

Endophytic fungi associated with Philippine endemic Annonaceae species yield extracts with anti-ESKAPE, antitubercular, and antioxidant activities

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Abstract

Rising cases of antibacterial resistance and oxidative stress-related disorders prompted the search for new compounds. In this study, endophytic fungi isolated from Philippine endemic Annonaceae plants were investigated as potential sources of antibiotic and antioxidant mycochemicals. Twelve isolates were cultured in four media (YM 6.3, ZM ½, Q6 ½, BRFT), extracted with ethyl acetate and methanol, yielding 48 crude extracts. Antibacterial activity was assessed using REMA for ESKAPE pathogens, and MABA MIC90 for *Mycobacterium tuberculosis* H37Rv, while antioxidant potential was determined via the DPPH assay. Twenty-three extracts were found to be inhibitory against ESKAPE pathogens, two against *M. tuberculosis* H37Rv, and 18 demonstrated free-radical scavenging properties. Morphological characterization and ITS-based phylogenetic analysis with MEGA X identified 11 distinct endophytic fungi as the sources of these bioactive extracts. Notably, the BRFT extract from *Chromolaenicola* sp. exhibited strong anti-ESKAPE activity (MIC = 250–7.81 µg/mL), while BRFT extracts from *Diaporthe longicolla* and *Endomelanconiopsis* sp. inhibited *M. tuberculosis* H37Rv by 90% and 96% at 64 µg/mL, respectively. In addition, the ZM extract from *Chaetomium* sp. (IC₅₀ = 56.03 µg/mL) exhibited radical scavenging activity comparable to BHT (IC₅₀ = 24.57 µg/mL), and ascorbic acid (IC₅₀ = 1.18 µg/mL). Among the tested media, BRFT supported the highest metabolite production. Mycochemical screening revealed anthrones, terpenes, sterols, and indoles as major constituents. This study provides the first documentation of *Chromolaenicola* sp. and *D. longicolla* from *O. cumingiana* and *Endomelanconiopsis* sp. from *A. cagayanensis*, representing novel records of these genera in Annonaceae, and as promising sources of novel antibiotics and antioxidants.

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Introduction

A major problem in global health is the lack of affordable and effective drugs available to fight many diseases^[1], including antibacterial resistance (AMR), and oxidative stress-associated diseases. Despite the availability of inexpensive antibiotics, due to AMR, these antibiotics are no longer effective against serious infections^[2]. Globally, it was estimated that about 1.27 million deaths in 2019 were attributed to infection with AMR bacteria^[3]. In early 2024, the World Health Organization (WHO) released a list of bacterial pathogens to be prioritized in research and development, and public health management to combat AMR^[4]. This includes the multi-drug resistant ESKAPE pathogens: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterobacter* species. In addition, the bacterium *Mycobacterium tuberculosis*, a causative agent of tuberculosis disease, is also a significant clinical disease burden due to its ability to develop AMR. In 2022, tuberculosis caused 1.3 million deaths, making it the top infectious disease killer globally^[5]. Furthermore, oxidative stress is another major problem, as it is associated with several kinds of diseases, such as reduced immune response to infections, neurodegenerative disorders^[6], cardiovascular disease, chronic pulmonary disease, chronic kidney disease, and cancer^[7].

Natural products have long played a critical role in addressing antibacterial and antioxidant diseases. The discovery of penicillin

in 1928 exemplifies how fungi have yielded transformative medicines^[8]. Over the decades, several fungal secondary metabolites or mycochemicals derived from fungi have been reported with biological activities including anticancer, antibacterial, anti-inflammatory, and antiarthritic activities^[9]. Mycochemicals can include a wide range of secondary metabolites produced by fungi or mushrooms, including terpenoids, lipids, polysaccharides, and proteins^[10]. Recently, there has been a growing interest in studies of fungi residing in plant tissues, known as endophytic fungi, due to their genetic diversity, which makes them a rich reservoir for the discovery of novel biologically active secondary metabolites^[11].

Endophytic fungi are known to produce secondary metabolites that help their host plants defend against herbivores and parasites, stimulate nutrient metabolism, and adapt to the environment^[12]. This phenomenon is thought to result from plant-fungi symbiosis, leading to mechanisms such as horizontal gene transfer, co-evolution, shared biosynthetic gene clusters, and chemical crosstalk^[12,13]. Hence, in exploring sources for biologically active compounds, medicinal plants were often targeted for isolation of biologically active secondary metabolites from endophytic fungi^[14].

To further expand the sourcing of novel and biologically active compounds from endophytic fungi, the OSMAC approach has been employed in several studies^[15]. This technique is conceptualized and referred to as 'one strain, many compounds' (OSMAC), based on the principle that a single microbial strain has the potential

to produce multiple compounds depending on specific growth conditions^[15]. This approach utilizes the defense mechanisms of endophytic fungi under competitive environments. Under stress, endophytic fungi release secondary metabolites which vary according to the necessity of their survival^[15,16]. Hence, modifications in the composition of the growth medium, pH levels, temperature, the inclusion of enzyme inhibitors, oxygen availability, or the type of culture vessel can result in the production of different sets of secondary metabolites^[16].

Recent efforts have underscored the antibacterial potential and diversity of secondary metabolites derived from the endophytic fungi of Annonaceae species^[17]. The Annonaceae, or the custard family, predominantly thrives in tropical and subtropical regions, encompasses around 135 genera and 2,500 species^[18]. Their medicinal properties include antimicrobial activities such as antibacterial, antimalarial, leishmanicidal, antiviral, trypanocidal, and antiprotozoal, as well as other uses such as antitumor, anticholinesterase, antioxidant, among others^[19]. These medicinal properties are attributed to phytochemicals produced by the plants, including flavonoids, alkaloids, acetogenins, and essential oil constituents^[19].

The Philippines has been recognized as having a very rich diversity that hosts about 13,500 plant species, of which 1,500 have been reported to have medicinal usage^[20]. Alkaloids, highly oxygenated cyclohexenoids, phenylpropanoids, and flavonoids isolated from several genera of Philippine Annonaceae, such as *Uvaria*^[21], *Goniothalamus*^[22], *Phaeanthus*^[23], and *Drepananthus*^[24] have shown potent antibacterial, antitubercular, cytotoxic, antidiabetic, and antiobesity activities. Despite this rich biodiversity, efforts to explore the biological activity of endophytic fungi extracts remain at an early stage. While there have been reports of the biological activities of endophytic fungi derived from Annonaceae species, none have focused so far on the endophytic fungi of endemic Annonaceae. By investigating these hosts, the present study highlights an untapped reservoir of microbial diversity with potential novel biologically active compounds. Among the Annonaceae species that were selected many have been recognized for their biologically active plant extracts and traditional medicinal uses. For instance, plant extracts from *Dasymaschalon clusiflorum* var. *megalanthum* Merr. have demonstrated antimicrobial properties^[25], while *Platymitra arborea* (Blanco) Kessler has shown anticancer potential^[26]. Meanwhile, as of the current publication date, no reports exist regarding the bioactivity or traditional use of *Orophea cumingiana* S. Vidal and *Artabotrys cagayanensis* Merr.

To combat the plight against AMR of ESKAPE pathogens and oxidative stress associated diseases, this study explored the antibiotic and antioxidant potential of secondary metabolites derived from the endophytic fungi of four Philippine endemic Annonaceae plants: *O. cumingiana*, *A. cagayanensis*, *P. arborea*, and *D. clusiflorum* var. *megalanthum*. This study represents the first report documenting both the identity and biological activity of endophytic fungi from Philippine endemic Annonaceae, underscoring their value in natural product-based drug discovery, and their potential contribution to addressing antimicrobial resistance and oxidative stress-related diseases.

Materials and methods

Reagents

The reagents used were all analytical grade, including culture media, bacterial strains, and antibiotics. Potato dextrose agar (PDA),

agar-agar powder, Mueller Hinton Broth (MHB), and malt extract powder were purchased from TMMedia (TMMedia, Rajasthan, India). Ampicillin, streptomycin, ciprofloxacin, amikacin, resazurin, D-mannitol, D-glucose, cottonseed flour, lactalbumin, and sodium tartrate were procured from Sigma-Aldrich (Sigma-Aldrich, Saint Louis, MO, USA). Yeast extract powder was purchased from Himedia (Himedia, Mumbai, India). Sucrose (saccharose) and potassium phosphate dibasic were obtained from Techno Pharmachem (Techno Pharmachem, Bahadurgarh, India). Glycerol, calcium carbonate, and sodium sulfate were acquired from Loba Chemie Pvt. Ltd. (Mumbai, India). Ammonium sulfate was obtained from Mallinckrodt (Mallinckrodt, Staines-upon-Thames, United Kingdom). Molasses, brown rice, and oatmeal were acquired from a local supermarket in the Philippines. The organic solvents used for extracting secondary metabolites, ethyl acetate (EtOAc), and methanol (MeOH), were also of analytical grade and procured from RCI Labscan (Bangkok, Thailand).

