum- host fungi

Hyperin is a major constituent produced by Hypericum perforatum L. (St. John’s-wort; Hypericaceae). It has a significant antidepressant activity mediated through the inhibition of dopamine β-hydroxylase action resulting in high dopamine levels along with antibiotic, antiviral and non-specific kinase inhibitor effects [38].
2008 and 2009, Kusari and his co-workers isolated the antidepressant emodin and hypericin \((1\text{ and }2)\) in \textit{in vitro} culture of the endophyte, \textit{Thielavia subthermophila}, isolated from stems of \textit{H. perforatum} [36,39](Fig. 3). Structures of all reported major AQs are listed in Fig. 5.

Depending on light, \textit{T. subthermophila} extract containing hypericin and emodin showed significant cytotoxic activity against human acute monocytic leukemia cell line (THP-1), with values 92.7 vs 4.9%, and 91.1 vs 1.0% viability at a concentration of 1.185 \(\mu\text{g}/\text{mL}\), in light and in the dark, respectively. The cytotoxic activity was enhanced by light indicating their photodynamic properties [36]. The results are constituents with previous reports indicating that hypericins possess minimal or no cytotoxicity in the dark [40]. In addition, hypericins accumulate significantly higher in cancer cells in comparison to surrounding normal cells [40]. Hence, natural photosensitizers hold substantially promise for improving the photodynamic therapy and diagnosis of various oncological diseases.

In the same line, isolated strains from \textit{H. perforatum} leaf; \textit{Epococcum nigrum} SZMC 23,769 and \textit{Alternaria} sp. (SZMC 23,771 and SZMC 23,772) showed the ability to produce emodin, and for \textit{E. nigrum} to produce both hypericin and emodin [41]. These metabolites exhibited moderate to high antimicrobial activity at the concentration (100 \(\mu\text{g}/\text{mL}\)) against \textit{E. coli}, \textit{P. aeruginosa}, \textit{Staphylococcus aureus}, \textit{B. subtilis}, \textit{Micrococcus luteus} and \textit{Streptomyces albus} with a growth inhibition of 65% – 92% and 60% – 78%, respectively. Hypericin displayed the highest antimicrobial activity against \textit{B. subtilis}, while emodin had the highest effect on \textit{P. aeruginosa}. Noticeably, the antimicrobial activity of hypericin was greater than that of emodin on all tested bacteria, except against \textit{E. coli} [41].

\textbf{Aloe vera-host fungi}

Emodin is one of the major AQs present in \textit{Aloe vera} plant [42], and to account for its laxative effect among other bioactives e.g., analgesic, anti-inflammatory, antibacterial, anti-allergic, antiviral and anticancer effects. A number of other pharmacological benefits were reported recently including, neuroprotective, anti-diabetic, immunosuppressive, anti-osteoporotic, and hepatoprotective activities. In addition, emodin was able in combination with chemotherapeutic agents to inhibit tumor cell growth. Recently, it has been isolated in its dimeric form, biemodin, for the first time from an \textit{in vitro} culture of \textit{Talaromyces wortmanii} derived from \textit{Aloe vera} (Fig. 3). Along with, several known AQs, emodic acid, skyrin, oxyskyrin, rugulosins A and B (3–7) have been identified in the same culture [43]. Skyrin and rugulosin A showed strong antibiotic activity against Gram-positive bacteria; Methicillin-resistant \textit{Staphylococcus aureus} (MRSA), \textit{S. epidermidis}, \textit{S. pneumonia}, and \textit{Enterococcus faecalis} with MIC values in the range of 4–16 \(\mu\text{g}/\text{mL}\). Further, biemodin exhibited considerable activity against the same Gram positive bacteria, however, it was less active compared to skyrin and rugulosin [43]. These results provide a basis for new antibacterial drugs development that needs further clinical trials to be conclusive whether a synergistic action can be observed in mixture of these AQs or with antibiotics to reduce their dose is an area that worth to be explored.

It is worth mentioning that, \textit{T. wortmanii} derived from \textit{Aloe vera} produced two previously unreported mixed dihydroanthracenone/anthraquinone dimers, Talaromannins A and B, as well as known AQs, emodin and skyrin [44]. All identified AQs showed inhibiting activity against \textit{S. aureus} strains including MRSA isolates with MIC values ranging from 4 to 8 \(\mu\text{g}/\text{mL}\). Notably, these compounds...
exhibited no cytotoxic activity, encouraging their further evaluation as potential leads for antibacterial drug development especially against resistant microbial strains like vancomycin-resistant S. aureus (VRSA) and MRSA.

**Rheum-host fungi**

*Rheum palmatum* L. (Chinese rhubarb) is a well-known traditional medicinal plant with dominant AQs including rhein (8), emodin, aloe-emodin (9), etc. [45]. Interestingly, *Fusarium solani* isolated from *R. palmatum* L. root was able to produce both rhein and emodin found mainly in *R. palmatum* L. [46] (Fig. 3). Moreover, a close relative of *Polyporales* sp. fungi hosting *R. emodi* (Indian rhubarb) was able to produce emodin [47]. The isolated emodin exhibited cytotoxic activity toward human cancer cell lines; THP-1 (Leukemia), A549 (Lung), NCI-H322 (Lung) and Colo-205 (colon) at a concentration of 70 and 100 μM [47]. Notably, the beneficial uses of *Rheum* plants are restricted to their high potassium and oxalate content which may lead to gastrointestinal problems, electrolyte disorders, and liver toxicity with long-term use [48]. The bioactive AQs level in rhubarb plants is usually small [49]. As a result, these endophytes have emerged as a potential alternative to this plant for safer and higher-yield of AQs production.

**Rumex-host fungi**

Several AQs have been identified in all *Rumex* sp. including emodin, chrysophanol, aloe-emodin, physcion and rhein, etc.[50]. Even though, these plants are safe with higher LD₅₀, the high exposure to their extracts for a long period of time may result in irreversible histopathological, biochemical and haematological changes of liver, lung and kidney [51]. Three AQ compounds were obtained by cultivating endophytic fungi derived from *R. gmelini* including emodin, rhein, and aloe-emodin. In details, emodin was isolated from 3 strains of *Penicillus* and *Cercospora* sp., rhein from *Alternaria* sp., and aloe-emodin from *Tolura, Eriocercospora, Fusarium* sp., with yields ranging from 683 to 688 μg/g [52] (Fig. 3).

