BMC Microbiology

RESEARCH Open Access

Investigating the antimicrobial and anticancer potential of culturable fungal endophytes isolated from the *stems* of *Kirkia acuminata* Oliv

Mfundo Magagula[1], Thabiso E. Motaung[2,3], Zukile Mbita[1] and Khumiso Dithebe[1*]

Abstract

Background Fungal endophytes produce various structurally and chemically diverse bioactive secondary metabolites including those that are similar to their host plants. However, fungal endophytes from South African medicinal plants are relatively under-explored. The medicinal plant, *Kirkia acuminata* Oliv., is on the decline in the natural environment due to overharvesting. This necessitates the search for novel alternatives to sustainably obtain the plant's bioactive metabolites. Thus, fungal endophytes may serve as suitable candidates as they can produce host-similar bioactive compounds.

Results Eighteen morphologically distinct fungal endophytes were isolated from the surface-sterilised stems of *K. acuminata* Oliv. Sequencing of the internal transcribed spacer (ITS) region revealed that the isolates were distributed among three genera, namely *Diaporthe*, *Neofusicoccum* and *Pseudofusicoccum*. The broth micro-dilution assay showed that 17 of the 18 ethyl acetate crude extracts exhibited inhibitory activity with minimum inhibitory concentration (MIC) values ranging from 0.31 to 2.5 mg/mL and 1.25 to 2.5 mg/mL against bacterial pathogens and *Candida albicans*, respectively. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay revealed that most of the crude extracts had dose-dependent cytotoxicity against non-cancerous human embryonic kidney (HEK-293) cells, with the crude extracts of the *N. parvum* KaS-3, *D. macadamiae* KaS-4, *P. olivaceum* KaS-5 and *D. neotheicola* KaS-6 isolates demonstrating safety against the non-cancerous cells. The alamarBlue assay revealed that the four non-cytotoxic crude extracts had moderate anticancer activity against cervical cancer ME-180 and melanoma A375 cancerous cell lines. Moreover, mycochemical analysis of the non-cytotoxic crude extracts using colourimetric quantification methods revealed that the observed cytotoxic effect could be attributed to the high total phenolic content in the crude extracts.

Conclusion The study highlights that the fungal endophytes inhabiting the stems of *K. acuminata* Oliv. produce secondary metabolites that may serve as leads for novel antimicrobial and non-toxic anticancer agents.

*Correspondence:
Khumiso Dithebe
Khumiso.Dithebe@ul.ac.za

Full list of author information is available at the end of the article

Keywords Antimicrobial resistance, Crude extracts, Cytotoxicity, Mycochemicals, Secondary metabolites, South African medicinal plants

Introduction

Antimicrobial resistance presents a significant global health concern due to the rise in antimicrobial resistant (AMR) pathogens. This exacerbates the spread of microbial infections and the subsequent rise in deaths associated with microbial infections [1]. This is due to the fact that AMR pathogens can survive and proliferate, leading to treatment failures and prolonged disease occurrence, which is associated with increased hospital stays, health-related financial burdens and ultimately, death [2]. Infections by AMR pathogens are difficult to treat and require expensive antimicrobials, which often have adverse side effects. Moreover, microbial infections induce inflammation, which has been linked with the development and progression of cancer [3]. As such, prolonged infections with AMR pathogens may result in chronic inflammation and ultimately increase the risk of cancer development [4]. A study by Shrestha et al. [5]. found that bacterial genotoxins, such as colibactin, produced during bacterial prostatitis can trigger inflammation and cause DNA damage. This process contributes to the formation of precancerous lesions and oncogenic gene fusions, ultimately promoting the early stages of prostate cancer development.

Cancer is regarded as a great cause for concern as it is estimated that it will globally affect approximately 32.6 million people and cause about 16.9 million fatalities by 2045 [6]. The rapidly increasing incidences of cancer are concerning, particularly since most of the conventional therapeutic drugs in use lack efficacy, selectivity and safety [7]. Additionally, the conventional treatment of cancer through antibiotic-assisted therapies is affected by infections with AMR pathogens as they minimise the efficacy of the anticancer therapeutics, further compromising cancer patients and leading to increased mortality and morbidity rates [8]. The fact that infections by AMR pathogens not only lead to treatment failures but may also lead to the propagation of cancer warrants the search for novel drug candidates that are highly effective against AMR pathogens and possess anticancer activity with minimal and/or no side effects.

Medicinal plants and their natural products have been extensively used to treat various ailments, including microbial infections and cancer [9]. The perception that plant-derived products possess little to no side effects has resulted in the increased consumer demand for these natural remedies with plant-derived natural products accounting for an estimated $165.66 billion in global medicine revenues [10, 11]. While medicinal plants may present a solution to the AMR crisis and associated ailments, the increased demand for plant-based medicine presents a potential threat of extinction for medicinal plants due to overharvesting and habitat destruction [12]. Additionally, factors such as slow growth, low levels and inconsistent production of bioactive secondary metabolites limit the use of medicinal plants as sustainable sources of bioactive compounds [13]. Accordingly, this necessitates the search for sustainable alternatives to produce plant-derived secondary metabolites. Interestingly, medicinal plants harbour fungal endophytes that can produce various secondary metabolites, including those that are the same or similar to the plant host [14]. Microbial secondary metabolites have showed increasing promise as novel agents for applications in pharmaceutical, agricultural and industrial fields [15]. Moreover, due to concerns over the severe toxicity of synthetic therapeutic agents, the search for novel antimicrobial and anticancer drugs has primarily focused on natural therapeutic agents [16]. Thus, fungal endophytes of medicinal plants represent a promising new source of therapeutic secondary metabolites for novel drug leads and may serve as alternative sources of natural products, which could assist in conserving the diminishing medicinal plant population.

Fungal endophytes are fungi that colonise and inhabit the internal tissues of healthy plants without causing any disease symptoms [17]. The potential of fungal endophytes as reservoirs of important bioactive compounds was highlighted by the discovery of *Taxomyces andreanae*, isolated from the plant, *Taxus brevifolia*, which produced the anticancer compound, Taxol [18]. Since then, fungal endophytes have been shown to produce secondary metabolites with various bioactivities, including antimicrobial, antioxidant, immunosuppressive, anticancer and anti-inflammatory activities [19]. This led to the discovery of several promising antimicrobial drugs, such as clavatol, sordaricin and jeserone, as well as prominent anticancer drugs, such as podophyllotoxin, vincristine and camptothecin [20, 21]. Interestingly, Zhang et al. [22]. reported that the endophytic fungal strain *Aspergillus ochraceus* XZC-1 produced secondary metabolites with significant antimicrobial activity, which selectively inhibited human lung carcinoma A549 and hepatoma HepG2 cancerous cell lines. Similarly, Mohamed and Alati [23] reported that 19 fungal endophytes from four Sudanese medicinal plants possessed antimicrobial activity against clinical pathogens, namely *Aspergillus niger*, *Bacillus subtilus*, *Candida albicans*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. Additionally, the authors reported that an unidentified isolate

produced trace amounts of Taxol, which had no cytotoxicity against the non-cancerous Vero cell line. These discoveries further elucidated the potential of fungal endophytes as alternatives for the sustainable production of novel therapeutic agents. However, there is a lack of knowledge on the bioactivity of fungal endophytes associated with the wide biodiversity of South African medicinal plants [24].

The medicinal plant, *Kirkia acuminata* Oliv., belonging to the *Kirkiaceae* family grows seasonally in the dry tropical areas of various Sub-Saharan African countries, including Angola, Botswana, Malawi, Namibia, South Africa and Zimbabwe, and is widely used by various communities in these regions as a primary means of treating numerous ailments [25]. The bark, roots and fruits are commonly used to treat toothaches, diarrhoea, malaria, cholera, dysentery and for wound healing [25]. Mongalo and Makhafola [26] noted the progressive decline of *K. acuminata* Oliv. in the natural environment in the Limpopo province of South Africa due to excessive harvesting by ethnomedicinal practitioners which is further exacerbated by the lack of replanting [27]. Owing to the decreasing availability of *K. acuminata* Oliv., there is a need to investigate the endophytic fungi associated with the plant for their therapeutic potential. The aim of this study was to isolate fungal endophytes from the stems of *K. acuminata* Oliv. and investigate the antimicrobial and anticancer potential of the fungal endophytes crude extracts as well as the cytotoxicity of the crude extracts on non-cancerous cells to assess whether the fungal endophytes of *K. acuminata* Oliv. can serve as alternative sources for secondary metabolites with therapeutic potential.

Materials and methods

Plant collection and isolation of endophytic fungi

Healthy, symptom-free scaffold branches of the stems of *K. acuminata* Oliv. were collected at the Lowveld National Botanical Garden (25°26′40″S, 30°57′58″E) in Nelspruit, Mpumalanga, South Africa with the assistance of the staff at the botanical garden and care was taken to avoid damaging the whole plant. The plant material was collected from randomly selected mature adult trees that were approximately 5 m from each other in March 2021. The plant material was packaged in clean plastic bags and transported to the University of Limpopo in a cooler box. The plant material was submitted to the Larry Leach Herbarium at the University of Limpopo to generate a voucher specimen (UNIN 1220027). The stems were washed under running tap water for 4 min to remove debris. The stems were then surface sterilised as described by Abdalla et al. [28]with minor modifications. Briefly, they were sterilised in series with 70% ethanol for 1 min, followed by 2.5% sodium hypochlorite for 4 min and then 70% ethanol for 30 s. Thereafter, the stems were rinsed three times with sterile distilled water for 1 min to remove residual sterilant. The surface sterilised samples were cut aseptically into 5 mm × 5 mm segments, which were further cut open to expose the inner layers of the stems, and placed in Petri dishes containing potato dextrose agar (PDA; Neogen, South Africa) supplemented with 30 µg/L chloramphenicol (Merck, South Africa) to prevent bacterial growth. Additionally, 100 µL of the final rinse water was inoculated on antibiotic-free PDA to assess the effectiveness of surface sterilisation. The plates were incubated at 28 °C until fungal growth was observed. Hyphal tips of newly growing endophytic fungi were continuously sub-cultured on antibiotic-free PDA to obtain pure isolates. The pure cultures were kept at 4 °C until further assessment. The isolates were also preserved at −80 °C in a potato dextrose broth (PDB; Neogen, South Africa)-glycerol solution (20 mL glycerol, 4.8 g PDB and 180 mL distilled water).