Isolation and fermentation of endophytic fungi

Orophea cumingiana S. Vidal, *Artabotrys cagayanensis* Merr., and *Platymitra arborea* (Blanco) Kessler were collected at the Northwestern University Ecotourism Park and Botanic Garden (18°9'4.28" N, 120°34'12.00" E), while *Dasymaschalon clusiflorum* var. *megalanthum* was collected from Kalbario Patapat Natural Park (18°30'19.51" N, 120°54'27.50" E) in September 2023. Voucher specimens were deposited in the Herbarium of Northwestern Luzon, Philippines (HNUL) for accession numbers. Leaves were sequentially immersed in 70% ethanol (1 min), sterile distilled water, and a final rinse of sterile distilled water. Stems underwent immersion in 70% ethanol (1 min), 3% sodium hypochlorite (NaClO) (10 min), and then 70% ethanol (30 s). Leaves were then cut into five small segments (5 mm × 5 mm), while stems were cut into four to five small portions (5 mm × 5 mm). These segments were individually transferred onto PDA plates (supplemented with 100 mg/L streptomycin sulfate and 100 mg/L ampicillin), and incubated at 25–30 °C for three to four weeks. This procedure was carried out in triplicate for each plant sample. Fungal isolates grown from the plate were purified by inoculating a small piece of fungal colony from the margin onto a new PDA culture medium^[27]. The endophytic fungal isolates used in this study were deposited at the UST Collection of Microbial Strains (UST-CMS) for accession numbers.

Selected endophytic fungi were fermented in three different liquid fermentation media such as YM 6.3 (malt extract 10 g/L, D-glucose 4 g/L, yeast extract 4 g/L), ZM ½ (molasses 5 g/L, oatmeal 5 g/L, saccharose 4 g/L, mannitol 4 g/L, D-glucose 1.5 g/L, calcium carbonate 1.5 g/L, lactalbumin 0.5 g/L, ammonium sulfate 0.5 g/L)^[20], Q6 ½ (D-glucose 2.5 g/L, glycerol 10 g/L, cotton seed flour 5 g/L)^[21], and one solid fermentation medium such as brown rice containing BRFT (yeast extract 1 g/L, sodium tartrate 0.5 g/L, K₂HPO₄ 0.5 g/L)^[22]. For the liquid medium, 200 mL of the solution was poured into a 500-mL culture flask, while for the solid medium, 56 g of brown rice and 200 mL of BRFT solution were poured into 500-mL culture flasks. The fermenting cultures were monitored daily for growth and glucose content with the use of glucose strips (Medi-Test, Macherey-Nagel, Düren, Germany).

Extraction of secondary metabolites

For cultures subjected to liquid-state fermentation, extraction was performed 72 h after the culture medium tested negative for glucose, indicating the end of the fermentation^[28]. The culture medium was filtered through cheesecloth to separate the mycelium

from the broth. The mycelium was discarded, and the culture filtrate was transferred into a separatory funnel. Exhaustive liquid-liquid extraction was then carried out by multiple extractions with ethyl acetate (EtOAc)^[29]. The obtained EtOAc extract was filtered with anhydrous sodium sulfate (Na₂SO₄) to ensure the complete removal of any remaining water^[29,30]. The EtOAc extract was then dried under reduced pressure using a rotary evaporator. Defatting of the extracts was performed using liquid-liquid extraction between 90% aqueous methanol and n-heptane^[31]. The methanol layer was collected, filtered, transferred into a vial, and dried until further use.

For brown rice cultures, the extraction process began by submerging the culture medium in EtOAc overnight, followed by filtration to separate the mycelia from the filtrate. This step was repeated three times. The filtered EtOAc extract was then dried under reduced pressure, and redissolved in 90% MeOH followed by extraction with n-heptane. The resulting aqueous methanol extract was dried using a reduce rotary evaporator^[28]. The same procedure was carried out for the fat removal, filtration, and drying of the methanolic layer.

The normality of extract yield across media was assessed using the Shapiro–Wilk test. Further statistical analysis was carried out using the Kruskal–Wallis test to assess differences in secondary metabolite production across culture media, followed by a multiple comparisons test via two-stage linear step-up procedure of Benjamini, Krieger, and Yekutieli (BKY) to identify specific group differences.

Antibacterial screening against ESKAPE pathogens

The antibacterial activity of selected methanol crude fungal extracts against ESKAPE bacteria was determined using resazurin microtiter assay (REMA), as described by de Jesús Dzul-Beh et al.^[32], with slight modifications. The antibacterial screening was performed in two stages: a primary screening to examine the antibacterial activity of the crude fungal extracts at 1,000 µg/mL, followed by the determination of the minimum inhibitory concentration (MIC) of the positive extracts using the same assay. Extracts with a yield of less than 7 mg were not considered for antibacterial investigation.

Test microorganisms such as *S. aureus* (USTCMS1182), *P. aeruginosa* (USTCMS10013), and *K. pneumoniae* (USTCMS1042) were acquired from UST-CMS. Other bacterial strains, including *A. baumannii* (ATCC 19606), antibiotic-resistant *E. faecium* (ATCC 27270), and *E. hormaechei* (ATCC 700323), were procured from Fil-Anaserve, Inc. An inoculum of the test microorganism from the glycerol stock was transferred into sterile MHB, and incubated at 37 °C. After 18–24 h, the optical density of the MHB culture was measured at 625 nm (OD₆₂₅) using a UV-Vis spectrophotometer. The MHB culture was then diluted with an appropriate volume of MHB to obtain an absorbance between 0.08 and 0.1, matching the 0.5 McFarland Standard (1.5 × 10⁸ CFU/mL).

The crude fungal extracts were dissolved in pure dimethyl sulfoxide (DMSO) and then diluted with sterile water to reach a working concentration of 6,000 µg/mL in 24% DMSO. For the positive control, 700 µg/mL of amikacin (for *E. faecium*), and ciprofloxacin (for other ESKAPE bacteria) were prepared in pure DMSO and then diluted with sterile water to reach a working concentration of 168 µg/mL in 24% DMSO. Meanwhile, 24% DMSO in sterile water was prepared as negative control, with a final well concentration of 4% as described in the assay method.

For the preliminary screening, 100 µL of the test culture was dispensed into separate wells of a clear 96-well microtiter plate

containing 20 µL of the test samples and controls. The final well concentration of the test samples was 1,000 µg/mL in 4% DMSO. A sterility control containing 120 µL of sterile MHB was also included. All samples and controls were tested in triplicate. Afterward, the plates were sealed and incubated at 37 °C. After 18 h of incubation, 20 µL of 0.02% resazurin solution was added to each well, and incubated at 37°C for another 2–4 h until the negative control showed a color change from blue to pink. In this assay, pink indicated bacterial growth, whereas blue indicated inhibition of bacterial growth. Samples exhibiting blue to dark violet color were considered to have positive antibacterial activity, and were further assayed to determine their MIC.

For the determination of the MIC of active extracts, a two-fold serial dilution of the test samples was initially performed, resulting in a concentration range from 1,000 to 7.8125 µg/mL. Then, the same assay procedure as the preliminary screening was followed for the incubation of samples and controls with the test culture. The lowest concentration of the extract that exhibited a blue color was recorded as the MIC of the sample.

DPPH assay for radical scavenging activity

The active extracts from the anti-ESKAPE screening were further subjected to antioxidant evaluation. Their free-radical scavenging activity was assessed using the DPPH assay, following the method of Bungihan & Matias^[33], with slight modifications. Stock solutions of the crude extracts and positive controls (BHT and ascorbic acid) were prepared in MeOH (3 mg/mL). A 0.1 mM DPPH solution was prepared and further diluted (1:10) with MeOH. In a 96-well microplate, 50 µL of each sample stock solution was mixed with 100 µL of the adjusted DPPH solution, resulting in a final concentration of 100 µg/mL per well. The plate was incubated at room temperature for 30 min, after which absorbance was measured at 490 nm using a microplate reader (Promega GloMax® Discover System). The radical scavenging activity (%RSA) was calculated using Eq. (1). Extracts with > 50% RSA at 1,000 µg/mL were further evaluated for dose-response activity using a two-fold serial dilution across eight concentrations (1,000–7.81 µg/mL) to determine IC₅₀ values. For ascorbic acid, a 12-point two-fold serial dilution at a lower concentration range (333–0.16 µg/mL) was prepared for IC₅₀ determination. IC₅₀ values for both extracts and controls were calculated using Graph-Pad Prism 9.

$$\% \text{ Inhibition} = \left[\frac{A_{\text{Control}} - A_{\text{sample}}}{A_{\text{Control}}} \right] \times 100 \quad (1)$$

Microplate Alamar Blue assay (MABA) against *Mycobacterium tuberculosis*

The active extracts from anti-ESKAPE screening were sent to the University of Illinois Chicago (Chicago, USA) for antitubercular activity screening using MABA. Stock solutions of the extracts were prepared in DMSO (2 mg/mL), and tested at a single concentration of 64 µg/mL. Test solutions were prepared in Middlebrook 7H12 medium (7H9 broth supplemented with 0.1% w/v casitone, 5.6 µg/mL palmitic acid, 5 mg/mL bovine serum albumin, and 4 mg/mL catalase, filter-sterilized) in a final volume of 100 µL per well in black 96-well microplates. A 100 µL inoculum of *M. tuberculosis* H₃₇R_v (2 × 10⁵ CFU/mL) was then added to each well, yielding a final volume of 200 µL. Plates were incubated at 37 °C for 7 d, after which 12.5 µL of 20% Tween 80 and 20 µL of Alamar Blue (Trek Diagnostic, Westlake, OH, USA) were added. Following 16–24 h of further incubation, fluorescence was measured (excitation 530 nm,

emission 590 nm). The percentage inhibition of bacterial growth was calculated relative to untreated bacteria-only controls using Eq. (2).

$$\% \text{ Inhibition} = \left[\frac{F_{\text{Control}} - F_{\text{sample}}}{F_{\text{Control}}} \right] \times 100 \quad (2)$$

Identification of the antibacterial-producing endophytic fungi

From the stock culture, a block of culture was transferred to the sterile PDA plates, and incubated for 7–10 d at 28 °C to observe the culture characteristics of the endophytic fungi. The culture characteristics such as color, surface structure, pattern, margin, growth, and others were observed and analyzed in the PDA plates^[24]. The microscopic morphology was carried out by using the transparent adhesive tape mount or tape-lift mount techniques^[25].