Interestingly, co-cultivation of plant tissues with their endophytic fungi may lead to a significantly higher production of their bioactive secondary metabolites and/or accumulation of novel metabolites that are not detected in the host plant. For example, *R. gmelini* tissues co-cultured with the endophytic fungi i.e., *Aspergillus, Fusarium*, and *Ramularia* sp. led to increased accumulation of chrysophaein, chrysophanol, emodin and physcion [53]. Additionally, musizin was found in co-cultured seedlings system; however, it was not detected in the control group. *Aspergillus* sp. showed the most significant enhancement of bioactive components in *R. gmelini* seedlings [53]. Accordingly, further techniques such as mixed fermentation along with co-cultivation should be examined in the future to construct economical production platforms of endophytes for high and sustainable production of valued AQs.

Furthermore, the utilization of fungi mixed culture results in higher dye yield and consequently affected the intensity of produced colour. *Aspergillus* and *Paecilomyces* in mixed culture provided maximum dye yield at pH 9, a temperature of 24 °C, with sucrose as a carbon source, and sodium nitrate as a nitrogen source [54]. It is noteworthy that most studies reported optimization parameters for maximal pigment yield from fungi. However, for the pharmaceutical industry more focus should be given towards improving culture conditions to increase the accumulation of AQs or for the production of novel chemicals.

**Endophytic fungi produce different AQs from their plant host**

Interestingly, several endophytic fungi may produce metabolites that are not similar to that of their hosts. Indeed, several new and important bioactive AQ compounds with antimicrobial, antiviral, insecticidal, and antitumor activities were obtained from the *in vitro* cultivation of the endophytic fungi [20]. Endophytes may protect their host plants by generating these metabolites with antiviral, antibacterial, insecticidal, antifungal, activities functioning to improve their fitness, to be detailed in that section.

**Endophytic fungi derived hydroanthraquinones**

Most of the tetrahydroanthraquinones were mainly isolated from endophytes, one of which has been derived from marine fungi and plants. Their significant cytotoxicity activity was reported in a relatively large number of publications, indicating their potential roles in developing or finding new anticancer drugs. Additionally, several hydroanthraquinone derivatives showed other biological actions including antimicrobial and antifungal activities [55].

Although, the genus *Alternaria* is commonly known as a plant pathogen, they can also be present as endophytic fungi. *Alternaria* sp. has emerged as privileged source of alterporriol and altersolanol compounds, which are tetrahydroanthraquinone derivatives with significant anticancer properties. Recently, several investigations have covered *Alternaria* sp. metabolites, alterporriols and altersolans, from terrestrial, mangrove, and marine sources and their potential as powerful AQ-based anticancer against various cancer cell lines [56,57].

Interestingly, endophytic fungi have been reported as a potential source of these compounds. Altersolanol B (10) and a new hexahydroanthraquinone named pleospdione (11) along with 5 other AQs (deoxybostrycin, dactylaril, 7-methoxy-2-methyl-3,4,5-trihydroxyanthraquinone, physcion, macrosporin) (12–16) were reported from the endophytic fungi, *Pleospora* sp., harbored in the normal stem of *Imperata cylindrica* [58]. Altersolanol B, deoxybostrycin and dactylaril displayed a strong cytotoxic activity against human colon cancer (SW1116) and leukemia K562 cell lines with IC₅₀ in the range of 0.8–58.8 μg/ml [58].

Other endophytic fungi have been reported as a potential source of these compounds. Altersolanol B (10) and a new hexahydroanthraquinone named pleospdione (11) along with five other AQs (deoxybostrycin, dactylaril, 7-methoxy-2-methyl-3,4,5-trihydroxyanthraquinone, physcion, macrosporin) (12–16) were reported from the endophytic fungi, *Pleospora* sp., harbored in the normal stem of *Imperata cylindrica* [58].

Additionally, altersolans A and J, alterporriols D and E (17–20) as well as 5 known AQs (macrosporin, macrosporin-7-O-sulfate, 3-O-methylalaternin, 3-O-methylalaternin-7-O-sulfate and ampeanol) were isolated from *Ampelomyces* sp. residing in the medicinal plant *Uroserpentum picroides* L. Among the isolated AQs, only 3-O-methylalaternin and altersolanol A that showed antimicrobial activity against *S. epidermidis, S. aureus*, in addition to *E. faecalis* for 3-O-methylalaternin. It was reported that altersolanol A inhibits bacterial growth by acting as an electron acceptor into the bacterial membrane [59].

Other bianthraquinone of alterporriols A (21), B (22), D, E, a mixture of alterporriols G and H, altersolanols A, K (23), L (24), J, 6-O-methylalaternin and macrosporin were isolated from an *in vitro* culture of *Stemphyllum globuliferum* isolated from stem tissues of *Mentha pulegium* (pennyroyal) [60]. Notably, alterporriols G and H represent the first dimers with a C-7-C-5 bond, 3-sulfate, 3-O-methylalaternin and altersolanol A that showed antimicrobial activity against *S. epidermidis, S. aureus*, in addition to *E. faecalis* for 3-O-methylalaternin. The use of alterporriol A inhibits bacterial growth by acting as an electron acceptor into the bacterial membrane [59].

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pure alterporriol G showed moderate antibacterial activity against only *S. pneumonia* [61].

Altersolanol A, tetrahydroaltersolanol B and macrosporin were produced by *S. globuliferum* isolated from the leaves of medicinal plant *Chenopodium album* grown in Egypt [62]. Similarly, alterporriols A, B, G, D and E, altersolanols A–C, dihydroaltersolanols B and C, acetylatedalterporriols D and E, macrosporin, and 6-O-methylalaternin were obtained from *S. globuliferum* harbouring *Juncus acutus* [63]. Comparison of dihydroaltersolanols B and C and acetylatedalterporriols D and E for their antibacterial activity against *S. aureus*, *E. coli*, and *P. aeruginosa* revealed that dihydroaltersolanol C having an extra hydroxyl group in B isomer exhibited moderate growth inhibition against *S. aureus* (MIC value of 49.7 μg/mL), whereas the other compounds were found inactive in this bioassay (IC50 > 64 μg/mL).