Morphological and molecular identification of endophytic fungi

Macromorphological characteristics such as colony shape, texture and pigmentation were used to distinguish the endophytic fungal isolates [29]. The isolates were then assigned codes: KaS-1 up to KaS-19. Thereafter, the nucleotide sequence of the internal transcribed spacer (ITS) region was sequenced to identify individual endophytic fungal isolates at Inqaba Biotechnical Industries (Pty) Ltd, South Africa. To do this, the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005) was used to extract genomic DNA from the cultures. Polymerase chain reaction (PCR) was performed to amplify the ITS1-5.8S Rrna-ITS2 region using primers ITS1 (5'-TCCGTAGGTGAACCTGCG G-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') by the protocol described by White et al. [30], with PCR amplification based on the following conditions: 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 50 °C for 30 s, 68 °C for 1 min and lastly a final step of 68 °C for 10 min. The integrity of the PCR amplicons was visualised on a 1% (w/v) agarose gel (CSL-AG500, Cleaver Scientific Ltd) stained with EZ–vision® Bluelight DNA Dye. The NEB Fast Ladder was used on all gels (N3238) as a size standard. Fragments were then enzymatically purified using the ExoSAP procedure (NEB M0293L; NEB M0371). Subsequently, amplicons were purified for sequencing using a DNA sequencing kit (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit™, Catalogue No. D4050) and sequenced in the forward and reverse direction (Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000) using the ABI 3730 XL Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific). Following this, FinchTV (https://finchtv.soft

ware.informer.com/1.4/) was used to view the raw chromatogram files (.abi) and CLC Bio Main Workbench was used to assemble the forward and reverse sequencing reads to form a consensus sequence for each sample. Thereafter, the basic local alignment search tool (BLAST) algorithm was used to find sequences similar to those obtained from fungal isolates. Hence, BLASTn analysis (with default parameters) [31] was performed on the National Center for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine if a sequence in the database matches the query sequence above a certain threshold (99% query coverage: 98% identity). The sequences of the isolates were deposited in the NCBI GenBank with accession numbers PQ867536 to PQ867553.

Fermentation of isolates and extraction of secondary metabolites

To produce secondary metabolites, five mycelial plugs (5 mm diameter) of 7-day-old actively growing PDA-grown fungal endophyte cultures were inoculated into 500 mL Erlenmeyer's flasks containing 200 mL potato dextrose broth (PDB; Neogen, South Africa). The flasks were then incubated for 21 days at 25 °C under stationary conditions [32]. Following incubation, the fermented broths were filtered using the Whatman no. 1 filter paper (Merck, South Africa) to remove fungal biomass. To extract secondary metabolites, the filtrates were mixed with equal volumes of ethyl acetate, shaken for 15 min and left for 8 h in a separation funnel to separate the organic layer from the aqueous layer [33]. The aqueous layer was collected and the extraction was repeated two more times. The organic solvent layer, of each isolate, containing the extracted secondary metabolites was pooled into a 500 mL round-bottom flask and the ethyl acetate was evaporated using a vacuum rotary evaporator at 45 °C. Thereafter, the dry crude extracts were re-suspended in dimethyl sulfoxide (DMSO; Sigma-Aldrich, South Africa) to a concentration of 10 mg/mL and stored at 4 °C in the refrigerator before the biological activities and mycochemical composition were analysed.

Antimicrobial activity of the crude extracts

Test organisms

The antimicrobial activity of the crude extracts was tested against clinically significant microorganisms; namely the Gram-positive *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212), the Gram-negative *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 15422) as well as the yeast *Candida albicans* (ATCC 10231). The strains are common causes of human infections and are essential for antimicrobial testing as they serve as model organisms for both bacterial and fungal pathogens [34]. For inoculum preparation, bacterial strains were plated on nutrient agar (Condalab, South Africa) and incubated for 24 h at 37 °C whilst the yeast strain was cultured on Sabouraud agar (Neogen, South Africa) for 48 h at 37 °C.

Antimicrobial screening

The minimum inhibitory concentration (MIC) of crude extracts was determined using the broth micro-dilution method described by Eloff [35] with *p*-iodonitrotetrazolium chloride (INT; Merck, South Africa) as a growth indicator. The bacterial test microorganisms were suspended in nutrient broth (Condalab, South Africa) and the yeast strain was suspended in Sabouraud dextrose broth (Condalab, South Africa). The optical density (OD) of the suspensions was measured and the turbidity was adjusted to an optical density at 625 nm (OD_{625nm}) of 0.08–0.1 to match a 0.5 McFarland standard. The 10 mg/mL crude extracts (100 µL) were serially diluted with 100 µL of respective growth media in 96-well microtiter plates to achieve a concentration gradient of 0.02–2.5 mg/mL. Thereafter, 100 µL of the prepared test cultures were added separately. Following this, the microplates were covered and incubated for 24 h at 37 °C for the bacterial cultures and 48 h at 37 °C for the yeast culture. After incubation, INT previously dissolved in sterile distilled water to a concentration of 0.2 mg/mL was added to the wells at 40 µL. The bacterial culture plates were then incubated for 40 min, whereas the yeast plates were incubated for 4 h. The incubation periods allowed for optimal colour development in the wells owing to the reduction of the INT by viable cells. The MIC was recorded as the lowest concentration of the crude extracts at which no colour change was observed. The assay was conducted in triplicate using biological replicates. Chloramphenicol and Amphotericin B were used as positive controls for bacteria and the yeast, respectively, while untreated wells and DMSO were separately regarded as negative controls.

Cytotoxicity of endophytic fungal ethyl acetate crude extracts

The non-cancerous embryonic human kidney (HEK-293; ATCC#: CCL-81) cells were used to test the safety and cytotoxicity of the endophytic fungal crude extracts using the 3-(4,5–dimethylthiazol–2-yl)-2,5-diphenyltetrazolium bromide assay (MTT) [ThermoFischer, United States of America (USA)]. The HEK-293 cells were cultured and maintained in accordance with the ATCC's instructions in Dulbecco's modification of eagle medium (DMEM) (Hyclone, USA) supplemented with 10% foetal bovine serum (FBS) (Hyclone, USA), 2mM L-glutamine (Hyclone, USA) and 1 x PSN (50 µg/mL penicillin, 50 µg/mL streptomycin and 100 µg/mL neomycin) (Biowest, USA). Thereafter, the cells were seeded into a 96-well

microtiter plate at 1×10^6 cell mL^{-1} in DMEM, followed by incubation for 24 h at 37 °C, 5% CO_2 and 95% humidity. The endophytic fungal ethyl acetate crude extracts were then added to each well at doses ranging from 15 to 1000 µg/mL and incubated for 24 h in a CO_2 incubator. Following this, 10 µL of a 5 mg/mL MTT solution (ThermoFischer Scientific, USA) was added to each well and the plates were incubated for an additional three hours. DMSO (100 µL) was used to dissolve the formazan crystals. The absorbance was then measured at 570 nm using a Glo-max multiplex (Promega, USA) microplate reader. The assay was conducted in triplicate using biological replicates. Curcumin was used as a positive control, whereas DMSO and untreated cells were considered negative controls. The concentration of the crude extract that inhibited more than 50% viability of the cells was labelled as the inhibitory concentration 50 (IC_{50}). The viability of the cells was calculated by the equation as previously reported by Makgoo et al. [36]:

$$\text{Cell viability } (\%) = \frac{\text{Absorbance of treated cells}}{\textit{Absorbance of untreated control cells}} \times 100$$

Anticancer activity of the crude extracts

The anticancer activity of the crude extracts displaying no cytotoxicity against the non-cancerous HEK-293 cells was tested against melanoma A375 and cervical cancer ME-180 cell lines using the alamarBlue assay, following a protocol previously published by Hamid et al. [37]. The density of the cancerous cells was determined with trypan blue exclusion assay by adding 1:1 cell to 1x trypan blue dye onto a countess slide and a countess 3 FL automated cell counter (ThermoFischer Scientific, USA), which was used to count viable vs. dead cells. Thereafter, cells were seeded at a density of 1×10^6 cells/mL in a 96 well plate containing complete media (DMEM + PSN + FBS) and allowed to grow to 90% confluence at 37 °C, 5% CO_2 and 95% humidity for 24 h. After incubation, the medium was discarded and cells were treated with varying concentrations of the crude extracts (1, 2 and 6 µg/mL) diluted with DMEM. The plates were then incubated at 37 °C, 5% CO_2 and 95% humidity for 24 h. After incubation, a stock solution of the alamarBlue reagent (ThermoFischer Scientific, USA) diluted 1:1 with assay medium was added at 20 µL/well to achieve a final concentration of 10% alamarBlue reagent in the test plates. The total volume of solution in the plates became 220 µL per well. Plates were then incubated and fluorescence was measured after 5 h with a Glo-max multiplex (Promega, USA) microplate reader at excitation/emission = 570/585 nm with an appropriate filter. The assay was conducted in triplicate using biological replicates. Curcumin was used as a positive control, while DMSO and untreated cells were considered negative controls. The percent viability was calculated by the equation as previously reported by Sharma et al. [38]:

$$\text{Cell viability } (\%) = \frac{\text{Fluorescence counts of treated cells}}{\textit{Fluorescence counts of untreated control cells}} \times 100$$

Mycochemical analysis of the crude extracts

The mycochemical composition of the non-cytotoxic crude extracts was carried out following the procedures detailed by Matotoka et al. [39]. The crude extracts were assessed for the presence of phenols, tannins and flavonoids. All analyses were performed in triplicates.