For the phylogenetic analysis, the endophytic fungi were sent to MacroGen Inc. (MacroGen Inc., Seoul, South Korea) for gDNA extraction, PCR amplification, and bidirectional sequencing of internal transcribed spacer (ITS), forward ITS5 (5'-GGAAGTAAAAGTCGTAACA AGG-3'), and reverse ITS4 (5'-TCCTCCGCTTATTGATATGC-3') region^[34]. The chromatogram (AB1 file) of the sequences was analyzed using Molecular Evolutionary Genetics Analysis across Computing Platform (MEGA X) software. The individual forward and reverse sequences were aligned using MUSCLE algorithm, and the contigs generated from the alignment were saved as a FASTA file. For preliminary identification, the BLASTN tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used, and sequences with ≥ 99% identity, and 99% query coverage were assigned to the genus level and was the basis for the retrieval of the reference sequencing from the National Center for Biotechnology information website (www.ncbi.nlm.nih.gov)^[35]. To construct the phylogenetic tree, the contigs of each endophytic fungus and its corresponding reference sequences were then aligned using MEGA X software. To search for the best-fit model for the phylogenetic analysis, the model with the lowest Bayesian Information Criterion (BIC) was used. Maximum likelihood trees were constructed with 1,000 bootstrap replicates^[26,27].

Chemical profiling of the active crude fungal extracts

Thin-layer chromatography (TLC) was employed to describe the chemical profile of the active crude fungal extracts. The presence of alkaloids, indoles, terpenes, and sterols was investigated using spray reagents of Dragendorff, Van Urk-Salowski, and Vanillin-sulfuric acid, respectively. Lastly, Bornträger reagent was used to detect the presence of anthraquinones, anthrones, and coumarins.

Results

Endophytic fungal isolates from Philippine endemic Annonaceae species

Forty-two endophytic fungi were isolated from the leaves and stems of four Annonaceae species in this study. From this, 12 isolates presented with distinct morphology and were investigated for their antibacterial activities (Table 1).

Effects of media on fungal growth and secondary metabolite production

The mass of secondary metabolites produced by the 12 fungal isolates varied significantly depending on the growth medium

Table 1. Endophytic fungi isolated from the leaves and stems of Philippine endemic Annonaceae species.

Plant code	Host plant	Plant part	Total number of isolates	Endophytic fungus code of selected isolates
001	<i>O. cumingiana</i>	Leaves	7	NA-001-Lv-C NA-001-Lv-D NA-001-Lv-E NA-001-Lv-F NA-001-Lv-G
		Stem	7	NA-001-St-B NA-001-St-D NA-001-St-H
002	<i>A. cagayanensis</i>	Leaves	6	NA-002-Lv-X
		Stem	6	NA-002-St-C
003	<i>P. arborea</i>	Leaves	3	–
		Stem	4	NA-003-St-C
017	<i>D. clusiflorum</i> var. <i>megalanthum</i>	Leaves	7	KA-017-Lv-A
		Stem	2	–
		Total	42	12

– indicates none.

(Fig. 1a). Among the four tested media, brown rice (BR) supported the highest yields, ranging from ~40 to 700 mg. In contrast, cultures grown in YM, ZM, and Q6 media produced markedly lower amounts, generally below 50 mg. Statistical analysis confirmed highly significant differences between BR and all other media (**** $p < 0.0001$). Moreover, comparison among the liquid media (Fig. 1b) revealed significant differences, with Q6 supporting higher metabolite production than ZM and YM (** $p < 0.01$).

Antibacterial activity of fungal extracts against ESKAPE

Of the 48 fungal extracts, six extracts were excluded from the antibacterial screening due to their low yield (less than 7 mg), or absence of mycelium (Table 2). Results from the antibacterial screening revealed 23 extracts with antibacterial activity in at least one ESKAPE pathogen. Notably, BR-001-St-D exhibited with the most promising antibacterial activity and presents high extract yield. This

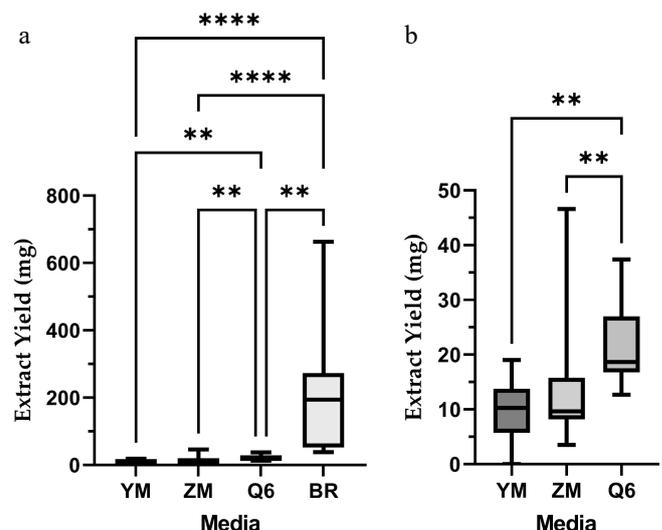


Fig. 1 (a) Mass of secondary metabolites produced by 12 fungal isolates cultivated in different media: YM = yeast malt medium; ZM = ZM ½ media; Q6 = Q6 ½ media; BR = brown rice media. (b) Enlarged view of panel (a) showing YM, ZM, and Q6. Statistical significance is denoted as follows: ** $p < 0.01$, **** $p < 0.0001$.

Table 2. Extract yield and minimum inhibitory concentrations (MIC, µg/mL) of selected endophytic fungal isolates of Annonaceae species against ESKAPE pathogens.

Isolate code	Extract code	Extract yield (mg)	MIC (µg/mL)					
			<i>Ef</i>	<i>Sa</i>	<i>Kp</i>	<i>Ab</i>	<i>Pa</i>	<i>Eh</i>
NA-001-Lv-C	YM-001-Lv-C-BE	7.3	–	–	–	–	–	–
	ZM-001-Lv-C-BE	3.5	u	u	u	u	u	u
	Q6-001-Lv-C-BE	37.4	–	–	–	–	1,000	62.5
	BR-001-Lv-C	54.3	–	62.5	–	–	–	–
NA-001-Lv-D	YM-001-Lv-D-BE	5	u	u	u	u	u	u
	ZM-001-Lv-D-BE	9.8	–	–	–	1,000	500	–
	Q6-001-Lv-D-BE	16.7	–	–	–	–	–	–
	BR-001-Lv-D	38.2	–	–	–	–	–	–
NA-001-Lv-E	YM-001-Lv-E-BE	No growth	u	u	u	u	u	u
	ZM-001-Lv-E-BE	46.6	–	–	7.81	1,000	–	–
	Q6-001-Lv-E-BE	16.9	–	–	–	–	–	–
	BR-001-Lv-E	195.7	–	–	–	–	–	–
NA-001-Lv-F	YM-001-Lv-F-BE	13.1	–	–	–	–	–	250
	ZM-001-Lv-F-BE	10.3	–	–	–	–	–	–
	Q6-001-Lv-F-BE	18.4	–	–	–	–	–	–
	BR-001-Lv-F	193.3	–	62.5	–	–	–	–
NA-001-Lv-G	YM-001-Lv-G-BE	5.2	u	u	u	u	u	u
	ZM-001-Lv-G-BE	9.1	–	–	–	–	250	–
	Q6-001-Lv-G-BE	17.1	–	–	–	–	500	–
	BR-001-Lv-G	41.9	–	–	–	–	–	–
NA-001-St-B	YM-001-St-B-BE	12.1	–	–	–	–	500	–
	ZM-001-St-B-BE	9.5	–	–	–	500	1,000	–
	Q6-001-St-B-BE	14.7	–	–	–	1,000	125	–
	BR-001-St-B	296.2	–	–	–	–	250	–
NA-001-St-D	YM-001-St-D-BE	14	–	1,000	–	250	1,000	–
	ZM-001-St-D-BE	17.2	–	1,000	–	7.81	62.5	–
	Q6-001-St-D-BE	18.9	–	–	–	500	62.5	–
	BR-001-St-D	202.3	–	125	15.63	250	7.81	250
NA-001-St-H	YM-001-St-H-BE	10.5	–	–	15.63	500	1,000	–
	ZM-001-St-H-BE	8.5	1,000	1,000	7.81	500	–	–
	Q6-001-St-H-BE	23.6	–	–	–	–	–	–
	BR-001-St-H	51.4	–	–	–	–	–	–
NA-002-Lv-X	YM-002-Lv-X-BE	18.3	1,000	125	–	–	1,000	–
	ZM-002-Lv-X-BE	7.9	–	–	–	–	–	–
	Q6-002-Lv-X-BE	12.7	–	–	–	–	–	–
	BR-002-Lv-X	122.5	–	–	–	–	–	–
NA-002-St-C	YM-002-St-C-BE	7.3	–	–	–	250	–	500
	ZM-002-St-C-BE	8.1	–	–	–	–	–	–
	Q6-002-St-C-BE	28.1	–	–	–	–	–	–
	BR-002-St-C	195.9	–	–	–	–	–	–
NA-003-St-C	YM-003-St-C-BE	28.2	500	62.5	–	125	1,000	1,000
	ZM-003-St-C-BE	13.7	–	–	–	500	500	–
	Q6-003-St-C-BE	14.5	–	62.5	–	250	1,000	–
	BR-003-St-C	196.4	–	–	–	–	–	–
KA-017-Lv-A	YM-017-Lv-A-BE	19.1	–	–	–	–	–	–
	ZM-017-Lv-A-BE	12	–	–	–	–	–	–
	Q6-017-Lv-A-BE	21.1	–	–	–	–	–	–
	BR-017-Lv-A	663.1	–	–	–	–	–	–