Two new naturally occurring hydroanthraquinones, nigrosporone A and B (26, 27), were isolated from *Nigrospora* sp. BCC 47,789 harbouring in the leaves of *Choeoprosnia axillaris* (Roxb). Nigrosporone B displayed a wide range of biological properties including, cytotoxic, anti-tubercular, antimalarial and antibacterial activities, as opposed to nigrosporone A which exhibited only cytotoxic activity. Further studies are needed to manifest the structure–function relations in which the AQs give the most cytotoxic activity [64].

The endophytic fungi *N. aurantiaca* CMUZY2045 isolated from the leaves of *Cinnamomum zeylanicum*, in Northern Thailand produced intense red pigment that was characterized as bostrycin, one of the tetrahydroanthraquinone compounds. The extracted fungal red pigment exhibited a high staining ability in dyeing of cotton fabrics with an excellent washing fastness. For the red pigment production, the optimum conditions were observed in the liquid medium containing glucose as a carbon source and yeast extract as a nitrogen source, at 27 °C and a pH value of 5.0. Notably, isolated red pigment did not exhibit a cytotoxic activity suggesting for its safety. Hence, *N. aurantiaca* has the potential of producing a safe natural dye to be used in textile processing [65].

In a further study, Huang et al. optimized conditions for bostrycin production from *N. aurantiaca* using submerged and solid-state fermentations. For submerged fermentations, *N. aurantiaca* fungi incubated at 30 °C and 150 rpm for 6 days in a medium with 1.0% cane molasses. While in solid-state fermentation using the ratio of 1: 2 between bagasse and water for 10 days increased the pigment yield. It is worth mentioning that bostrycin showed antibacterial activity in meat samples reaching 91% against *S. aureus*. Whether other bactericidal effects for bostrycin exist posing it as food preservative has yet to be determined. Besides, bostrycin had the ability to color meat with a red color stable for 24 h and posing it as a natural dyes for food processing [66].

**Structure-cytotoxic activity relationship of fungal derived AQs**

The chemical structure features of AQs are emerging as essential prerequisite to disclose their biological effects, especially that of cytotoxic activity and to be presented in that subsection and as summarized in Fig. 4 showing the major structure-cytotoxic activity relationship analysis of AQ derivatives. Generally, AQs with *para*-quinone moiety exhibit strong cytotoxic activity as in case of altersolanol A mediated by the presence of *p*-quinone moiety in its chemical structure. This structure showed 50% inhibition of human chronic myeloid K562 leukemia and A549 lung cancer cells after 24 h of treatment at a concentration of 4.2–29.5 μM [67]. The reduction of one of the carbonyl groups as in natural AQs exemplified in case of tetrahydroaltersolanol B and amelanol compounds rendered them inactive against K562 and A549 cells [67] (Fig. 4A).

Altersolanol K and J represent another examples of fungal-derived AQs with an oxidized C-9 and a reduced C-10, to exhibit weak activities against LS178Y cells compared to altersolanol A (IC50 1.5 μg/mL), that would emphasize the pivotal role of *para*-quinone moiety in AQs cytotoxic activity [60].

Distinctly, lipophilicity is a parameter that plays a crucial role in determining absorption, distribution, excretion, and metabolism in determining AQs biological activity. Generally, sulfonation of AQs may result in a decrease in cytotoxic activity due to the formation of more polar derivatives that could reduce lymphoma cells uptake. For example, the cytotoxic activities of 6-O-methylalaternin and macrosporin, respectively, against LS178Y cells (IC50 4.2 and 6.9 μg/mL) have been reduced by the presence of sulfate substitution in macrosporin-2-O-sulfate and 6-O-methylalaternin-2-O-sulfate, respectively. They both displayed weak activities against LS178Y cells compared to their parent compound macrosporin and 6-O-methylalaternin [59]. However, the acetylation of altersolanol A did not improve its activity against tested cell lines though expected to increase its lipophilicity. Hydroxyl derivatization in AQs by acetylation of altersolanol A (tetraacetylateslolanol A) did not indeed enhance altersolanol A bioactivity against K562 and A549 cells. The results strongly demonstrated that the consequent increase of lipophilicity or masking of hydroxyl groups did not impact altersolanol A bioactivity, however, compound lipophilicity should still be considered as a critical factor affecting drug bioavailability after oral administration [67] (Fig. 4A &B).

In addition, the ortho-dihydroxy substitution pattern of AQs was reported as remarkable structural motif in the cytotoxic activity. In vitro, 6-O-methylalaternin showed significant cytotoxic activity against LS178Y cells (IC50 4.2 μM), while emodin and macrosporin showed moderate and no activity, respectively [68]. In contrast, there was no change in altersolanol A and C cytotoxic activities upon derivatization by 1,2- and 1,3-diols protecting group: acetonide, indicating that substituents at the aliphatic ring in tetrahydroanthraquinone derivatives are not essential structural motifs for their activity, with further OH groups at the 2- and 3-positions not to much impact the cytotoxicity of altersolanol-type derivatives [68] (Fig. 4B).

Axial chirality is another structural feature that plays a modifying role in enhancing or reducing the cytotoxic activity of AQs. For example, in case of tetrahydroanthraquinone dimers, (αR) axial chirality of acetylatedporriol E and alterporriol E exhibited marked cytotoxic activity against LS178Y cells with IC50 values of 10.4 and 6.9 μM, respectively, whereas their (αS) congeners exhibited no activity [68]. These results suggest that the axial chirality of tetrahydroanthraquinone dimeric derivatives plays a crucial role for the mode of action of these compounds (Fig. 4E). Furthermore, the arrangement of tricyclic aromatic systems in AQs molecules played another fundamental role of improving the cytotoxicity of AQs. “L”-like shape of alterporriols G and H mixture was found to possess considerable cytotoxic activity against L5178Y cells (IC50 2.7 μM), while other alterporriols i.e., A, B, D and E displayed only moderate to weak activity. This might be attributed to that the bicyclic aromatic systems of alterporriols G and H are arranged more or less perpendicular to each other, while the dimer units in the remaining alterporriols are arranged parallel [59] (Fig. 4C).