Total phenolic content

The fungal endophyte crude extracts were assessed for total phenol content (TPC) using a modified Folin-Ciocalteu reagent method by Matotoka et al. [39]. Firstly, 10 µL of the 10 mg/mL crude extracts was diluted with 490 µL of distilled water and mixed with 0.25 mL of the Folin-Ciocalteu reagent (Merck, South Africa) in a test tube. Following this, 1.25 mL aqueous sodium carbonate (7%) was added and the mixture was incubated for 30 min in the dark at 25 °C. The absorbance of the samples and a similarly prepared blank were measured at 725 nm. Varying concentrations of gallic acid (0.08–1.25 mg/mL) were used to construct a standard curve. The TPC was expressed in milligrams of gallic acid equivalent (GAE) per gram of crude extract. The regression equation ($y = 0.6814x + 0.0057$ with $R^2 = 0.9983$) was used to estimate the TPC content of the crude extracts.

Total tannin content

The total tannin content (TTC) of the crude extracts was assessed using the Folin-Ciocalteu reagent method described by Matotoka et al. [39]. To begin, 100 µL of 10 mg/mL crude extract was diluted with 7.5 mL of distilled water, mixed with 0.5 mL of the Folin-Ciocalteu reagent (Merck, South Africa) in a test tube and then carefully mixed using a vortex. Following this, 10 mL of a 35% sodium carbonate solution was added to the mixture and the mixture was transferred to a 10 mL volumetric flask and the volume was brought to 10 mL using distilled water. Following mixing, the mixture was incubated at 25 °C for 30 min in the dark. A set of reference standard solutions of tannic acid ranging from 0.625 to 1 mg/mL were similarly prepared as described earlier. The absorbance was measured at 725 nm and the TTC was expressed in milligrams of tannic acid equivalent (TAE) per gram of crude extract. The regression equation ($y = 3.8869x - 0.023$ with $R^2 = 0.993$) was used to estimate the TTC content of the crude extracts.

Total flavonoid content

The total flavonoid content (TFC) of the crude extracts was assessed using the aluminium chloride colourimetric method described by Matotoka et al. [39]. Firstly, 100 µL of the 10 mg/mL crude extracts were mixed with 4.9 mL of distilled water in a test tube. Thereafter, 300 µL of 5% sodium nitrite dissolved in distilled water was added to the mixture and incubated at 25 °C for 5 min. After incubation, 300 µL of 10% aluminium chloride solution prepared by dissolving in distilled water was added to the mixture. The reaction was allowed to continue for 5 min before 2 mL of 1 M sodium hydroxide was added. Varying concentrations of quercetin ranging from 0.016 to 0.125 mg/mL were used to construct a standard curve. The absorbance was measured at 510 nm and TFC was expressed in milligrams quercetin equivalent (QE) per gram of crude extract. The regression equation ($y = 3.4379x + 0.0017$ with $R^2 = 0.996$) was used to estimate the TFC content of the crude extracts.

Statistical analysis

The GraphPad Prism Version 8.0 was used to analyse graphical data, which was reported as mean ± standard error of the mean. The data was assessed for normality using the Shapiro-Wilk test and statistical significance was verified using the One-Way analysis of variance (ANOVA) Tukey-Kramer Multiple Comparison Test, with asterisks (*), (**), (***) and (****) indicating $p < 0.05$, $p < 0.001$, $p < 0.0005$ and $p < 0.0001$, respectively. Moreover, the statistical analysis of mycochemical contents in the non-cytotoxic crude extracts was assessed row-wise using ANOVA, followed by Tukey's multiple comparison post hoc test. The results are represented as values that have different lowercase superscripts ($^{a, b,c\cdots}$), indicating significant differences at $p < 0.05$.

Results

Morphological and molecular identification of endophytic fungi

A total of 18 fungal endophytes were isolated from the stems of *K. acuminata* Oliv. The fungal endophyte isolates were differentiated based on their colony morphological features (Fig. 1). Thereafter, molecular identification by sequencing of the ITS region revealed that the endophytic fungal isolates belonged to 11 different species which were distributed among three genera; namely, *Neofusicoccum*, *Diaporthe* and *Pseudofusicoccum* (Table 1). The highest number of isolates belonged to the

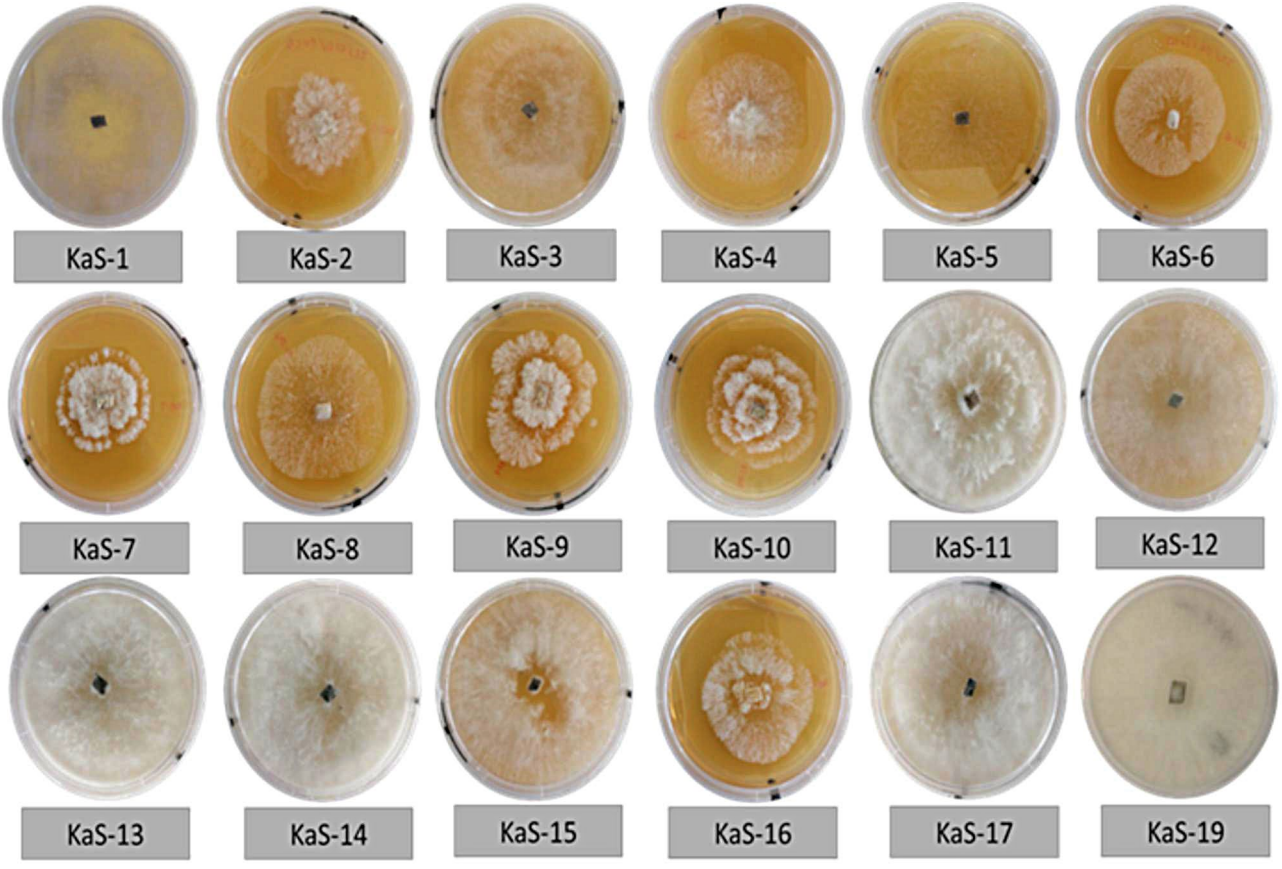

Fig. 1 Morphological characteristics of endophytic fungi from *Kirkia acuminata* Oliv. grown on potato dextrose agar (PDA)

Table 1 Colony morphological features and molecular identification of fungal endophytes of *Kirkia acuminata* Oliv stems