Culture media: YM = yeast malt medium; ZM = ZM ½ media; Q6 = Q6 ½ media; BR = brown rice media. ESKAPE pathogens: *Ef* = *E. faecium*; *Sa* = *S. aureus*; *Kp* = *K. pneumoniae*; *Ab* = *A. baumannii*; *Pa* = *P. aeruginosa*; *Eh* = *E. hormaechei*; – = no antibacterial activity; u = untested; Amikacin (28 µg/mL) was used as positive control for *E. faecium* and ciprofloxacin (28 µg/mL) was used as positive control for other ESKAPE pathogens. The extract with the most promising antibacterial activity is in bold.

extract exhibited antibacterial activity against five ESKAPE pathogens, with MIC values ranging from 7.81 to 250 µg/mL.

Antioxidant activity of fungal extracts

Extracts that showed antibacterial activity against ESKAPE were further evaluated for their antioxidant activity. From the 23 anti-ESKAPE active extracts, 18 extracts exhibited antioxidant properties as well, with radical scavenging activity ranging from 50% to 99%, as presented in Table 3. ZM-001-Lv-E was the most potent antioxidant

with an IC₅₀ of 56.03 µg/mL, and with %RSA comparable to antioxidant drugs—ascorbic acid and BHT. A dose-response curve was not performed for extracts with less than 50% RSA at 1,000 µg/mL. For extracts with undetermined IC₅₀, a different concentration is recommended to identify their IC₅₀ values.

Antitubercular activity of crude fungal extracts

In determining the antitubercular activity of the crude fungal extracts, those with antibacterial activity against ESKAPE were

Table 3. IC₅₀ values (µg/mL), and radical scavenging activity of anti-ESKAPE active extracts (at 1,000 µg/mL), AA, and BHT in DPPH assay.

Extract code	IC ₅₀ (µg/mL)	%RSA ± SD
Q6-001-Lv-C-BE	–	13.52 ± 2.28
BR-001-Lv-C	–	31.26 ± 5.99
ZM-001-Lv-D-BE	102.7	72.31 ± 0.94
ZM-001-Lv-E-BE	56.03	77.06 ± 0.27
YM-001-Lv-F-BE	–	48.77 ± 2.23
BR-001-Lv-F	–	28.36 ± 2.92
ZM-001-Lv-G-BE	65.85	68.74 ± 1.74
Q6-001-Lv-G-BE	87.47	58.67 ± 2.86
YM-001-St-B-BE	ND	54.34 ± 0.58
ZM-001-St-B-BE	ND	56.65 ± 0.62
Q6-001-St-B-BE	200.9	58.02 ± 4.49
BR-001-St-B	ND	52.04 ± 0.82
YM-001-St-D-BE	ND	62 ± 1.67
ZM-001-St-D-BE	133.6	61.46 ± 0.52
Q6-001-St-D-BE	205.2	74.28 ± 1.34
BR-001-St-D	443.6	82.31 ± 1.28
YM-001-St-H-BE	435.7	88.99 ± 2.12
ZM-001-St-H-BE	ND	53.16 ± 0.89
YM-002-Lv-X-BE	213.5	98.85 ± 0.23
YM-002-St-C-BE	121	59.85 ± 0.94
YM-003-St-C-BE	ND	91.73 ± 1.11
ZM-003-St-C-BE	–	11.33 ± 3.31
Q6-003-St-C-BE	63.29	61.32 ± 4.39
AA	1.18	76.79 ± 1.04
BHT	24.57	78.59 ± 0.82

– = no antioxidant activity; ND = not determined; BHT= Butylated hydroxytoluene; AA= Ascorbic acid The extract with the most promising antioxidant activity is in bold.

screened against *M. tb.* H₃₇R_v. Promising extracts, which showed at least 90% of growth inhibition of the viral bacteria, were BR-001-Lv-F and YM-002-Lv-X-BE (Table 4). However, some extracts were not screened for antitubercular activity due to the limited amount of samples.

Identification of endophytic fungi with antibiotic potential

The 11 active endophytic fungal isolates that produced antibacterial extracts against ESKAPE were identified using their morphology (Fig. 2), and phylogenetic tree analysis through MEGA X software. The active endophytic fungi from *O. cumingiana* effective against ESKAPE pathogens consist of five genera, including *Diaporthe* (NA-001-Lv-C, NA-001-Lv-F), *Fusarium* (NA-001-Lv-D, NA-001-Lv-G, NA-001-St-B), *Chaetomium* (NA-001-Lv-E), *Chromolaenicola* (NA-001-St-D), and *Corynespora* (NA-001-St-H). The endophytic fungal isolate NA-001-Lv-C was identified as *D. tectonendophytica*, showing 99% ML support in the phylogenetic tree. *D. tectonendophytica* is characterized by white colonies on both the front and reverse sides, with aerial hyphae, a radiate pattern, and an irregular margin. Moreover, the endophytic fungal isolate NA-001-Lv-F was identified as *D. longicolla* with 98% ML support based on its phylogenetic tree results. It was described as having white colonies with furrowed, zonate, irregular, spreading growth, aerial hyphae, and no reproductive cells. Endophytic fungal isolates NA-001-Lv-D and NA-001-Lv-G were identified as *Fusarium* species with ML support of 87% and 89%, respectively. Both *Fusarium* isolates were described as having white colonies on the front and reverse sides, a circular pattern, and a smooth margin. However, NA-001-Lv-D had a larger colony after 7 d, and exhibited chlamydospores and ellipsoidal conidia, while NA-001-Lv-G had a smaller colony and wedge-shaped macroconidia. Both *Fusarium* species had aerial mycelia. NA-001-St-B was

Table 4. Percent inhibition activity of anti-ESKAPE fungal extracts (64 µg/mL) against *M. tb.* H₃₇R_v in MABA.

Extract code	% Inhibition
Q6-001-Lv-C-BE	0
BR-001-Lv-C	0
ZM-001-Lv-D-BE	16%
ZM-001-Lv-E-BE	24%
YM-001-Lv-F-BE	34%
BR-001-Lv-F	90%
ZM-001-Lv-G-BE	NT
Q6-001-Lv-G-BE	32%
YM-001-St-B-BE	22%
ZM-001-St-B-BE	17%
Q6-001-St-B-BE	NT
BR-001-St-B	16%
YM-001-St-D-BE	20%
ZM-001-St-D-BE	21%
Q6-001-St-D-BE	2%
BR-001-St-D	0
YM-001-St-H-BE	4%
ZM-001-St-H-BE	NT
YM-002-Lv-X-BE	96%
YM-002-St-C-BE	NT
YM-003-St-C-BE	21%
ZM-003-St-C-BE	11%
Q6-003-St-C-BE	19%

NT= not tested; Most promising extracts are highlighted in bold.

identified as *F. decemcellulare* with 100% ML, and is described as having red colonies at the center, white colonies at the edges, aerial hyphae, a circular pattern, a smooth margin, and the presence of microconidia.

Endophytic fungal isolate NA-001-Lv-E, with 66% ML, was identified as *Chaetomium* sp., characterized by white to brown colonies on the front and yellow to orange colonies on the reverse, aerial hyphae, and limoniform ascospores as reproductive cells. NA-001-St-D was identified as *Chromolaenicola* sp. with 98% ML support. It was described as having white to pale brown colonies on both the front and reverse sides, growing slowly with an irregular pattern, and containing germinated conidium from the hyphae. NA-001-St-H was identified as *C. cassicola* with 98% ML support in the phylogenetic tree. It is characterized by yellowish-gray to white colonies on both the front and reverse sides, featuring a cottony texture and smooth margins, with no reproductive cells present.