Glicosylation of alizarin, 2-amino-3-hydroxyanthraquinone and anthraflavic acid enhanced their anticancer activities against three different cancer cell lines (gastric cancer-AGS, uterine cervical cancer-HeLa and liver cancer-HepG2 cells). The glycosylated AQs compounds exhibited up to 60% inhibition of cell growth at concentration between 50 and 100 μM. Among them, alizarin-2-O-β-D-glucoside showed the strongest activity against all tested cell lines by more than 90% inhibition of cell growth [62] (Fig. 4D). These results also emphasized for the role of ortho-dihydroxy substitution in increasing the cytotoxic activity of AQs.
Endophytic fungi derived dimeric and trimeric anthraquinones

Several new homo- and heterodimers of AQs with rare chemical skeleton were produced by endophytic fungi. Two new bisanthraquinones named (+)-epicytoskyrin and (+)-1,10-bislunatin were isolated from the culture of *Diaporthe phaseolorum* var. sojae, associated with a tea plant [69]. (+)-1,10-bislunatin, a dimeric form of lunatin, that showed moderate antimicrobial activity against 7 bacterial strains (*E. coli*, *P. mirabilis*, *S. aureus*, *Micrococcus luteus*, *B. subtilis*, *Shigella flexneri*, and *Proteus vulgaris*) with MIC value in the range of 32–64 \( \mu \text{g/mL} \). Exposure to (+)-1,10-bislunatin caused morphological changes of *E. coli* and *B. subtilis* cells including degeneration of cell membrane [70]. Additionally, two new heterodimers, diaporthemins A and B were isolated from *D. melonis* found in *Annona squamosa* L. Both of them were not active against tested *S. pneumonia* and MRSA strains [71].

Further, another new asymmetrical dimeric anthraquinone, 3-demethyl-3-(2-hydroxypropyl)-skyrin together with 4 known monomer compounds (skyrin, oxyskyrin, emodin and 1,3,6-trihydroxy-8-methyl-anthraquinone) were produced by Talaromyces spp. YE 3016 isolated from roots of *Aconitum carmichaelii* [72]. Xanthoquinodins are heterodimers of xanthone and anthraquinones monomers connected in an ‘end to body’ fashion. A new xanthoquinodin B9 (28) as well as chrysophanol (29), emodin, alatinone (30), xanthoquinodin A1 (31) and A3 (32) were isolated from the endophytic fungus *Chaetomium globosum*, from *Rhapis cochinchinensis* (Lour.) Mart. [73]. Compounds xanthoquinodin A1, A3 and B9 exhibited cytotoxicity against KB, MCF-7 and NCI-H187 cancer cell lines, though with cytotoxic activity towards a normal cell line (Vero cell) with IC\(_{50}\) values ranging from 0.04 to 3.86 \( \mu \text{M} \) [73], suggestive of possible side effects as in case of doxorubicin [12].

Another source of xanthoquinodins is provided by several species of *Cytospora* comprising endophytes, saprobes and plant pathogens on a wide range of woody hosts [74]. Xanthoquinodins A4–A10 and B4–B8, cytosporanthraxanthone, ketoxanthoquinodin A6, and spiroxanthoquinodins A and B were isolated from *Cytospora eugeniae* (Valssaceae) harboured in the petiole of *Arenia pinnata* (Wurmb) Merr. [75]. Xanthoquinodins A4, A6, B4, and B5 (33–36; Fig. 5) and ketoxanthoquinodin A6 showed cytotoxicity.
against both cancer (MCF-7, KB, NCI-H187; IC50 between 2.91 and 10.36 μM) and non-cancer (Vero) cells with IC50 value in the range of 6.9–9.6 μM [75].

Additionally, xanthoquinodins A6, B4, and B5 displayed significant antimalarial activities against *Plasmodium falciparum*, K1 strain with IC50 values ranging from 0.52 to 0.92 μM as well as antibacterial activity against *B. cereus* (MIC of 1.56 μg/mL). While xanthoquinodin A6 exhibited additional fungicidal effect against *Curvularia lunata* with MIC of 3.1 μg/mL [75].

Two novel naturally occurring trimeric AQs, stemphyllanthranols A and B ([37, 38]), were produced from endophytic fungi, *Stemphylium globuliferum*, isolated from *Juncus actus* found to exhibit no cytotoxic activity against L5178Y cell line or antibacterial activities against *S. aureus*, *E. coli*, and *P. aeruginosa* [55].

Fig. 5. Structures of AQs identified from terrestrial host-living fungi.
Endophytic fungi derived hydroxyanthraquinone

Hydroxyanthraquinone (HAQ) derivatives have long been recognized for their laxative effects such as emodin, rhein, physcion, chrysophanol and aloe emodin found in several plant species [76]. Recent studies revealed that endophytic fungi synthesize these bioactive metabolites. Chrysophanol and emodin were successfully produced in the fermentation broth of the endophyte *P. oxalicum* isolated from the medicinal plant *Curcuma wenyujin* [77]. The solid phase culture of *P. restrictum*, isolated from stems of milk thistle (*Silybum marianum*) plant, produced distinct red guttates. Several HAQs were identified from this guttates including emodin, *α*-hydroxyemodin, emodic acid, and their derivatives; (+)-2′S-isorhodoptilometrin, 2-hydroxyemodic acid, 1′-hydroxyisorho doptilometrin, 1′-hydroxy-2′-ketoisorhodoptilometrin and desmethyl dermoquinone, 2-chloroemodic acid [78]. Moreover, emodin and 1,2,8-trihydroxyanthraquinone were isolated and purified from the ethyl acetate extract of *Diaporthe lithocarpus* isolated from the leaves of *Artocarpus heterophyllus* [79].

Several HAQ pigments are used in many industries e.g. textile dyeing, food processing or in cosmetics. Examples of natural AQs used at industrial scale include Arpink red from *P. oxalicum* and carminic acid from insect. Fungi have the potential to serve as a source of natural pigments that could be used for the production of different dyestuffs. The orange-yellow pigment, pachybasin (39), was produced as a major AQ by *Coelomycetes* sp. isolated from yellow moonsheed (*Archangelisia flava* (L.) Merr.) [80]. Further, *Coniothyrium* sp. isolated from *Salsola oppositifolia* yielded pachybasin and the yellow pigment phomarin (40) [81]. While, the purple dye anthrapurpurin, 1,2,7-trihydroxyanthracene-9,10-dione, (41) was isolated from two *Colletotrichum* spp. (TI 2 and TI 3) obtained from *Tragia insuavis* leaf [82]. This purple dye is used in histologic staining for the detection of calcium in tissues [83].