Isolate code	Colony description	Endophytic fungi	NCBI Genbank best match	Percent (%) ID
KaS-1	Irregular form, raised elevation, undulate margin, smooth surface and an initially white colony that turns greyish green over time. Reverse colony was dark grey to black.	*Neofusicoccum parvum*	KU997560.1	100
KaS-2	Irregular form, umbonate elevation, filiform margin, wavy surface and a cream-white colony. Reverse colony was creamy-white to yellow.	*Diaporthe* sp.	KY962982.1	98.28
KaS-3	Filamentous form, raised elevation, undulate margin, fluffy surface and an initially white colony that turns greyish green over time. Reverse colony was olivaceous to dark-grey.	*Neofusicoccum parvum*	KU997560.1	100
KaS-4	Filamentous form, umbonate elevation, filiform margin, wavy surface and cream-white mycelium with patches of green spots around the centre. Reverse colony was greenish-yellow.	*Diaporthe macadamiae*	NR_168240.1	98.43
KaS-5	Filamentous form, flat elevation, filiform margin, smooth surface and dark grey colony. Reverse colony was olivaceous to grey-olivaceous.	*Pseudofusicoccum olivaceum*	MH863433.1	99.83
KaS-6	Irregular and filamentous form, umbonate elevation, filiform margin, wavy and fluffy surface, with an orange colony. Reverse colony was creamy-white to orange.	*Diaporthe neotheicola*	KC145902.1	97.57
KaS-7	Irregular form, flat elevation, undulate margin, rough surface and a peach-coloured colony with a green centre. Reverse colony was creamy-white to yellow.	*Diaporthe* sp.	MT355681.1	99.31
KaS-8	Filamentous and fluffy form, umbonate elevation, filiform margin, smooth surface and cream-white colony. Reverse colony was creamy-white.	*Diaporthe parapterocarpi*	NR_168152.1	99.30
KaS-9	Irregular form, flat elevation, undulate margin, rough and wavy surface and a yellow colony. Reverse colony was creamy-white to yellow.	*Diaporthe arengae*	NR_111843.1	98.58
KaS-10	Irregular form, flat elevation, undulated margin, rough surface and a peach colony. Reverse colony was creamy-white to yellow.	*Diaporthe* sp.	MT355681.1	99.31
KaS-11	Circular form, raised elevation, undulated margin, smooth surface and an initially white that turns to dark grey over time. Reverse colony was olivaceous to dark-grey.	*Neofusicoccum parvum*	KU997399.1	100
KaS-12	Irregular form, raised elevation, undulate margin, smooth surface and an initially white colony which later turns to a dark grey colour. Reverse colony was dark-grey to black.	*Neofusicoccum parvum*	KU997560.1	100
KaS-13	Circular form, raised elevation, entire margin, fluffy surface and a grey colony. Reverse colony was olivaceous to dark-grey.	*Neofusicoccum parvum*	KU997399.1	100
KaS-14	Circular form, raised elevation, entire margin, fluffy surface and an initially white colony which later changes to a grey-greenish colony. Reverse colony was olivaceous to dark-grey.	*Neofusicoccum parvum*	KU997399.1	100
KaS-15	Irregular form, raised elevation, undulated margin, fluffy surface and grey colony. Reverse colony was dark grey to black.	*Neofusicoccum parvum*	KU997560.1	100
KaS-16	Irregular form, umbonate elevation, undulate margin, wavy and fluffy surface and white-orange colony. Reverse colony was creamy-white to orange.	*Diaporthe vangueriae*	KJ869137.1	99.65
KaS-17	Circular form, raised elevation, entire margin, fluffy surface and an initially white colony that turns grey in time. Reverse colony was olivaceous to dark-grey.	*Neofusicoccum parvum*	KU997399.1	100
KaS-19	Circular form, flat elevation, entire margin, fluffy surface and grey colony. Reverse colony was dark-grey to black.	*Neofusicoccum kwambonambiense*	KU997386.1	100

genus *Neofusicoccum* (50%), followed by *Diaporthe* (44%) and *Pseudofusicoccum* (6%).

Antimicrobial activity of the endophytic fungal crude extracts

The antimicrobial activity of the crude extracts of the 18 endophytic fungal isolates was assessed using the broth micro-dilution assay to determine the MICs. The crude extracts displayed varying antimicrobial activity against the test pathogens, with the exception of *P. olivaceum* KaS-5 which did not exhibit any inhibitory activity. The MIC values of the crude extracts ranged from 0.31 to 2.5 mg/mL against the bacterial cultures and from 1.25 to 2.5 mg/mL against *C. albicans* (Table 2). The isolates *N. parvum* KaS-11, *N. parvum* KaS-13 and *N. parvum* KaS-14 displayed high inhibitory activity against all the bacterial pathogens with MICs of 0.63 mg/mL. However, these extracts were less effective against *C. albicans* with MICs of 2.5 mg/mL. Interestingly, the crude extract of the *N. parvum* KaS-17 isolate displayed high potency against *S. aureus* with an MIC value of 0.31 mg/mL.

Cytotoxicity effects of fungal endophyte crude extracts

The cytotoxic profiles of the crude extracts of the fungal endophytes were assessed against the non-cancerous embryonic kidney HEK–293 cell line using the MTT assay. The *in vitro* analysis revealed that the crude extracts had both dose-dependent cytotoxicity (Fig. 2) and non-cytotoxic and insignificant effects (Fig. 3) against the HEK–293 cell lines. Higher concentrations of the crude extracts (≥ 62.5) significantly ($p < 0.0001$) inhibited the viability of HEK-293 cells, whilst lower

Table 2 The minimum inhibitory concentrations (MIC) of the endophytic fungal crude extracts (mg/mL)

Endophytic fungi	*S. aureus*	*E. coli*	*E. faecalis*	*P. aeruginosa*	*C. albicans*
Neofusicoccum parvum KaS-1	1.25	2.5	1.25	2.5	2.5
Diaporthe sp. KaS-2	1.25	2.5	1.25	2.5	2.5
Neofusicoccum parvum KaS-3	1.25	2.5	1.25	2.5	2.5
Diaporthe macadamiae KaS-4	1.25	2.5	1.25	2.5	2.5
Pseudofusicoccum olivaceum KaS-5					
Diaporthe neotheicola KaS-6	1.25	1.25	1.25	1.25	1.25
Diaporthe sp. KaS-7	1.25	1.25	1.25	1.25	2.5
Diaporthe parapterocarpi KaS-8	1.25	1.25	1.25	1.25	1.25
Diaporthe arengae KaS-9	1.25	1.25	1.25	1.25	2.5
Diaporthe sp. KaS-10	1.25	2.5	1.25	1.25	2.5
Neofusicoccum parvum KaS-11	0.63	0.63	0.63	0.63	2.5
Neofusicoccum parvum KaS-12	1.25	0.63	1.25	1.25	2.5
Neofusicoccum parvum KaS-13	0.63	0.63	0.63	0.63	2.5
Neofusicoccum parvum KaS-14	0.63	0.63	0.63	0.63	2.5
Neofusicoccum parvum KaS-15	1.25	1.25	1.25	1.25	1.25
Diaporthe vangueriae KaS-16	1.25	1.25	1.25	1.25	1.25
Neofusicoccum parvum KaS-17	0.31	0.63	0.63	1.25	1.25
Neofusicoccum kwambonambiense KaS-19	1.25	1.25	1.25	1.25	1.25
Chloramphenicol	0.039	0.020	0.039	0.020	
Amphotericin B					0.020
DMSO	25%	25%	25%	25%	25%

*: undetected activity at tested concentrations (mg/mL)

concentrations showed little to no cytotoxicity against these non-cancerous cells.

The crude extract of *N. parvum* KaS-12 was shown to be the most cytotoxic ($p < 0.0001$), with an IC_{50} of 125 µg/mL. The crude extracts of both *N. parvum* KaS-1 and *N. parvum* KaS-11 had an IC_{50} of 500 µg/mL, while the crude extracts of *Diaporthe* sp. KaS-2, *N. parvum* KaS-14 and *N. parvum* KaS-17 had an IC_{50} of 1000 µg/mL. Notably, only the crude extracts of isolates *N. parvum* KaS-3, *D. macadamiae* KaS-4, *P. olivaceum* KaS-5 and *D. neotheicola* KaS-6 showed no cytotoxic effects against the HEK-293 cells and were therefore, regarded as safe.

Anticancer activity of selected crude extracts

The anticancer potential of the non-cytotoxic crude extracts of the isolates *N. parvum* KaS-3, *D. macadamiae* KaS-4, *P. olivaceum* KaS-5 and *D. neotheicola* KaS-6, were assessed against cervical cancer ME-180 and melanoma A375 cell lines using the alamarBlue assay. The crude extracts exhibited antiproliferative activity against the cervical ME-180 (Fig. 4) and melanoma A375 (Fig. 5) cancerous cells.

The best activity was exhibited by the crude extract of *D. macadamiae* KaS- 4, which significantly ($p < 0.05$) reduced the viability of ME-180 cervical cancer cells at 2 µg/mL. However, the crude extracts of *P. olivaceum* KaS-5 and *D. neotheicola* KaS-6 had a dose-dependent inhibitory action against the ME-180 cervical cancer cells, with increasing concentrations showing higher antiproliferative activity.

The cell viability of the cancerous cells ranged from 60 to 78%, revealing that none of the fungal endophyte crude extracts inhibited 50% of the ME-180 cervical cancer cell line's growth. The crude extract of *P. olivaceum* KaS-5 exhibited significant ($p < 0.05$) inhibitory action against A375 melanoma cells at 6 µg/mL. In comparison, the crude extracts of the *N. parvum* KaS-3, *D. macadamiae* KaS-4 and *D. neotheicola* KaS-6 isolates were less cytotoxic against the A375 melanoma cells. Moreover, none of the crude extracts inhibited 50% of the A375 melanoma cells as the cell viability of the cancer cells ranged from 60 to 82%.