The two endophytic fungal isolates from *A. cagayanensis* were identified as *Endomelanconiopsis* sp. (NA-002-Lv-X) and *Lasiodiplodia* sp. (NA-002-St-C). *Endomelanconiopsis* sp. has only 18% ML support, and is described as having white, dark green to black colonies on both the front and reverse sides, with aerial hyphae, a furrowed, zonate pattern, and an irregular margin. *Lasiodiplodia* sp. has 65% ML support and is described as having white to gray colonies on both the front and reverse sides, aerial hyphae, a thick cottony surface, a radiate pattern, and a smooth margin. Both endophytic fungal isolates have sterile mycelia, with no reproductive cells present. The endophytic fungal isolate from *P. arborea* (NA-003-St-C) was identified as *D. eschscholtzii* with 98% ML support and is described as having white to gray colonies, a raised surface structure, a radiate pattern, an irregular margin, spreading growth, and the presence of ellipsoidal conidia.

Chemical profile of the active extracts

Mycological screening via TLC was employed to describe the chemical profiles of the active extracts. Extracts with the same

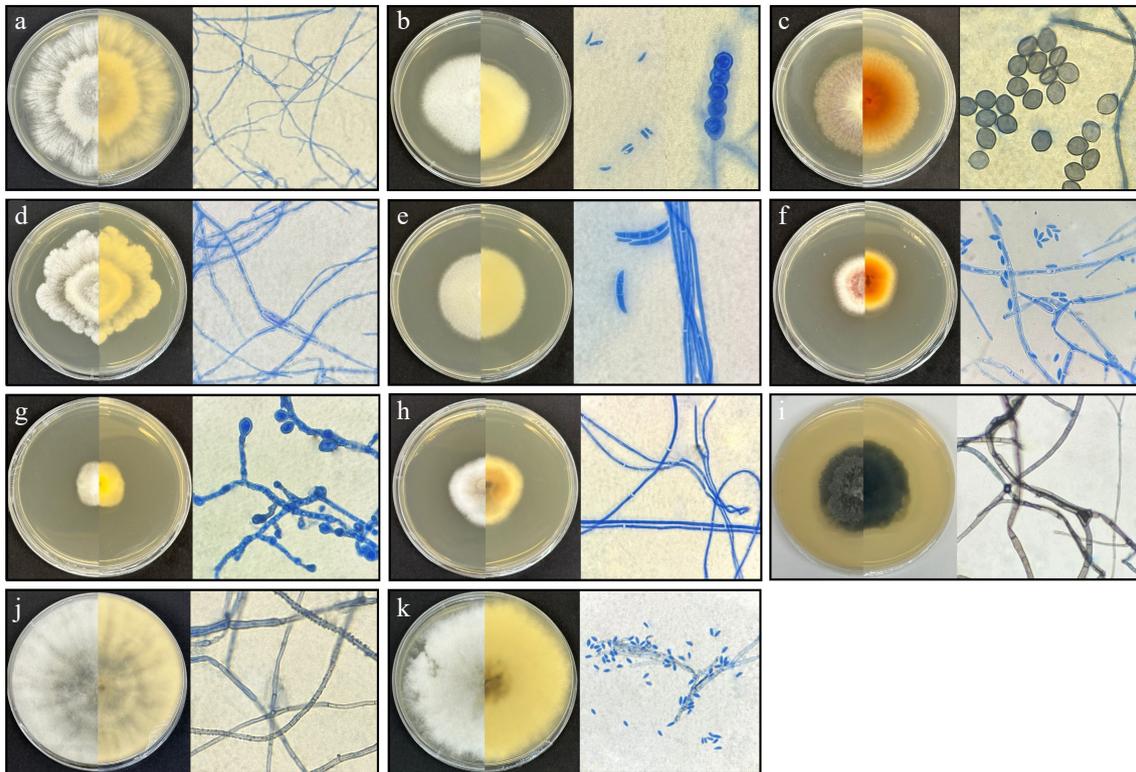


Fig. 2 Culture morphology and microscopic morphology of active endophytic fungi observed under a compound light microscope at 1,000× magnification (oil immersion objective). (a) NA-001-Lv-C. (b) NA-001-Lv-D. (c) NA-001-Lv-E. (d) NA-001-Lv-F. (e) NA-001-Lv-G. (f) NA-001-St-B. (g) NA-001-St-D. (h) NA-001-St-H. (i) NA-002-Lv-X. (j) NA-002-St-C. (k) NA-003-St-C.

fungus isolates showed small variations with respect to the present mycoconstituents (Table 5). Results from the mycochemical analysis of active extracts indicate the presence of anthrones, coumarins, terpenes and sterols, and indoles. Notably, chemical diversity was apparent in the TLC profile of the active extracts (Fig. 3).

Discussion

In the present study, 48 fungal extracts were isolated from four Annonaceae species. From these, 12 distinct fungal species were selected and cultivated in four different media (YM 6.3, ZM 1/2, Q6 1/2, and BRFT), which produced 23 crude fungal extracts with antibacterial activity against ESKAPE pathogens (Table 2). The potency of the active crude extracts was classified based on their MIC values following the criteria established by de Jesús Dzul-Beh et al., wherein weak activity was defined as MIC 1,000–500 µg/mL, moderate activity as 500–250 µg/mL, and strong activity as ≤ 125 µg/mL^[32]. Most of the extracts were found to be active against *P. aeruginosa*, *A. baumannii*, and *S. aureus*, with varying range of antibacterial strength from weak to strong. Additionally, five extracts were found active against *E. hormaechei* with a varying antibacterial strength (weak to strong) while three extracts were revealed to have weak antibacterial activity against *E. faecium*.

Because previous studies have demonstrated overlap between antioxidant and antibacterial mechanisms^[36–38], the active anti-ESKAPE extracts were further evaluated for antioxidant and antitubercular properties. In the DPPH assay, most anti-ESKAPE extracts also displayed antioxidant activity. The dual activity is consistent with reports highlighting the role of reactive oxygen species (ROS) in antibacterial mechanisms, particularly in mediating bacterial cell death^[36,38]. For instance, May et al. emphasized the repurposing

Table 5. Mycoconstituents present in the active crude fungal extracts.

Extract code	ALK	ANQ	ANT	C	TS	IN
Q6-001-Lv-C-BE	–	–	–	–	–	–
BR-001-Lv-C	–	–	+	–	++	++
ZM-001-Lv-D-BE	–	–	–	–	–	–
ZM-001-Lv-E-BE	–	–	++	–	+	++
YM-001-Lv-F-BE	–	–	–	–	–	+
BR-001-Lv-F	–	–	+	–	+	+
ZM-001-Lv-G-BE	–	–	–	–	–	–
Q6-001-Lv-G-BE	–	–	–	–	–	–
YM-001-St-B-BE	–	–	–	–	+	–
ZM-001-St-B-BE	–	–	–	–	–	–
Q6-001-St-B-BE	–	–	–	–	–	–
BR-001-St-B	–	–	–	–	+	–
YM-001-St-D-BE	–	–	–	+	–	–
ZM-001-St-D-BE	–	–	+	–	–	–
Q6-001-St-D-BE	–	–	–	–	–	–
BR-001-St-D	–	–	++	–	–	–
BR-001-St-E	–	–	–	–	–	–
ZM-001-St-H-BE	–	–	–	+	–	–
Q6-001-St-H-BE	–	–	–	–	–	–
YM-002-Lv-X-BE	–	–	+	–	–	–
YM-002-St-C-BE	–	–	–	+	–	–
YM-003-St-C-BE	–	–	+	–	+	–
ZM-003-St-C-BE	–	–	+	–	+	–
Q6-003-St-C-BE	–	–	+	–	+	–

ALK = alkaloids; ANQ = Anthraquinones; ANT = Anthrones; C = Coumarins; TS = Terpenes and sterols; IN = indoles; + indicates the relative abundance of certain mycoconstituent; – = absence; The most promising extract is highlighted in bold.

potential of antioxidant drugs such as Auranofin and Ebselen, which inhibit thioredoxin reductase in *M. tuberculosis* and *S. aureus*^[39]. Thioredoxin reductase is a critical enzyme for bacterial redox