The orange-red quinizarin (42) is produced abundantly by *Epicoccum nigrum* isolated from fresh leaves of *Entada abyssinica* [84], posing it as potential source of this dye from fungal sources. Synthetic quinizarin, 1,4-dihydroxyanthraquinone, is used in colouring
resins and plastics, and further as an intermediate for the manufacture of several dyes e.g., disperse blue, brilliant blue, reduced brown BR, etc. or in 1,4-dihydroxyanthraquinone-2-sulfonic acid production [85].

Additionally, endophytic fungi showed the potential as a source for new HAQs not reported in planta. For example, neobulgarone G (43), a new HAQ was isolated from Penicillium sp. derived from fresh healthy stem tissues of Limonium tubiformum (Rutaceae) [86]. Another one is 1,3-dihydroxy-2,8-dimethoxy-6-methylanthaquinone produced in the culture of Colletotrichum spp. grown on potato dextrose agar along with three known AQs (1,2,3-trihy droxy-6-methyl-8-methoxanthraquinone, 1-hydroxy-2,3,8-trime thoxy-6-methylanthaquinone and 1,2-dihydroxy-3,8-dimethoxy-6-methylanthraquinone) [87].

Trichoderma asperellum was the first endophytic fungus reported to produce 1-hydroxy-8-methoxyanthraquinone [88]. However, it was previously isolated from the phytopathogenic fungus Leptographium wagneri. 1-Hydroxy-8-methoxanthraquinone did not show any antibacterial activity against E. coli, P. aeruginosa and S. aureus [88], in addition to exhibiting no growth inhibition effect against Chinese hamster ovary (CHO) and Mus musculus skin melanoma (B16F10) cell lines [88].

Nigrospora is a fungal species that is commonly reported as a plant pathogen, endophytes or saprobes that has potential for the discovery of novel metabolites. Several novel HAQs including 1,2,8-trihydroxyanthraquinone, 1,3,8-trihydroxyanthraquinone, rheoemodin, 1,3,6-trihydroxy-8-methylanthraquinone, aloe-saponarin II are first time to be reported from Nigrospora sp. living inside the roots of Aconitum carmichaeli [54] that has potential to be assessed for other biological effects. Whether these novel chemicals can be produced from other plant sources encompassing Nigrospora sp. should now be examined.

Endophytic fungi derived steroidal / glycosylated anthraquinones

Until now, endophytic fungi represent the unique source for natural anthraquinone–steroids, being absent from plant sources. Two novel anthraquinone–steroids, evanthrasterol A (44) and B (45) were isolated from Emericella variecolor (also called, Aspergillus variecolor) derived from the leaves of Croton oblongifolius. Evanthrasterol A and B did not show cytotoxicity against human cancer cell lines (BT 474, Chago, Hep-G2, KATO-III and SW620) [89]. This result may inspire the search for other fungi species producing natural AQ-steroids or for other biological effects to be further investigated for these steroidal anthraquinones.

A new glycosylated anthraquinone, 1-O-methyl-6-O-(α-D-ribofuranosyl)-emodin was obtained from Gaumannomyces sp. endophyte of Phragmites communis along with 1-O-methylemodin that both showed anti-inflammatory activity against lipopolysaccharide (LPS)-stimulated BV-2 microglia cells without causing cell death. 1-O-methylemodin and its glycosylated...
derivative decreased nitric oxide synthesis in LPS-stimulated microglia cells by 43 and 31%, respectively [90]. There is increasing need to identify novel anti-inflammatory agents with less toxicity and selective action due to the side effects of steroidal and non-steroidal anti-inflammatory agents [91].

**Anthraquinone-producing lichen**

Although that lichen is a complex form of two separate organisms; fungi and algae, in a symbiotic relationship, fungi represent a dominant partner that gives lichens their characteristics [92]. Up to 800 secondary metabolites are produced by these unique organisms including, dibenzofuranes, phenolics, depsidones, depsides antirrhinones, and xanthones [92]. Many of which to exhibit significant biological activities such as anti-inflammatory, antibacterial, antioxidant, anti-plasmodial and anticancer [92]. In this section, we shall focus on the known and novel AQs isolated from lichens and their biological activities (Fig. 5).

**Methodoxy-anthraquinones from lichens**

Of all AQ subclasses, methoxy-AQs are the most dominant class in lichens. Despite that methoxy-AQs are widespread in plants, lichens continue to yield a diverse chemical structure of methoxy-AQs with a broad range of pharmacological activities. Five methoxylated AQs, erythroglauca, xanthorin, fallaccinal and teloschistin (fallacinal) [46-49], physcion, as well as emodin were identified in three species of the lichen genus Xanthoria (X. fallax, X. elegans and X. polcarpa) [93]. All of the isolated AQs displayed broad-spectrum fungicidal activity against non-lichenized A. niger, T. viride, P. verrucosum and Doratomyces stemonitis. Furthermore, they exhibited selective antibacterial activity with growth inhibition effect between 5 and 25% against P. fluorescens, P. glicinea and P. phaseolicola, while they were inactive against B. mycoides [93]. Besides the previous species, X. parietina produced 2-methoxy-4,5,7-trihydroxy-anthraquinone [94]. Similarly, two methoxy-AQs; draculone and haematommone (50, 51) were isolated from the corticophil lichen Melanotheca cruenta [95].

7 Methoxy-AQs derivatives including parietin (52), 1-O-methylparietin and 8-O-methylparietin, erythroglauca, fallaccinal, fallaciola, parietinic acid (53) along with emodin were produced by the three lichen species named Caloplaca lactea, C. critina, and C. schaereri [96]. Further investigation of two more species of genus Caloplaca; C. schaereri and C. dalmatica led to the identification of 1,8-dihydroxy-6-methoxy-3-methyl-9,10-anthraquinone; 1,8-dihydroxy-3-formyl-6-methoxy-9,10-anthraquinone; 1,8-dihydroxy-3-hydroxymethyl-6-methoxy-9,10-anthraquinone and 1,3,8-trihydroxy-9,10-anthraquinone. The contents of AQs in each were at 0.79 and 0.65 g/100 g dried thallus, respectively [97].