Mycochemical composition of the crude extracts

The mycochemical composition of the non-cytotoxic crude extracts was assessed to determine the presence of polyphenols associated with both antimicrobial and anticancer activities. The analysis revealed the presence of phenols, tannins and flavonoids in all the crude extracts (Table 3). The TPC ranged from 12.71 to 63.59 mgGAE/g, whilst the TTC and TFC ranged from 3.41 to 14.44 mgTAE/g and 1.30 to 4.28 mgQE/g, respectively.

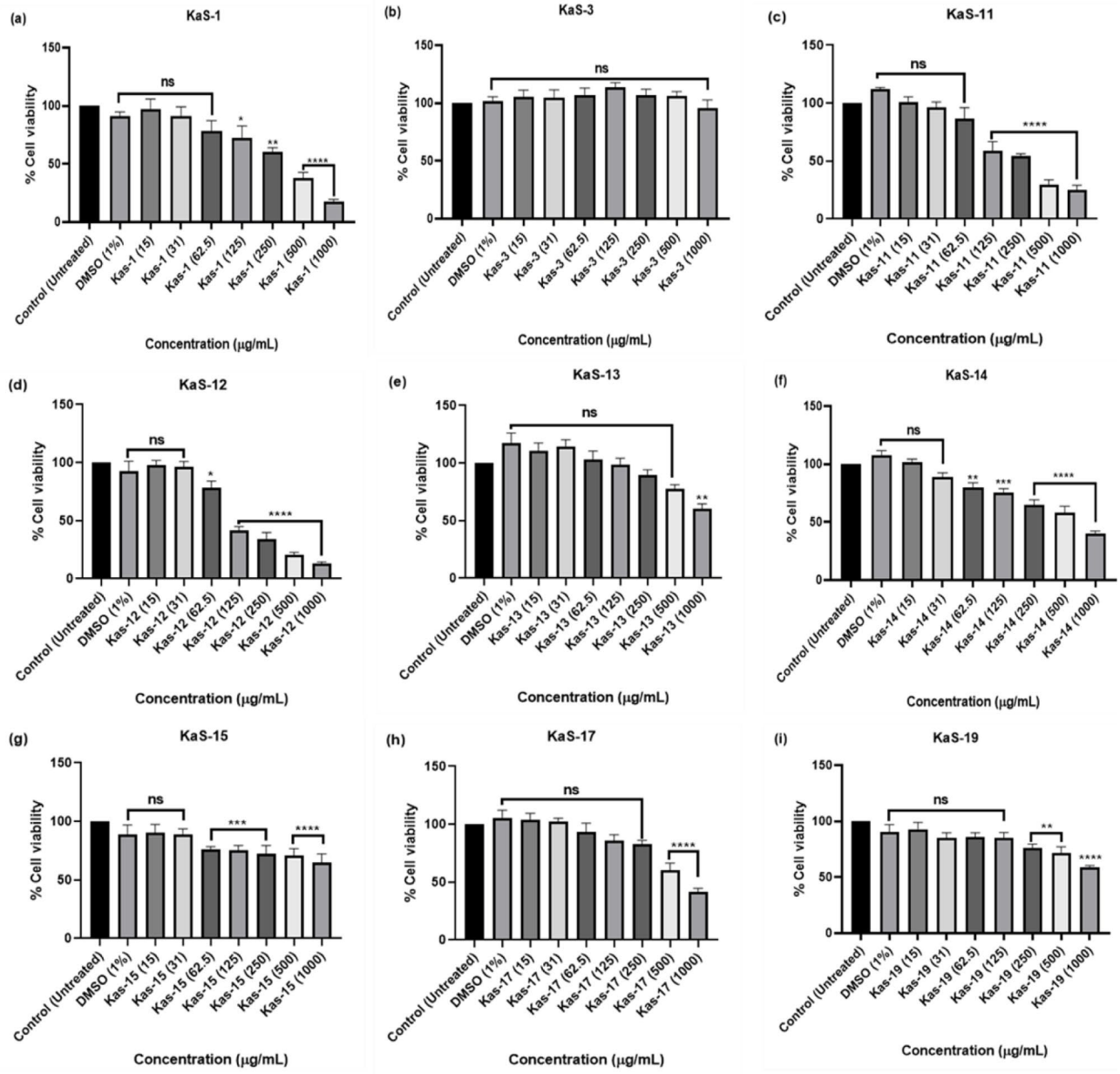

Fig. 2 Cytotoxicity of the crude extracts from *Neofusicoccum* isolates against HEK-293 cells. (**a**) KaS-1, (**b**) KaS-3, (**c**) KaS-11, (**d**) KaS-12, (**e**) KaS-13, (**f**) KaS-14, (**g**) KaS-15, (**h**) KaS-17 and (**i**) KaS-19. The percentage mean ± standard error of the mean of the data from three replications is represented in this figure. The difference in the cell viability was statistically analysed and presented as either ns: non-significant, *: Significant at $p < 0.05$, **: at $p < 0.001$, ***: at $p < 0.0005$ or ****: at $p < 0.0001$

Discussion

Isolation and identification of fungal endophytes

Fungal endophytes of medicinal plants have been widely investigated for the production of bioactive compounds with therapeutic potential owing to their ability to produce structurally and chemically diverse secondary metabolites [40]. However, the diversity and pharmacological potential of fungal endophytes associated with the large biodiversity of medicinal plants used in South Africa remains relatively under-explored [24]. In this study, 18 morphologically distinct fungal endophytes were isolated from the stems of *K. acuminata* Oliv. Interestingly, all the isolated endophytic fungi belonged to the phylum Ascomycota. Fungal endophytes belonging to the phylum Ascomycota are the most widely isolated, with reports suggesting that these microorganisms have an enhanced ability to inhabit the internal tissues of plants [41, 42]. Various species of fungal endophytes belonging to the genera *Diaporthe*, *Neofusicoccum* and *Pseudofusicoccum* have been previously isolated from various host

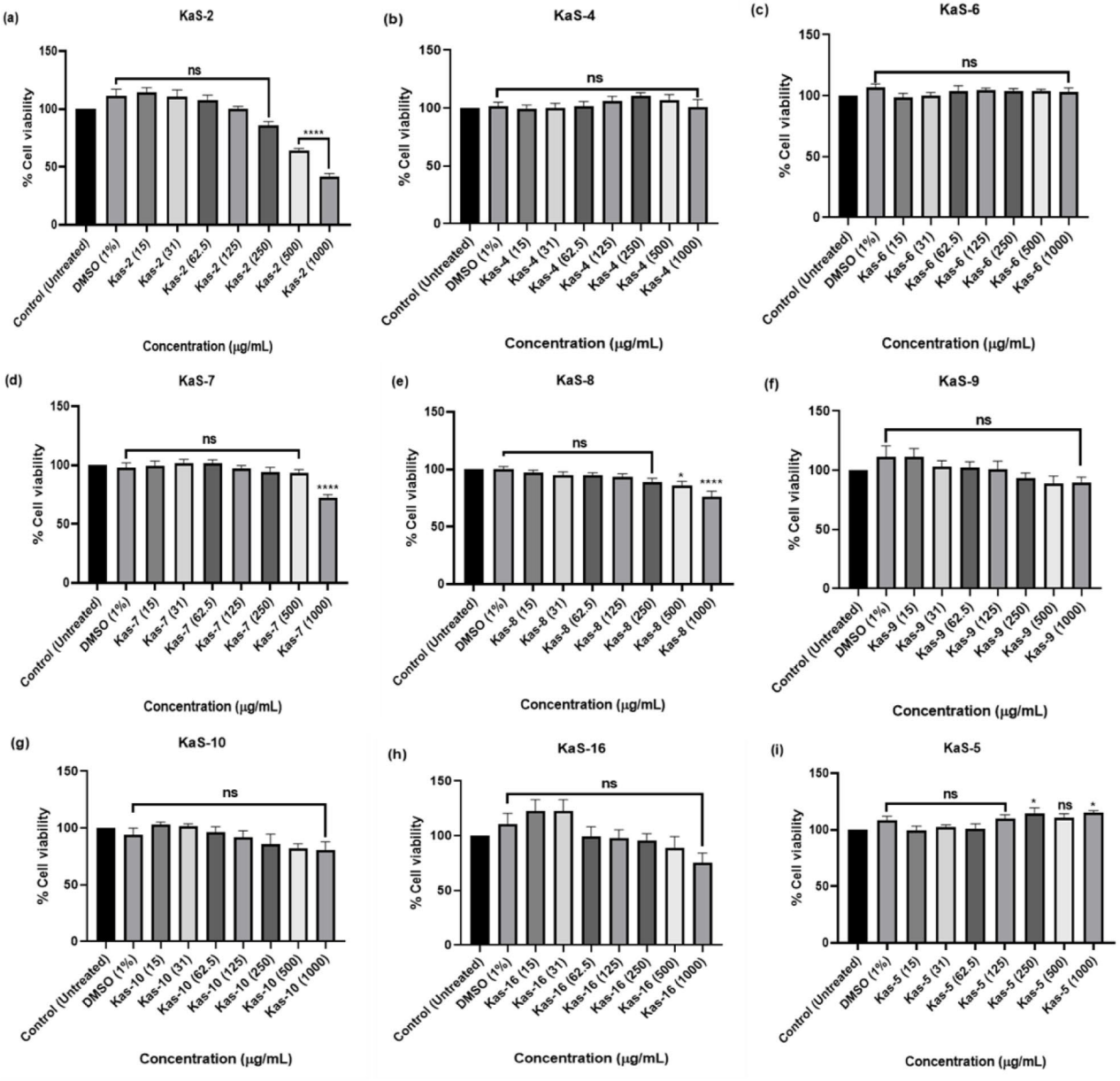

Fig. 3 Cytotoxicity of the crude extracts from *Diaporthe* (**a**-**h**) and *Pseudofusicoccum* (**i**) isolates against HEK-293 cells. (**a**) KaS-2, (**b**) KaS-4, (**c**) KaS-6, (**d**) KaS-7, (**e**) KaS-8, (**f**) KaS-9, (**g**) KaS-10, (**h**) KaS-16 and (**i**) KaS-5. The percentage mean ± standard error of the mean of the data from three replications is represented in this figure. The difference in the cell viability was statistically analysed and presented as either ns: non-significant, *: Significant at $p < 0.05$, **: at $p < 0.001$, ***: at $p < 0.0005$ or ****: at $p < 0.0001$