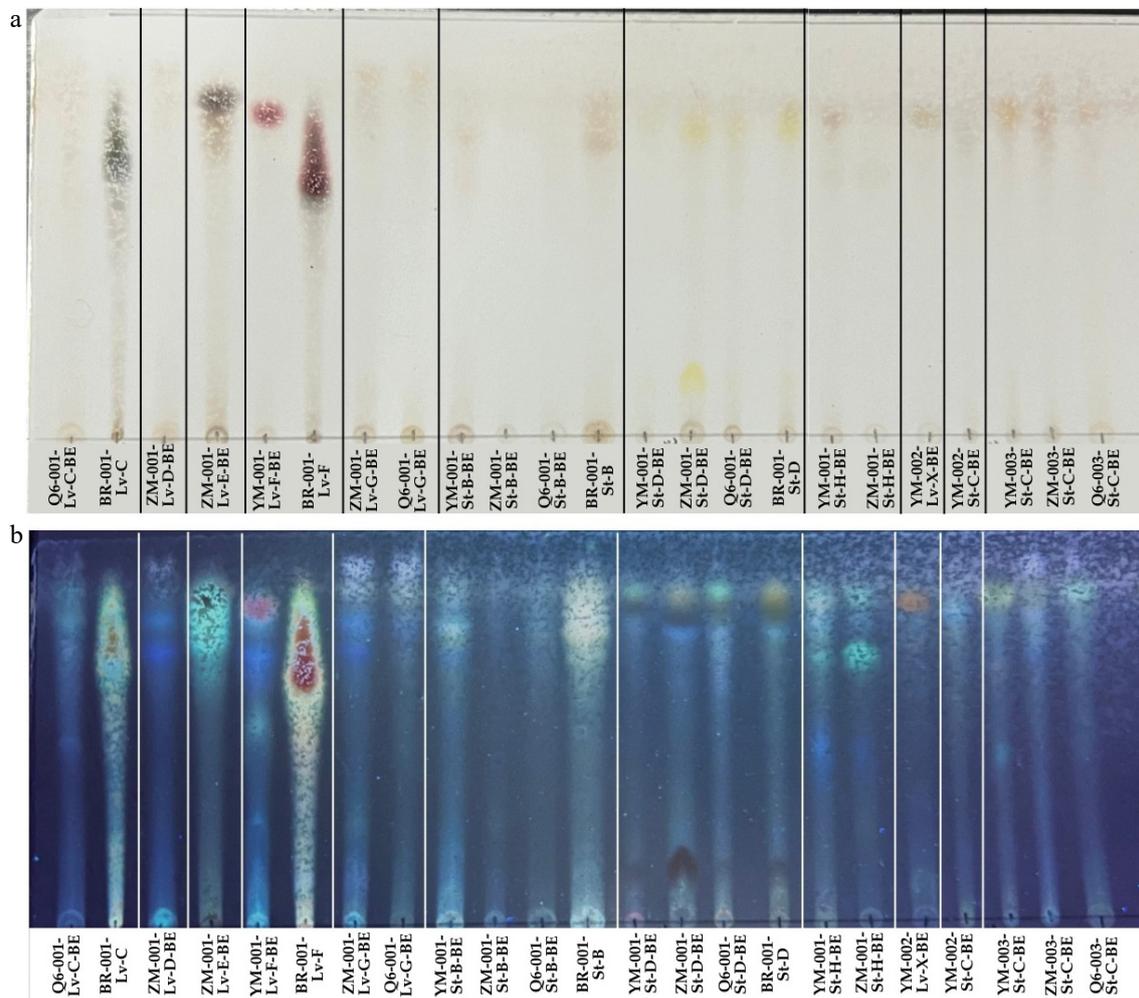


Fig. 3 TLC profile of active crude fungal extracts. The TLC plate was viewed under (a) visible light, and (b) 365 nm using 7:2 EtOAc-MeOH solvent system and ethanol sulfuric acid spray reagent for derivatization. Active extracts were separated per fungal isolates by solid lines.

balance, as many bacteria lack glutathione synthase, and rely instead on the thioredoxin system to counteract ROS from host immunity or antibiotic stress. Similarly, Naqvi et al. showed evidence showing that natural product-derived antioxidants can act as 'natural antibiotics', with antibacterial mechanisms including impairment of membrane integrity, disruption of nucleic acid synthesis, and inhibition of bacterial respiration^[38]. In fact, in the study by Bag & Chattopadhyay, synergistic interactions on antibacterial and antioxidant efficacy of essential oils were observed^[40].

For targeting ESKAPE pathogens, the most promising extract was BR-001-St-D, which conferred strong to moderate antibacterial activity against five ESKAPE pathogens (MIC = 250–7.81 µg/mL). BR-001-St-D was isolated from *Chromolaenicola* sp. However, since the present identification was based solely on ITS sequencing, we were only able to identify this fungal isolate up to its genus level. Notably, all extracts in our studies derived from *Chromolaenicola* sp. demonstrated inhibitory activity against *S. aureus*, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*. This aligns with a recent report in which three *Chromolaenicola* species (*C. chiangraiensis* MFLUCC 24-0058, *C. siamensis* MFLUCC 24-0057, *C. thailandensis* MFLUCC 24-0056) were shown to possess antibacterial and antifungal activity against *Bacillus subtilis* and *Mucor plumbeus*^[41].

Of particular interest, the present extract was found to contain anthraquinones, a class of compounds well-documented for their

antibacterial potential. In a study by Vidal et al., an anthraquinone compound—1,8-dihydroxyanthraquinone (danthron)—was isolated from a marine *Chromolaenicola* sp. (HL-114-33-R04)^[42]. While danthron was initially characterized by its antioxidant and angiogenic properties^[43], a study by Wei et al. has demonstrated its direct antibacterial effects^[44]. Specifically, danthron was shown to exert potent activity against *S. aureus* through cell membrane disruption, leading to loss of bacterial viability^[44].

For targeting tuberculosis, the most promising extracts were YM-002-Lv-X-BE and YM-001-Lv-F, derived from *Endomelanconiopsis* sp. and *D. longicolla*, respectively. Chemical profiling revealed the presence of terpenes and sterols, anthraquinones, and indoles in these extracts. Previous studies have established the antibacterial potential of compounds isolated from *D. longicolla*^[45,46]. For instance, Liu et al. reported nine biologically active metabolites from this fungus, including a novel terpene, Longidacid A, which exhibited antitubercular activity by inhibiting an *M. tb.* protein enzyme at 50 µM^[45]. Similarly, extracts of *Endomelanconiopsis* sp. have been documented for antibacterial activity against *S. aureus* and antifungal activity against *Candida albicans*^[47], as well as antioxidant properties^[48]. In a study by Pushpavathi & Krishnamurthy, dereplication of the ethyl acetate extract of *Endomelanconiopsis endophytica* identified several antibacterial metabolites, including Butylparaben, Sakuranetin, Tenuazonic acid, and 3,4-dimethoxycinnamic acid^[48]. Notably,

3,4-dimethoxycinnamic acid, a phenylpropanoid derivative and a common precursor of coumarins, has been shown to inhibit bacterial filament formation by targeting the FtsZ protein, ultimately leading to impaired cell division and proliferation^[49]. However, to date, we are the first to report antitubercular activity from this genus.

Other noteworthy antibacterial fungal isolates identified in this study include *C. cassiicola*, *D. tectonendophytica*, and *F. decemcellulare*. Extracts from *D. tectonendophytica* (MIC = 1,000–62.5 µg/mL) and *F. decemcellulare* (MIC = 1,000–125 µg/mL) remain largely unexplored, making this the first report to demonstrate their antibacterial potential. Previous work by Gagana et al., showed that ethyl acetate extracts of *C. cassiicola* were active against *Salmonella typhi* but inactive against *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*^[50]. In contrast, the present findings revealed that *C. cassiicola* grown in ZM ½ and Q6 ½ media exhibited strong activity against *K. pneumoniae*, as well as weak activity against *E. faecium*, *S. aureus*, *A. baumannii*, and *P. aeruginosa*. This discrepancy may stem from differences in culture media, since Gagana et al. used potato dextrose broth. Such variation in media composition likely triggered the biosynthesis of distinct secondary metabolites in the present active extracts, which would not have been produced under potato dextrose broth. Importantly, this underscores the value of the OSMAC strategy in enhancing the chemical diversity of fungal metabolites. Accordingly, chemical profiling of the active extracts (Fig. 3, Table 5) showed that even extracts derived from the same fungal isolate displayed notable differences in metabolite composition.

Focusing on the novelty of the present findings, *Chromolaenicola* sp., *Endomelanconiopsis* sp., and *D. longicolla*, are reported here for the first time as endophytic fungi associated with endemic Philippine Annonaceae. *Chromolaenicola* sp. has previously been isolated from various plant families, including Asteraceae, Bromeliaceae, Fabaceae, Ranunculaceae, and Spindaceae^[41], and the marine-derived *Chromolaenicola* sp. HL-114-33-R04, which has been noted for its antioxidant properties^[42]. However, there have been no prior reports of *Chromolaenicola* sp. isolated from Annonaceae, specifically from the endemic Philippine plant *O. cumingiana*. Similarly, this study is the first to report *D. longicolla* that was isolated from *O. cumingiana*. Although this endophytic fungus has also been reported from *Cananga odorata* (Annonaceae)^[51], *Glycine max* (Fabaceae), and *Pyrus* sp. (Rosaceae)^[52]. The endophytic fungus *Endomelanconiopsis* sp. has been documented in multiple plant families, including Malvaceae, Olacaceae, Ochnaceae^[53], Fagaceae^[54], and Lauraceae^[55], but we are the first to record *Endomelanconiopsis* sp. isolated from *A. cagayanensis*.