Currently, all known naturally halogenated AQs were isolated from lichens or non-lichenized fungi. From the lichen Nephroma laevigatum, 7-chloro-1-O-methyl-hydroxyemodin, 7,7'-dichlorohypericin, 2,2',7,7'-tetrachlorohypericin, 7-chloroemodin and 7-chloro-1-O-methylemodin have been isolated (67–71; Fig. 5) [106]. The last two and 5,7-dichloroemodin were isolated also from the lichens N. laevigatum and Heteroderma obscura, along with two chlorinated bianthrone; flavoobscurin A and flavoobscurin B (72,73). Further, more common compounds; emodin, 6-O-methylemodin, skyrin, endocrocin, chrysophanol, and for the first time hypericins from lichens were isolated [107]. Hypericins are potential CNS affecting drugs in planta and should be pursued further for their biogenetic origin in lichens [108]. 5,7-Dichloroemodin hypericin, 7,7'-dichlorohypericin, 7-chloroemodin and 7-chloro-1-O-methylemodin exhibited significant inhibitory activity against herpes simplex virus type 1 (HSV-1). Among all isolated AQs, 5,7-dichloroemodin showed the most inhibitory activity, whereas, 6-O-methylemodin, skyrin, endocrocin, chrysophanol, flavoobscurin A, flavoobscurin B, 7-chloro-1-O-methyl-5-hydroxyemodin were found inactive. Antiviral activity appears to be associated with the increased number of chlorine substitutions in the AQ structures [107], that needs further studies to draw the final conclusions and to prove a clear picture of structure–activity relationship among these rather unique halogenated AQs. Furthermore, 7-chloroemodin, valsarin (7-chloro-5-hydroxyemodin), 5-chloro-1-O-methylemodin and 5-chloro-1-O-methyl-5-hydroxyemodin were isolated from the rare Southern lichen Lasallia papulose (Ach.) in their natural habitat [109]. Most reports of AQs from lichens are more than 10 years old, hence, still worth more studies to be conducted for the identification of more AQs from lichens. Probing of the biosynthetic pathways leading to the generation of these halogenated AQs in lichens can also aid in their biotechnological production.

**Pathogenic fungus**

Intuitively, pathogenic fungi have gained tremendous attention because of their ability to produce a diverse range of bioactive metabolites including AQs [94] to aid increase their colonization inside the host. These metabolites further could be exploited in medicine for the discovery of novel drugs.
Plant pathogenic fungi- derived AQs of different origin

*Fusarium sp.*

*Fusarium oxysporum* can cause drastic damage in many agricultural crops, in the field or during storage. 2-Acetyl-3,8-dihydroxy-6-methoxy anthaquinone and 3-acetyl-2,8-dihydroxy-6-methoxy anthaquinone were isolated from *F. oxysporum* infecting the roots of citrus trees causing rot disease. The ecological function of these AQs has yet to be fully elucidated. Dyeing wool with isolated AQs showed high colour strength and good fastness properties [110], likely due to that AQs form a covalent bond with the fibers. Consequently, they exhibit excellent wash fastness compared to simple dyeing methods. A marked advantage of reactive dyes over direct dyes is that, their chemical structures are often much simpler, with brighter dyeing properties [111].

*Cladosporium fulvum*

*Cladosporium fulvum* fungus is a causal agent of tomato leaf mould. For the first time, cladofulvin was isolated from the in vitro culture of *C. fulvum* to yield a bianthraquinone homodimer composed of two nataloe-emodin moieties linked by an aryl-aryl bond [112]. Strikingly, synthesised cladofulvin by *C. fulvum* did not induce any necrosis on Solanaceae plants. Besides, cladofulvin did not demonstrate antimicrobial activity against *P. fluorescens*, *S. coelicolor*, *B. cinerea* and *Hansfordia pulvinata* [112]. No biological activities or functions of cladofulvin have so far been determined. However, Griffiths et al, demonstrated that the production of cladofulvin play a vital role for the survival of *Ascochyta lentis* caused complete growth inhibition of *B. cinerea* inside tomato plant [113].

*Ascoscytha lentis*

*Lentisone* (74) was isolated for the first time from *Ascoscytha lentis*, a pathogen of Lentil, as well as the known AQ i.e., pachybasin [114]. Lentisone is highly toxic for *A. lentis* host plant, however, its toxicity extremely depends on light [114]. In a further study, three novel AQs, named lentiquinones A (75), B and C (76) along with the known lentisone, pachybasin, ω-hydroxy-pachybasin, 1,7-dihydroxy-3-methylnanthracene-9,10-dione, and phomarin were isolated from *A. lentis*. Among all isolated AQs, lentiquinones A, B, and C and lentisone caused complete growth inhibition of *B. subtilis*. Only lentiquinone A exhibited antifungal activity against *Verticillium dahlia*, *Penicillium alii*, *Rhizoctonia sp.* and *Phoma exigua* [115].

*Ramularia sp.*

The phytopathogenic fungus, *Ramularia collo-cygni*, is the causal agent of barley leaf-spot disease [116]. Rubellins A-E (77–81; Fig. 5) were the AQs derivatives isolated from *R. collo-cygni*, and to cause necrosis on barley leaves in concentration- and light-dependent manners [117–119]. Additionally, chrysophanol, caeruleorulinarin (82) and uredinorubellins I and II (83, 84) were isolated from liquid culture of *R. uredinicola*. Like rubellins, uredinorubellins also exhibited photodynamic activity, in contrast to chrysophanol and caeruleorulinarin [121]. Today, identification of natural metabolites with photodynamic efficiency has gained interest due to their potential in cancer treatment. They are nontoxic and photosensitizer compounds accumulate in tissues or cells. Upon irradiation with light of certain wavelength, these compounds exhibit cytotoxic activity by providing reactive molecular species [122].