plants. Owing to their ability to occupy multiple hosts, members of the genus *Diaporthe* are amongst the most isolated fungal endophytes occurring in the stems of various host plants in different environments [43–45]. It is important to note that the low yields and diversity of fungal endophytes obtained in the study may have been influenced by factors such as environmental conditions, sampling period, geographical location, surrounding vegetation and surface sterilisation method used [46]. In the current study, the different *Diaporthe* species accounted for 44% of the isolates. The genera *Neofusicoccum* and *Pseudofusicoccum*, both of which belong to the family *Botryosphaeriaceae*, are usually present in many woody plants [47, 48]. de Almeida et al. [49]. revealed the prevalence of endophytic *Neofusicoccum* species in asymptotic twigs, petioles, roots and offshoots of *Vitis vinifera* L. and other *Vitis* species. Similarly, Sessa et al. [50]. reported the presence of these fungal species in twigs of *Malus domestica* Borkh., *Prunus persica* (L.) Batsch., *Pyrus communis* L. and *Vaccinium corymbosum* L. In our study, *Neofusicoccum* species accounted for 50% of the fungal endophytes isolated. Interestingly, the eight *N. parvum*

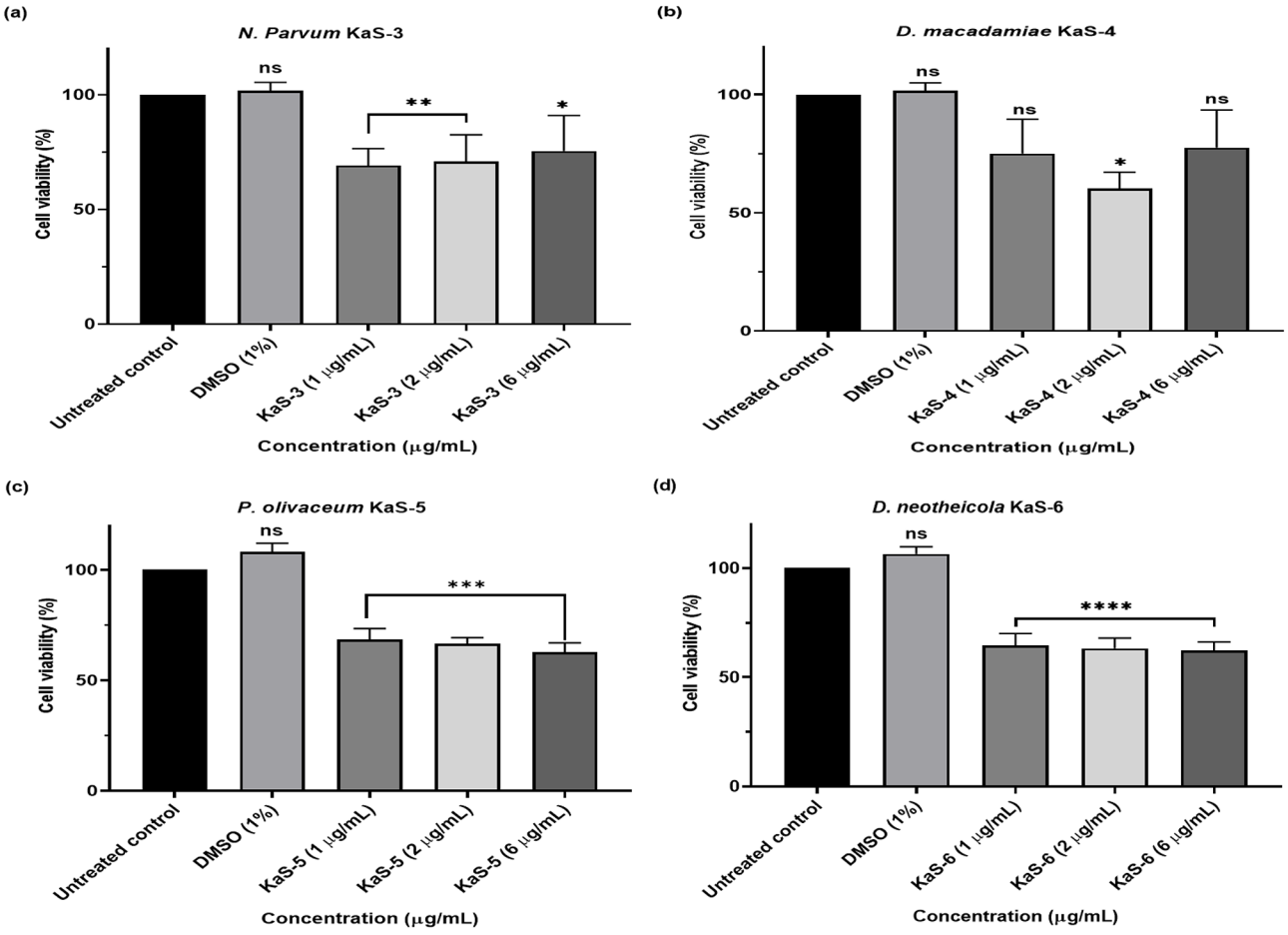

Fig. 4 The effect of non-cytotoxic endophytic fungal crude extracts against the cervical cancer ME-180 cell line. (**a**) *N. parvum* KaS-3, (**b**) *D. macadamiae* KaS-4, (**c**) *P. olivaceum* KaS-5 and (**d**) *D. neotheicola* KaS-6. Data from four different experiments were shown as ± standard error of the mean, with significant differences presented as either ns: non-significant, *: Significant at $p < 0.05$, **: at $p < 0.001$, ***: at $p < 0.0005$ or ****: at $p < 0.0001$

isolates showed 100% genetic similarity but they exhibited varied morphological features in PDA. This highlights that genetic similarity of isolates does not directly correlate to morphological uniformity. Moreover, it is important to note that the analysis of a single molecular marker used for ITS sequencing has the potential to underestimate genuine species diversity in closely related or enigmatic species [51], which may have led to the different strains being incorrectly grouped or identified as the same species. Despite the *Pseudofusicoccum* species generally possessing a wide host range of woody plants [48], only one isolate belonging to the genus was obtained in this study. As in the current study, different South African plants have been reported to host various fungal endophytic species belonging to the genera *Neofusicoccum* [52] and *Pseudofusicoccum* [53].

Antimicrobial action of fungal endophyte extracts

The ability of fungal endophytes to produce structurally and chemically diverse secondary metabolites with the potential to aid in the battle against the global challenge of increasing AMR has piqued the interest of the scientific community [54]. In this study, 17 of the 18 fungal endophyte crude extracts exhibited antimicrobial activity toward *S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa* and *C. albicans*. In contrast to the high frequency of fungal endophytes exhibiting antimicrobial activity in the current study, Nurunnabi et al. [55]. reported that only 50% of ethyl acetate crude extracts from 12 fungal endophytes of *Sonneratia apetala* Buch.-Ham possessed antimicrobial activity against more than one tested human pathogenic strain. Similarly, Gurgel et al. [56]. reported that a much lower percentage (17%) of the ethyl acetate crude extracts of fungal endophytes from the stems of *Arrabidaea chica* Bonpl. exhibited inhibitory activity against the tested pathogens. Interestingly, Mabadahanye et al. [57] reported that the methanol, acetone, dichloromethane and ethyl acetate extracts obtained from the stems of *K. acuminata* Oliv. lacked inhibitory activity against *E. coli* while displaying MICs of 1.25 mg/mL against *S. aureus*. Notably, the inhibitory activities reported for the plant extracts are much lower than the inhibitory