In addition to revealing antibacterial potential, the present study highlights the importance of culture medium selection in optimizing both metabolite diversity and yield. Among the media tested, brown rice proved particularly effective in providing the highest crude extract yields (Fig. 1). Brown rice is rich in phenolics, flavonoids, γ -oryzanol, vitamins, and minerals that serve as biosynthetic precursors or inducers for fungal metabolism^[56]. Under solid-state fermentation, these nutrients are more readily available, stimulating enhanced fungal growth and secondary metabolite production^[57]. Moreover, an increase in production of biologically active compounds such as phenolic acids, flavonoids, and coumaric acids was observed in several fungi after fermentation with brown rice^[57]. Previous studies have consistently shown that solid substrates such as rice outperform liquid broths, sometimes producing tenfold to hundredfold higher yields. For instance, VanderMolen et al. compared six liquid broths and five solid-state substrates and reported that, across three fungal strains, solid media—particularly rice—yielded significantly greater metabolite output^[58].

It is noteworthy, however, that higher extract yields in brown rice did not necessarily correspond to stronger antibacterial activity. In several cases, extracts from liquid media, although produced in smaller quantities, exhibited greater potency. This suggests that liquid media may induce the biosynthesis of unique or more biologically active metabolites absent in brown rice cultures. For example, Hewage et al. demonstrated that *Dothidiomycetes* sp. CR17 produced azaphilone derivatives, tricyclic polyketides, and austidol in potato dextrose broth, while in Czapek malt medium, the same fungus produced four novel polyketides, 4-hydroxybenzaldehyde, and austidol^[59]. Such findings emphasize that the choice of medium not only influences metabolite yield but also determines the chemical diversity and bioactivity of the compounds produced. Accordingly, further purification and profiling of the present extracts are necessary to determine the optimal culture conditions for specific biologically active metabolites.

Conclusions

This study highlights the untapped potential of extracts from endophytic fungi isolated from Philippine endemic Annonaceae species as sources of novel antibacterial and antioxidant compounds. It is the first to report the antibacterial potential of fungal endophyte extracts from *O. cumingiana*, *A. cagayanensis*, *P. arborea*, and *D. clusiflorum* var. *megalanthum*, which are sources of extracts with diverse bioactivities. These findings underscore the long-term value of endophytic fungi from the Annonaceae family as sources of biologically active compounds that could help combat antibacterial-resistant bacterial diseases and oxidative stress-related disorders, which are global health problems that remain unresolved due to limited resources for lead compound discovery. The identified endophytic fungi of notable importance include *Chromolaenicola* sp., with anti-ESKAPE activities; *Chaetomium* sp., with antioxidant properties; and *Diaporthe longicolla* and *Endomelanconiopsis* sp., with anti-tuberculosis activity. Furthermore, although brown rice as a medium could result in a high yield of extract, it does not guarantee bioactivity, as certain media may only produce compounds with bioactivity. Further purification of the active extracts or dereplication is recommended to determine the identity of biologically active compounds and their mechanism of action. Moreover, species-level identification of the fungi is recommended to determine which endophytic fungal species hold the greatest potential for the treatment of various diseases.

Author contributions

The authors confirm their contributions to the paper as follows: conceptualization, supervision, project administration and writing—review and editing: Macabeo AP, Bungihan ME; methodology and validation: Macabeo AP, Bungihan ME, Fajardo MT, Mendoza G, Arturo HC, Suyu LR; formal analysis and investigation: Fajardo MT, Mendoza G, Arturo HC, Suyu LR; data curation: Mendoza G, Arturo HC; writing—original draft preparation: Fajardo MT, Mendoza G; visualization: Bungihan ME. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article and are available from the corresponding author on reasonable request.

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Conflict of interest

The authors declare that they have no conflict of interest.

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References

- [1] Garrett L. 2007. The challenge of global health. *Foreign Affairs* 86:14–38
- [2] Genilloud O. 2019. Natural products discovery and potential for new antibiotics. *Current Opinion in Microbiology* 51:81–87
- [3] Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, et al. 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet* 399:629–655
- [4] Sati H, Carrara E, Savoldi A, Hansen P, Garlasco J, et al. 2025. The WHO bacterial priority pathogens list 2024: a prioritisation study to guide research, development, and public health strategies against antimicrobial resistance. *The Lancet Infectious Diseases* 25:1033–1043
- [5] Vu A, Glassman I, Campbell G, Yeganyan S, Nguyen J, et al. 2024. Host cell death and modulation of immune response against *Mycobacterium tuberculosis* infection. *International Journal of Molecular Sciences* 25:6255
- [6] Teleanu DM, Niculescu AG, Lungu II, Radu CI, Vladăncenco O, et al. 2022. An overview of oxidative stress, neuroinflammation, and neurodegenerative diseases. *International Journal of Molecular Sciences* 23:5938
- [7] Liguori I, Russo G, Curcio F, Bulli G, Aran L, et al. 2018. Oxidative stress, aging, and diseases. *Clinical Interventions in Aging Volume* 13:757–772
- [8] Kardos N, Demain AL. 2011. Penicillin: the medicine with the greatest impact on therapeutic outcomes. *Applied Microbiology and Biotechnology* 92:677–687
- [9] Ślusarczyk J, Adamska E, Czerwik-Marcinkowska J. 2021. Fungi and algae as sources of medicinal and other biologically active compounds: a review. *Nutrients* 13:3178
- [10] Cateni F, Gargano ML, Procida G, Venturella G, Cirlincione F, et al. 2022. Mycochemicals in wild and cultivated mushrooms: nutrition and health. *Phytochemistry Reviews* 21:339–383
- [11] Deshmukh SK, Verekar SA, Bhavé SV. 2015. Endophytic fungi: a reservoir of antibacterials. *Frontiers in Microbiology* 5:715
- [12] Kusari S, Hertweck C, Spitteller M. 2012. Chemical ecology of endophytic fungi: origins of secondary metabolites. *Chemistry & Biology* 19:792–798
- [13] Alam B, Li J, Gě Q, Khan MA, Gōng J, et al. 2021. Endophytic fungi: from symbiosis to secondary metabolite communications or vice versa? *Frontiers in Plant Science* 12:791033
- [14] Kaul S, Gupta S, Ahmed M, Dhar MK. 2012. Endophytic fungi from medicinal plants: a treasure hunt for bioactive metabolites. *Phytochemistry Reviews* 11:487–505
- [15] Romano S, Jackson SA, Patry S, Dobson ADW. 2018. Extending the "one strain many compounds" (OSMAC) principle to marine microorganisms. *Marine Drugs* 16:244
- [16] Schwarz J, Hubmann G, Rosenthal K, Lütz S. 2021. Triaging of culture conditions for enhanced secondary metabolite diversity from different bacteria. *Biomolecules* 11:193
- [17] de Silva NI, Hyde KD, Lumyong S, Phillips AJL, Bhat DJ, et al. 2022. Morphology, phylogeny, host association and geography of fungi associated with plants of Annonaceae, Apocynaceae and Magnoliaceae. *Mycosphere* 13:955–1076
- [18] Moghadamtousi SZ, Fadaeinasab M, Nikzad S, Mohan G, Ali HM, et al. 2015. *Annona muricata* (Annonaceae): a review of its traditional uses, isolated acetogenins and biological activities. *International Journal of Molecular Sciences* 16:15625–15658
- [19] Veras VLS, Furtado PVOS, Costa PS, Rai M, Feitosa CM, et al. 2023. Annonaceae family: characteristics, properties and applications. In *Bioprospection of Co-products and Agro-industrial Wastes*, eds. Freire JAP, Medeiros SRA, Feitosa CM, Rai M. 1st Edition. Boca Raton: CRC Press. 292 pp. doi: 10.1201/9781003239994
- [20] Clemen-Pascual LM, Macahig RAS, Rojas NRL. 2022. Comparative toxicity, phytochemistry, and use of 53 Philippine medicinal plants. *Toxicology Reports* 9:22–35
- [21] Notarte KIR, Quimque MTJ, Macaranas IT, Khan A, Pastrana AM, et al. 2023. Attenuation of lipopolysaccharide-induced inflammatory responses through inhibition of the NF- κ B pathway and the increased NRF2 level by a flavonol-enriched *n*-butanol fraction from *Uvaria alba*. *ACS Omega* 8:5377–5392
- [22] Macabeo APG, Lopez ADA, Schmidt S, Heilmann J, Dahse HM, et al. 2013. Antitubercular and cytotoxic constituents from *Goniothalamus gitingensis*. *Records of Natural Products* 8:41–45
- [23] Manzano JAH, Llamas LCJ, Macabeo APG. 2024. Tetrahydrobisbenzylisoquinoline alkaloids from *Phaeanthus ophthalmicus* inhibit target enzymes associated with type 2 diabetes and obesity. *Journal of Applied Pharmaceutical Science* 14:230–237
- [24] Macabeo APG, Rubio PYM, Alejandro GJD, Knorn M. 2015. An α -glucosidase inhibitor from the Philippine endemic Annonaceae plant, *Drephananthus philippinensis*. *Procedia Chemistry* 14:36–39
- [25] Thinh BB, Thin DB, Ogunwande IA. 2024. Chemical composition, antimicrobial and antioxidant properties of essential oil from the leaves of *Vernonia volkameriifolia* DC. *Natural Product Communications* 19:1–8
- [26] Bugayong MLG, Jacinto SD. 2017. *In-vitro* cytotoxicity of *Wrightia pubescens* (Blanco) Merr., *Aphanamixis polystachya* (Wall.) Parker, and *Platymitra arborea* (Blanco) against selected human cancer cell lines. *International Journal of Biosciences* 11:204–213
- [27] Anoumedem EGM, Mountessou BYG, Kouam SF, Narmani A, Surup F. 2020. Simplicilones A and B isolated from the endophytic fungus *Simplicillium subtropicum* SPC3. *Antibiotics* 9:753
- [28] Matio Kemkuignou B, Lambert C, Stadler M, Kouam Fogue S, Marin-Felix Y. 2023. Unprecedented antimicrobial and cytotoxic polyketides from cultures of *Diaporthe africana* sp. nov. *Journal of Fungi* 9:781
- [29] Hassan K, Chepkirui C, Llanos-López NA, Matasyoh JC, Decock C, et al. 2022. Meroterpenoids possibly produced by a bacterial endosymbiont of the tropical basidiomycete *Echinochaete brachypora*. *Biomolecules* 12:755
- [30] Phukhamsakda C, Macabeo APG, Huch V, Cheng T, Hyde KD, et al. 2019. Sparticolins A–G, biologically active oxidized spirodioxynaphthalene derivatives from the sscormycete *Sparticola junci*. *Journal of Natural Products* 82:2878–2885
- [31] Phutthacharoen K, Toshe R, Khalid SJ, Llanos-López NA, Wennrich JP, et al. 2024. Lachnucic acids A–F: ambuic acid congeners from a saprotrophic *Lachnum* species. *Chemistry & Biodiversity* 21:e202400385
- [32] de Jesús Dzul-Beh A, Uc-Cachón AH, González-Sánchez AA, Dzib-Baak HE, Ortiz-Andrade R, et al. 2023. Antimicrobial potential of the Mayan medicine plant *Matayba oppositifolia* (A. Rich.) Britton against antibiotic-resistant priority pathogens. *Journal of Ethnopharmacology* 300:115738
- [33] Bungihan ME, Matias CA. 2013. Determination of the antioxidant, phytochemical and antibacterial profiles of flowers from selected ornamental plants in Nueva Vizcaya, Philippines. *Journal of Agricultural Science and Technology* 3:833–841