*Phoma sp.*

The genus *Phoma* is known to produce a number of phytoxins belonging to several classes of natural metabolites including AQs [123]. Emodin and crysophanol, pachybasin and phomarin were produced by *Phoma foevata*, the causal agent of gangrene in potatoes [124]. Furthermore, *P. herbarum* obtained from the weed *Parthenium* produced anhydroseudphelegmacin-9,10-quinoine-3′-amino-8′-O-methyl ether that exhibited a herbicidal activity against several weeds of central India i.e., *Hypitís suaveolens*, *Lantana camara*, *Parthenium hysterophorus*, and *Sida acuta* [125].

Human pathogenic fungi- producing AQs

Questin (85) was detected as a major metabolites of *A. fumigatus* spores. Currently, *A. fumigatus* has been considered as the most common airborne fungal pathogen, that can severely infect immunosuppressed individuals causing aspergillosis disease [126]. This result opens new promising avenues for research of AQs in human fungal pathogens.

Insect-pathogenic fungi produc AQs

Entomopathogenic fungi specifically attack insects [127]. The infection cycle of entomopathogenic fungi starts with the attachment of infective spores, onto the outer surface of the insect integument. After germination, the hyphae directly penetrate the cuticle of the host, and rapidly proliferate as yeast-like cells in the hemolymph, eventually killing the host [127]. Two known AQ dimers, (+) rugulosin and skyrin were identified in the extract of the fungus *Aschersonia samoensis*, being the first report on the presence of AQ dimers in insect-pathogenic fungi [128]. The purified (+) rugulosin and skyrin exhibited selective cytotoxic effect against the insect cell line *Sf 9* (ovarian cells of the fall armyworm, *Spodoptera frugiperda*) with respective ID50 values of 1.2 and 9.6 μg ml−1, but with no activity against *C6* or *36 s* (unspecified cells of mosquito larvae, *Aedes albopictus*) or mammalian test cell lines. Considering that the safety of biopesticides is dependent on their selective activity towards insects rather than mammals, *Aschersonia* sp. could be used as biological pesticides considering being not toxic to mammalian cells [128]. Besides, the preferential cytotoxic activity against insect species would be an additional safety feature. Nevertheless, such results are still preliminary and need to be confirmed using in vivo animal and insect models to ensure their efficacy and safety.

Additionally, *Aschersonia* species recognized as a rich source of anthaquinone glycosides. Versicolorin-β-6-(4-O-methyl)-β-D-glucopyranosyl, 1’-hydroxyversicolorin-β-6-(4-O-methyl)-β-D-glucopyranosyl, nidurufin-6-(4-O-methyl)-β-D-glucopyranosyl were detected in the extract of *Aschersonia coffeae* Henn. fungus isolated from a Homoptera scale insect, along with versicolorin B, averufin (86) and nidurufin (87) (Fig. 5) [129]. Furthermore, the scale insect fungi *A. marginata* produced nidurufin-2’-(4-O-methyl)-β-D-glucopyranosyl and nidurufin-6-(4-O-methyl)-β-D-glucopyranosyl as well as AQ aglycones i.e., 10-hydroxy versicolorin B, nidurufin, paeciloquinone A, averufin [130].

Nidurufin-6-(4-O-methyl)-β-D-glucopyranosyl and 1’-hydroxy versicolorin-β-6-(4-O-methyl)-β-D-glucopyranosyl exhibited moderate antimalarial activity (*IC50* 1.60 and 3.88 μg/mL respectively) against *P. falciparum* K1 and cytotoxicity activity against NCI-H187 cells (*IC50*: 5.12 μg/mL) [129,130]. While, nidurufin-2’-(4-O-methyl)-β-D-glucopyranosyl had no biological activity. These results suggest that the position of the sugar unit may be important for cytotoxic activity [130], and whether its aldehyde exhibits a stronger effect should be pursued. It is worth mentioning that AQ glycosides are less suitable for the textile dyeing process due to their water solubility compared to their aglycone [131].

The pathogenic fungi *Torrbuiella* sp. has attracted a great deal of attention because of their dimeric AQs with rare chemical skeleton named torrubiellins A and B. The two compounds along with three known AQs i.e., chrysophanol, aloe-emodin, and emodin were produced by *Torrbuiella* sp. isolated from a leafhopper (Hemiptera). The presence of AQ monomers suggests that torrubiellins A and B may be derived from two AQ molecules. Although that several
AQS dimers were identified, the linkage at C-4-C-5’ and C-11-C-10α in the new dimers torrubiliens, is entirely rare. It is worth mentioning that, uredinorubellins I and II isolated from the plant pathogenic fungi Ramularia uredinicola are closely related compounds to torrubiliens [132].

**Advances of fungal endophytic metabolomics**

Several scientific disciplines incorporating metabolomics as one of their “omics” platforms have led to a paradigm shift in drug discovery. Compared to classical analytical methods targeting a specific class of compounds, metabolomics is a potential technology that allows for the multi-targeted analysis of the great number of metabolites. Metabolomics provides a (semi) quantitation of the multi-parametric metabolic response of living systems that has also been reported in the field of fungal biology [133], especially fungal metabolism considering its complexity. A complete inventory of metabolomes can now be achieved by using several techniques including nuclear magnetic resonance (NMR), mass spectrometry (MS) coupled to chromatography [4].

This section is intended to demarcate the different applications of metabolomics for elucidation of fungal metabolism in context to anthraquinones biosynthesis.

Metabolomics approaches have been used as an effective tool for chemotaxonomic based profiling of fungal taxa in order to find out their distinctiveness from other species based on metabolites abundance. Qualitative tandem liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS/MS) profiling of Penicillium setosum, an endophyte isolated from of Withania somnifera was employed to identify their distinctiveness from other members of section Lanata-divaricata, revealed that its clustering along section Lanata-Divaricata members was attributed to the production of quinalizarin, while its discrepancy was dominated by its ability to produce mycotoxin, patulin [134].

Another study employed liquid chromatography coupled to mass spectrometry (LC-MS) to distinguish between the mutant Aspergillus flavus AF70 from its wild parent. Mutation affected the production of 4 major anthraquinones including asparasone A (358 Da), and related anthraquinones with masses of 316, 340 and 374 Da. These latter mass weights exhibited similar fragmentation pattern to that of asparasone A [135], highlighting the powerful tool of metabolomics in identifying novel compounds.