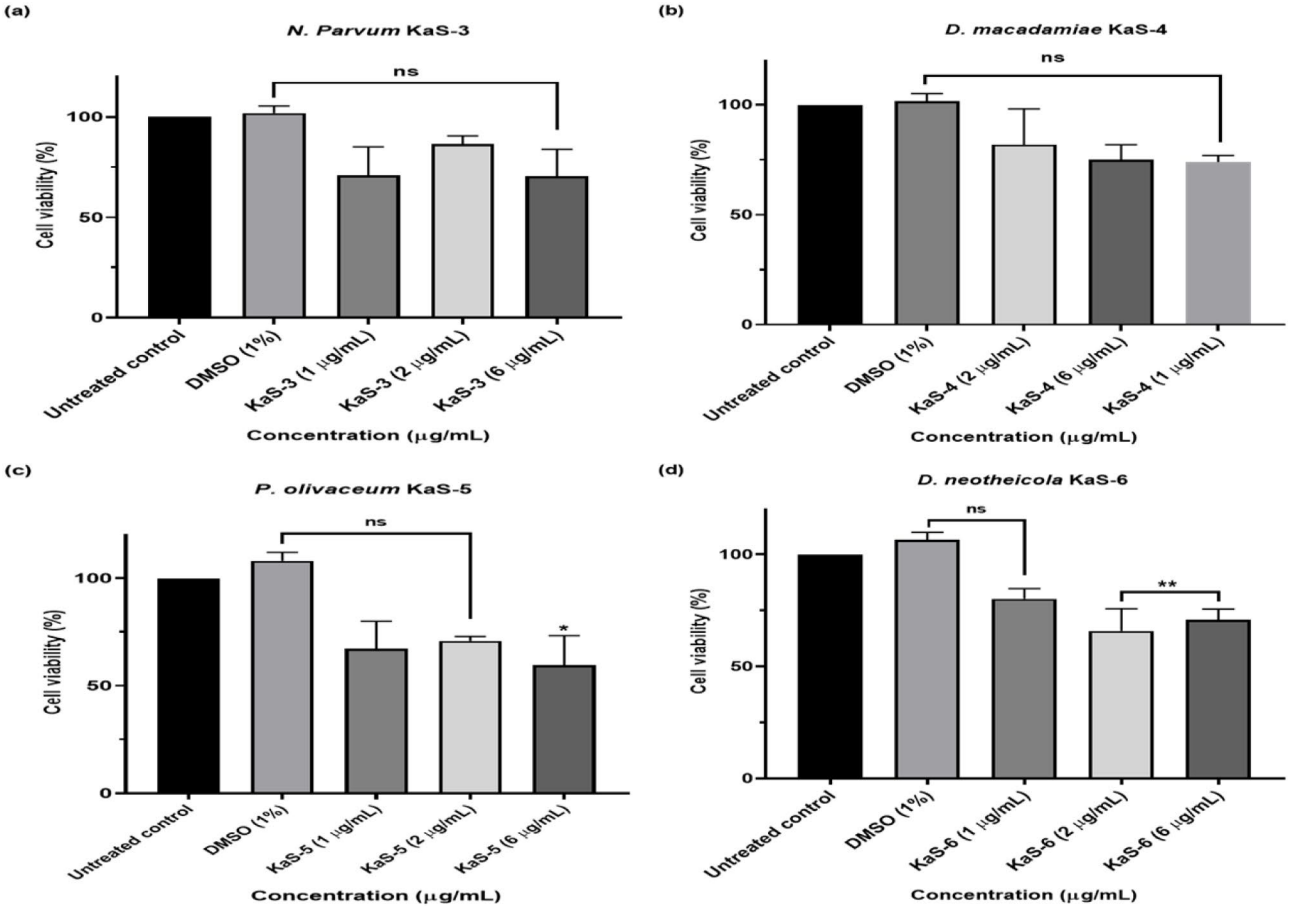

Fig. 5 The effect of non-cytotoxic endophytic fungal crude extracts against the A375 melanoma cell line. (**a**) *N. parvum* KaS-3, (**b**) *D. macadamiae* KaS-4, (**c**) *P. olivaceum* KaS-5 and (**d**) *D. neotheicola* KaS-6. Data from four different experiments were shown as ± standard error of the mean, with significant differences presented as either ns: non-significant, *: Significant at $p < 0.05$ or **: at $p < 0.001$

Table 3 Quantitative mycochemical composition of the non-cytotoxic crude extracts

Endophytic fungi	TPC (mgGAE/g)	TTC (mgTAE/g)	TFC (mgQE/g)
Neofusicoccum parvum KaS-3	14.18 ± 0.92 [a]	3.41 ± 0.23 [b]	2.11 ± 0.26 [c]
Diaporthe macadamiae KaS-4	12.71 ± 1.29 [a]	3.66 ± 0.32 [b]	1.30 ± 0.28 [c]
Pseudofusicoccum olivaceum KaS-5	63.59 ± 2.28 [a]	14.44 ± 0.87 [b]	4.28 ± 0.17 [c]
Diaporthe neotheicola KaS-6	26.56 ± 1.09 [a]	6.71 ± 0.22 [b]	2.06 ± 0.10 [c]

Data is expressed as a mean of three replicates ± standard deviation. GAE: Gallic acid equivalent, QE: Quercetin equivalent, TAE: Tannic acid equivalent, TFC: Total flavonoid content, TPC: Total phenol content and TTC: Total tannin content. Values sharing the common lowercase superscript ([a], [b], [c]) are statistically non-significant row-wise at $p < 0.05$ (Tukey's test)

activities observed for the crude extracts of the fungal endophytes used in the current study. This was more apparent for the ethyl acetate extract of the plant which completely lacked inhibitory activity against the tested bacterial pathogens [57]. Furthermore, the majority of the crude extracts tested in this study displayed higher inhibitory activity against the Gram-positive bacteria than the Gram-negative bacteria. This may be a consequence of the presence of the unique outer membrane of Gram-negative bacteria, which limits the exchange of molecules between the cell and the exterior environment and contributes to the high levels of inherent multidrug resistance of these bacteria [58–60]. It is also possible that the difference in inhibitory activity of the crude extracts is due to the differences in metabolites produced by the fungal endophytes which maybe be more effective against the Gram-positive bacteria than the Gram-negative bacteria. This necessitates the characterisation and identification of the bioactive compounds within the crude extracts to ascertain their potentials as antimicrobial agents. It is important to note that the crude extracts displayed inhibitory against *E. coli* and *C. albicans*, both of which are commensal microorganisms of the human body [61, 62]. As such, it may be necessary to investigate

their mode of action and effects on virulence factors, to develop targeted and safer antimicrobials from these crude extracts [63]. Previous studies have reported on the antimicrobial potential of fungal endophytes belonging to the *Pseudofusicoccum* [64, 65], *Diaporthe* [66, 67] and *Neofusicoccum* [68, 69] genera. de Carvalho et al. [70]. reported that the ethanol crude extracts of the *Diaporthe* sp. 1 UFMGCB 7696, *Diaporthe* sp. 14 UFMGCB 7927, *D. miriciae* UFMGCB 7646 and *D. miriciae* UFMGCB 7719, isolated from *Copaifera pubiflora* Benth., displayed inhibitory activity against *E. coli* and *S. aureus* with MICs ranging from 1.25 to 2.5 mg/mL. Cadamuro et al. [71]. reported that the methanolic/dichloromethane crude extracts of fungal endophyte *N. parvum* isolated from *Avecinnia schaueriana* Stapf & Leechm. ex Moldenke displayed antibacterial activity at 0.5 mg/mL against *Listeria monocytogenes* and *E. coli*, with no inhibitory activity against *C. albicans*. Interestingly, the most significant antimicrobial activity in this study was observed with the crude extracts of *N. parvum* KaS-11, *N. parvum* KaS-13, *N. parvum* KaS-14 and *N. parvum* KaS-17, which inhibited all the tested pathogens. Mishra et al. [72]. previously reported MICs of 5 mg/mL for the ethyl acetate crude extracts of an endophytic *Pseudofussicoccum adansoniae* isolated from *Tinospora cordifolia* (Willd.) Miers against eight clinical pathogens. This was in contrast to the results obtained in this study wherein the *P. olivaceum* KaS-5 isolate lacked inhibitory activity against all the tested pathogens. The differences in antimicrobial activity may be attributed to the differences in concentrations used with Mishra et al. [72]. using higher concentrations than the ones used in the current study. It is also possible that the isolate is a passive endophyte that does not directly confer any beneficial biological activities to the host plant [73] or it may serve a different important ecological role within the host plant tissues.

Cytotoxic effects of the crude extracts

The search for new therapeutic agents demands the discovery of optimal drugs that selectively target diseased cells while having no harmful effect on host cells [74]. Even though natural products from both plants and endophytic fungi are regarded as safe, these products may have adverse cytotoxic effects against non-cancerous cells [75]. This highlights the need to screen the safety of crude extracts to detect and rule out cytotoxic compounds. In this study, only the crude extracts of *N. parvum* KaS-3, *D. macadamiae* KaS-4, *P. olivaceum* KaS-5 and *D. neotheicola* KaS-6 displayed no cytotoxicity against the non-cancerous HEK-293 cells. Similarly, the ethyl acetate crude extracts of the endophytic fungi, *Talaromyces purpureogenus* [76] and *Paraconiothyrium brasiliense* [77] have been reported to be non-cytotoxic against non-cancerous HEK-293 cells. According to Naveen et al. [78], the safety of the crude extracts against HEK-293 cells may be due to the presence of natural antioxidant compounds within the crude extracts that promote the viability of the non-cancerous cells. However, the safety of the crude extracts may also be ascribed to their selective toxicity towards different cell types other than the HEK-293 cells. The crude extracts of *Neofusicoccum parvum* [71] and *Diaporthe* spp. 94 strain [79] have been reported to have adverse cytotoxic effects against the non-cancerous VERO monkey kidney fibroblasts and human skin fibroblasts from the HFF-1 cell line, respectively. Thus, there is a need to purify and identify the active non-cytotoxic compounds within the crude extracts to develop novel and safer therapeutic agents.