- [34] White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols*, eds. Innis MA, Gelfand DH, White TJ. Amsterdam: Elsevier. pp. 315–322 doi: [10.1016/b978-0-12-372180-8.50042-1](https://doi.org/10.1016/b978-0-12-372180-8.50042-1)
- [35] Drapisa HJC, Llamas LC, Abellanos EA, Vidar WS, Macabeo APG. 2024. Differential induction of fusaric acid in the endophytic fungus, *Fusarium* sp. (UST-UVG10) by three different media results to enhanced, variable anti-staphylococcal and antimycobacterial activity. *Studies in Fungi* 9:1–8
- [36] Ren X, Zou L, Holmgren A. 2020. Targeting bacterial antioxidant systems for antibiotics development. *Current Medicinal Chemistry* 27:1922–1939
- [37] Delghandi PS, Soleimani V, Fazly Bazzaz BS, Hosseinzadeh H. 2023. A review on oxidant and antioxidant effects of antibacterial agents: impacts on bacterial cell death and division and therapeutic effects or adverse reactions in humans. *Naunyn-Schmiedeberg's Archives of Pharmacology* 396:2667–2686
- [38] Naqvi SAR, Nadeem S, Komal S, Ali Asad Naqvi S, Samee Mubarak M, et al. 2019. Antioxidants: natural antibiotics. In *Antioxidants*, ed. Shalaby E. London, UK: IntechOpen 418 pp. doi: [10.5772/intechopen.77838](https://doi.org/10.5772/intechopen.77838)
- [39] May HC, Yu JJ, Guentzel MN, Chambers JP, Cap AP, et al. 2018. Repurposing auranofin, ebselen, and PX-12 as antimicrobial agents targeting the thioredoxin system. *Frontiers in Microbiology* 9:336
- [40] Bag A, Chattopadhyay RR. 2015. Evaluation of synergistic antibacterial and antioxidant efficacy of essential oils of spices and herbs in combination. *PLoS One* 10:e0131321
- [41] Htet ZH, Mapook A, Chethana KWT. 2024. Molecular taxonomy reveals new records of *Chromolaenicola* (Didymosphaeriaceae, Pleosporales) and potential antibacterial properties. *Studies in Fungi* 9:e006
- [42] Vidal I, Torres-Vargas JA, Sánchez JM, Trigal M, García-Caballero M, et al. 2023. Danthron, an anthraquinone isolated from a marine fungus, is a new inhibitor of angiogenesis exhibiting interesting anti-tumor and antioxidant properties. *Antioxidants* 12:1101
- [43] Masi M, Evidente A. 2020. Fungal bioactive anthraquinones and analogues. *Toxins* 12:714
- [44] Wei Y, Liu Q, Yu J, Feng Q, Zhao L, et al. 2015. Antibacterial mode of action of 1, 8-dihydroxy-anthraquinone from *Porphyra haitanensis* against *Staphylococcus aureus*. *Natural Product Research* 29:976–979
- [45] Liu Z, Chen Y, Li S, Wang Q, Hu C, et al. 2020. Bioactive metabolites from the deep-sea-derived fungus *Diaporthe longicolla* FS429. *Marine Drugs* 18:381
- [46] Nishad JH, Singh A, Gautam VS, Kumari P, Kumar J, et al. 2021. Bioactive potential evaluation and purification of compounds from an endophytic fungus *Diaporthe longicolla*, a resident of *Saraca asoca* (Roxb.) Willd. *Archives of Microbiology* 203:4179–4188
- [47] Ramos-Garza J, Rodríguez-Tovar AV, Flores-Cotera LB, Rivera-Orduña FN, Vásquez-Murrieta MS, et al. 2016. Diversity of fungal endophytes from the medicinal plant *Dendropanax arboreus* in a protected area of Mexico. *Annals of Microbiology* 66:991–1002
- [48] Pushpavathi D, Krishnamurthy YL. 2023. Molecular characterization and metabolite profiling of *Endomelanconiopsis endophytica* E.I. Rojas & Samuels, an Endolichenic fungal isolate. *Journal of Mycopathological Research* 61:481–487
- [49] Tripathy S, Sahu SK. 2019. FtsZ inhibitors as a new genera of antibacterial agents. *Bioorganic Chemistry* 91:103169
- [50] Gagana SL, Kumaraswamy BE, Shivanna MB. 2020. Diversity, antibacterial and antioxidant activities of the fungal endophytes associated with *Schleichera oleosa* (Lour.) Merr. *South African Journal of Botany* 134:369–381
- [51] Toghueo RMK, Zabalgoeazcoa I, Vázquez de Aldana BR, Boyom FF. 2017. Enzymatic activity of endophytic fungi from the medicinal plants *Terminalia catappa*, *Terminalia mantaly* and *Cananga odorata*. *South African Journal of Botany* 109:146–153
- [52] Dissanayake AJ, Phillips AJL, Hyde KD, Yan JY, Li XH. 2017. The current status of species in *Diaporthe*. *Mycosphere* 8:1106–1156
- [53] Rojas El, Herre EA, Mejía LC, Arnold AE, Chaverri P, et al. 2008. *Endomelanconiopsis*, a new anamorph genus in the Botryosphaeriaceae. *Mycologia* 100:760–775
- [54] Xie Y, Shi L, Cheng K, Li Y, Yu S. 2023. Host recognition and specific infection of *Endomelanconiopsis endophytica* during early infection. *Journal of Fungi* 9:1040
- [55] Rodrigues JGC, Cardoso FV, Duvoisin SJ, Machado NT, Albuquerque PM. 2024. *Endomelanconiopsis endophytica* lipase immobilized in calcium alginate for production of biodiesel from waste cooking oil. *Energies* 17:5520
- [56] Henderson AJ, Ollila CA, Kumar A, Borresen EC, Raina K, et al. 2012. Chemopreventive properties of dietary rice bran: current status and future prospects. *Advances in Nutrition* 3:643–653
- [57] Zhang D, Ye Y, Tan B. 2022. Comparative study of solid-state fermentation with different microbial strains on the bioactive compounds and microstructure of brown rice. *Food Chemistry* 397:133735
- [58] VanderMolen KM, Raja HA, El-Elimat T, Oberlies NH. 2013. Evaluation of culture media for the production of secondary metabolites in a natural products screening program. *AMB Express* 3:71
- [59] Hewage RT, Aree T, Mahidol C, Ruchirawat S, Kittakoop P. 2014. One strain-many compounds (OSMAC) method for production of polyketides, azaphilones, and an isochromanone using the endophytic fungus *Dothideomycete* sp. *Phytochemistry* 108:87–94



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