MS based metabolites profiling was reported to assess the effects of different culture conditions on fungal metabolism. For example, cultivation of Aspergillus nidulans under N-limiting conditions led to the specific induction of polyketide biosynthesis genes that were previously regarded as silent under normal conditions. Two novel complex polyketide metabolites have been identified in A. nidulans culture namely sanghaspirodins A and B. For the first time, the interaction of emodin with an orsellinic acid-derived oxanthrene has been demonstrated in polyketide biosynthetic pathways of spiroanethrones [136].

The metabolite profile of Aspergillus oryzae KCCM 12.698 under different culture conditions i.e., SSF (solid-state fermentation) and submerged fermentation (SmF) were annotated using the ultrahigh performance liquid chromatography-linear trap quadrupole-ion trap-mass spectrometry (UHPLC-LTQ-IT-MS/MS). The results showed distinct between the metabolite contents of the two primary cultivations i.e., SSF and SmF in the case of A. oryzae. Indeed, SSF extracts showed higher production rates of anthraquinonoid, whereas terpenoids were abundant in SmF [137]. Whether such pattern can be observed in other related fungi has yet to be determined and should be pursued.

Metabolomics has been increasingly applied for elucidating secondary biosynthetic pathways in planta as part of system biology and less evidenced in fungal systems.

The production mechanism of hypericin and emodin in the endophytic fungus T. subthermophila, isolated from H. perforatum was studied in submerged axenic culture. The other main proposed precursors of hypericin and emodin in planta, emodin anthrone and protohypericin, could not though be detected at any stage of T. subthermophila fermentation. Besides, identification of some intermediates based on high performance liquid chromatography with tandem mass detection (HPLC-MS/MS) suggests that endophytic biosynthetic pathway might be different and/or governed by a different molecular mechanism than the host plant or host cell suspension cultures [36].

Likewise, another metabolomic approach delineated a semi-natural biosynthetic pathway of carminic acid, in a heterologous production host cell, e.g, a recombinant Aspergillus production host cell. As known, the natural pigment carminic acid is one of the most widely used colorants of textiles, cosmetics, food and drugs, which can be produced in vivo from scale insect (Dactylopius coccus). Targeted liquid chromatography-high resolution mass spectrometry (LC-HRMS) analysis of A. nidulans extract detected carminic acid along with several polyketide precursors, flavokermesic acid anthrone, kermesic acid, flavokermesic acid involved in carminic acid biosynthesis [138].

Metabolomics as presented offer an innovative advanced route for both qualitative and quantitative investigation of all metabolites in fungal complex extracts or fractions. In addition, chromatographic techniques have proved its worth as a tool of bioassay-guided lead discovery from natural products for rapid identification of the bioactive compounds of interest. However, separation of the bioactive metabolites from complicated fungal culture remains still creates problems and may need expertise. Additionally, insufficient quantities may be obtained in most cases. Further, the identification of metabolites in the natural product is another impediment that needs to be undertaken. Hence, it is necessary to develop different sample preparation protocols and data processing tools to detect leads even in a small amount. In the future, there is a vast room to improve fungal metabolites production and identification of new bioactive compounds. In addition, the establishment of combinatorial fungal databases and the combining of metabolomics with genomics approaches are new challenges in fungal secondary metabolites discovery.

**Conclusion**

Several efforts have already been made in recent years towards identifying alternatives for plant supply of biologically active metabolites from other resources. Additionally, as a result of the pacing advent of multidrug-resistant pathogens and the emergence of new diseases, discovery of novel, safe and affordable molecules should now follow. Today, natural AQ derivatives represent exceptionally an effective class used widely in laxative, anti-inflammatory, antimicrobial and anticancer drugs clinically besides from their industrial use in the food and textile industries. Host-living fungi have proved to be a prolific source of biologically active compounds for many different chemical classes of AQS. The diversity and distribution of AQSs in host-living fungi living in terrestrial ecosystems are covered in this review. Additionally, the relevant information regarding the AQS biosynthesis pathway in fungi is provided.

The ability of some endophytes to produce valuable bioactive molecules of AQS, originally characteristic of the host plant was the most exciting discovery in this area of research. Chief examples include the identification of endophytic fungi producing hypericin, emodin, chrysophanol, aloë-emodin, physcion and rhein and harbouring H. perforatum, R. palmatum and Rumex sp. Clearly, the possible microbiological fermentation could provide stable, robust and
reproducible yields from batch to batch due to the well controllable cultivation parameters.

Identification of biologically active metabolites produced by pathogenic fungi is critical for revealing their double-face activities; detrimental and beneficial effects. Several valuable AQs with antimicrobial, insecticidal, cytotoxic, and antitumor activities have been successfully obtained from pathogenic fungi. In fact, the novel AQs with rare chemical skeleton e.g. torrubriellins A and B open future venues for investigation of their biological activities.

Despite that lichen fungi exhibit the most diversity and distribution among fungal communities, they are least studied among examined ecosystems. Lichens are still unique source of numerous bioactive classes of AQs e.g. halogenated AQs. Hence, exploring the metabolites of the lichen-associated fungi is considered a promising perspective in the future.

The photosensitizing potential of several natural AQ compounds; rubellins, uredinorubellins and hypericin may help reduce toxicity and side effects of synthetic anti-cancer drug and inspire the discovery for more photosensitive compounds from other natural sources. AQs exhibit a wide range of in vitro cytotoxicity depending largely on their chemical structures. Ortho-dihydroxy substitution, p-quinone moiety, and glycosylation in their structures in addition to the axial the axial chirality of AQs compounds appear to play intrinsic roles in their cytotoxic activity. Hence, SAR of anti-cancer AQs could improve drug development and guide chemosynthetic approaches in novel drug design. The recent developments in biotechnology and co-culture experiments could further aid in discovering new AQ compounds with higher specificity and more importantly, reduced toxicity besides the production of the known biologically active AQs. Nevertheless, there is still a need for studying the optimization tools to increase the final production yield and to transform these processes at an industrial scale. The use of elicitors well exploited for the production of plant bioactives is far less understood in case of fungal endophytes culture and should be pursued.

Withal, advances in omics i.e., proteomics, genomics, mass spectrometry and other analytical techniques may play an important role in the investigation of diverse biologically active metabolites in the fungi holobiome and to better understand its regulation for better exploitation under laboratory conditions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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