Anticancer action of the crude extracts

The increasing incidence of cancer in the global population is a concerning risk factor that threatens global health. The anticancer drugs which are currently used have limitations as they fail to eradicate cancer cells, have detrimental side effects on patients and are becoming less effective due to the multidrug resistance development of tumours [80]. Owing to this, the anticancer potential of fungal endophytes has been widely assessed [81]. In this study, the non-cytotoxic crude extracts of the *N. parvum* KaS-3, *D. macadamiae* KaS-4, *P. olivaceum* KaS-5 and *D. neotheicola* KaS-6 isolates were tested against cervical cancer ME-180 and melanoma A375 cancerous cell lines. The four crude extracts exhibited variable inhibitory action against both the ME-180 and A375 cancerous cell lines. However, the lack of an IC_{50} for all of the crude extracts against both of the tested cancerous cell lines suggests that they possess moderate anticancer activity. In contrast, Liu et al. [82]. reported on the strong anti-cervical cancer activity of ethyl acetate crude extracts from two *Fusarium proliferatum* fungal endophyte isolates associated with *Ginkgo biloba* L.; namely, J-1 and J-3 strains, which inhibited 76.2% and 65% of the treated cervical cancer HeLa cells, respectively. However, these strains had no activity against lung cancer A549 cells. In their study, Pedra et al. [83]. reported on the anticancer activity of a fractionated dichloromethane (F_{DCM}) extract from *Achyrocline satureioides* (Lam.) DC-associated endophytic fungus, *Biscogniauxia* species, against A375 melanoma cells wherein only 30% of the cell viability was reduced by the extract. The authors also reported a dose- and time-dependent anticancer activity of the F_{DCM} extract as an IC_{50} of 10.34 and 6.89 µg/mL against the viability and proliferation of the cancerous A375 cells was observed after 72 h of treatment, respectively. Thus, the crude extracts from the current study may also have an increased potency against the tested cancerous cells after prolonged exposure. It is important to note that alamarBlue allows for only the metabolic activity of the

ME-180 and A375 cancerous cells to be measured [37]. Thus, the use of staining techniques [84], microscopy [85] and flow cytometry [86] could help characterise the cytological effects of the crude extracts on the cancerous cells. The ethyl acetate crude extracts of fungal endophytes have been reported to induce apoptosis in HeLa cervical cancer cell lines [87] and MCF-7 breast cancer cells [88]. These observations suggest that the antiproliferative activity of the fungal endophyte crude extracts in the current study may be due to an inhibitory mechanism that interrupts the cell cycle and activates apoptotic pathways. Thus, the mechanism of action of the crude extracts against the cancerous cell lines should also be investigated.

Mycochemical analysis of the crude extracts

Fungal endophytes are considered biochemical synthesisers in their host plants with the ability to produce various secondary metabolites [89]. In this study, the mycochemical analysis revealed the presence of phenols, tannins and flavonoids in the ethyl acetate crude extracts of fungal endophytes. The acetone and ethyl acetate crude extracts [57] as well as the methanol and dichloromethane crude extracts [90] of the *K. acuminata* Oliv. bark have been reported to possess phenols, flavonoids and tannins. This elucidates the ability of fungal endophytes to produce host-similar secondary metabolites. The detected polyphenols contain hydroxyl groups that have different pharmacological effects, including antibacterial, antifungal and anticancer activities, due to their ability to interact with enzymes, receptors and signalling pathways based on the number, arrangement and configuration of their hydroxyl groups [91–95]. Interestingly, the *P. olivaceum* KaS-5 crude extract, which possessed the highest polyphenol contents lacked antimicrobial activity against all the tested pathogens. This suggests that the antimicrobial activity may have been masked by the presence of antagonistic compounds in the crude extracts [96]. The anticancer activities of the endophytic fungus *Penicillium decumbens* MBS 3.2 against the human colorectal HCT116 cancerous cell line [97] and endophytic fungus *Penicillium chrysogenum* against the MCF-7 breast cancerous cell [98] have been ascribed to the high polyphenolic content of their ethyl acetate crude extracts. Interestingly, the four non-cytotoxic crude extracts of the fungal endophytes possessed high phenolic content, which may explain their selective activity against cervical cancer ME-180 and melanoma A375 cancerous cell lines. This was more evident in the crude extracts of *P. olivaceum* KaS-5 and *D. neotheicola* KaS-6 as their high polyphenol contents correlated to their increased activities on the tested cancerous cells. However, the moderate anticancer activities observed may have been due to a low concentration of the bioactive compounds in the fungal crude extracts due to unfavourable culture conditions in laboratory settings [99]. Thus, the influence of culture conditions on the production of bioactive metabolites needs to be investigated. Additionally, other complementary activities, such as antioxidant and anti-inflammatory activities, of the crude extracts should be assessed further to determine their pharmacological potential against chronic and degenerative diseases.

Conclusion

In this study, the *K. acuminata* Oliv. plant hosts a diverse group of fungal endophytes, highlighting the underexplored potential of South African medicinal plants as valuable sources of fungal endophyte strains with the ability to produce unique bioactive secondary metabolites. This diversity highlights the complex fungal community associated with *K. acuminata* Oliv. which may have important ecological roles within the plant. However, the community structure and interactions of the fungal endophytes need to be assessed to fully understand their ecological significance in the host plant. Notably, the fungal endophytes of *K. acuminata* Oliv. showed promising potential as sustainable sources of secondary metabolites with antimicrobial and selective anticancer properties that are crucial in drug discovery. The four non-cytotoxic crude extracts of the *N. parvum* KaS-3, *D. macadamiae* KaS-4, *P. olivaceum* KaS-5 and *D. neotheicola* KaS-6 isolates make them promising candidates for the discovery of therapeutic agents that do not have adverse effects on healthy cells. However, the respective mechanisms of action of the crude extracts against the tested bacterial and yeast pathogens as well as the cancerous cells should be investigated. The effect of growth conditions on the production and bioactivities of the secondary metabolites of the fungal endophytes should be investigated. Furthermore, identification and quantification of the bioactive compounds present in the crude extracts need to be further investigated to determine their potential as novel drug leads for pharmaceutical application.

Abbreviations

AMR	Antimicrobial resistant
ANOVA	Analysis of Variance
BLAST	Basic local alignment search tool
DMEM	Dulbecco's modification of eagle medium
DMSO	Dimethyl sulfoxide
FBS	Foetal bovine serum
GAE	Gallic acid equivalent
HEK-293	Human embryonic kidney
IC_{50}	Inhibitory concentration 50
INT	*p*-iodonitrotetrazolium violet
ITS	Internal transcribed spacer
KaS	*Kirkia acuminata* Oliv. stems
MIC	Minimum inhibitory concentration
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCBI	National Center for Biotechnology Information
OD_{625nm}	Optical density at 625 nm

PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PSN	Penicillin, streptomycin and neomycin
QE	Quercetin equivalent
TAE	Tannic acid equivalent
TFC	Total flavonoid content
TPC	Total phenol content
TTC	Total tannin content

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03964-y.

Supplementary Material 1

Acknowledgements
The authors would like to thank Dr Mashilo Matotoka for providing assistance during the antimicrobial screening and Mr Thabang Marema for the assistance during the cytotoxicity screening.

Author contributions
M.M. conducted the experimental work, analysed the results and wrote the initial manuscript. T.E.M. and Z.M. helped design the study, supervised the research and edited the manuscript. K.D. conceptualised the study, acquired the funding, supervised the research and edited the manuscript. All authors read and approved the final manuscript.

Funding
This work is based on the research supported wholly by the National Research Foundation (NRF Thuthuka) of South Africa [Grant Number: 138274] and the University of Limpopo. All the findings, conclusions and recommendations expressed in this publication are those of the authors, and the NRF accepts no liability whatsoever in this regard.

Data availability
All data generated during this study are included in this manuscript. The ITS sequence data have been deposited in the National Center for Biotechnology Information (NCBI) GenBank with accession numbers PQ867536 to PQ867553.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo, Private Bag X1106, Sovenga, Polokwane, Limpopo 0727, South Africa
[2]Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Private Bag X20, Hatfield, Pretoria, Gauteng 0028, South Africa
[3]Forestry and Agricultural Biotechnology Institute, University of Pretoria, Hatfield, Pretoria, Gauteng 0083, South Africa

Received: 13 January 2025 / Accepted: 14 April 2025
Published online: 30 May 2025

References

1. Mittal RP, Rana A, Jaitak V. Essential oils: an impending substitute of synthetic antimicrobial agents to overcome antimicrobial resistance. Curr Drug Targets. 2019;20:605–24.
2. Saeed U, Insaf RA, Piracha ZZ, Tariq MN, Sohail A, Abbasi UA, et al. Crisis averted: A world united against the menace of multiple drug-resistant superbugs -pioneering anti-AMR vaccines, RNA interference, nanomedicine, CRISPR-based antimicrobials, bacteriophage therapies, and clinical artificial intelligence strategies to safeguard global antimicrobial arsenal. Front Microbiol. 2023;14:1270018.
3. van Elsland D, Neefjes J. Bacterial infections and cancer. EMBO Rep. 2018;19:46632.
4. Wang J, Yao Y, Zhang Q, Li S, Tang L. Inflammatory responses induced by *Helicobacter pylori* on the carcinogenesis of gastric epithelial GES-1 cells. Int J Oncol. 2019;54:2200–10.
5. Shrestha E, Coulter JB, Guzman W, Ozbek B, Hess MM, Mummert L, et al. Oncogenic gene fusions in nonneoplastic precursors as evidence that bacterial infection can initiate prostate cancer. Proc Natl Acad Sci USA. 2021;118:2018976118.
6. WHO, Cancer Tomorrow, World Health Organization. World Health Organization. 2025. https://gco.iarc.who.int/tomorrow/en/dataviz/isotype?types=126single_unit=500000 (accessed March 23, 2025).
7. Sun D, Gao W, Hu H, Zhou S. Why 90% of clinical drug development fails and how to improve it? Acta Pharm Sin B. 2022;12:3049–62.
8. Nanayakkara AK, Boucher HW, Fowler VG, Jezek A, Outterson K, Greenberg DE. Antibiotic resistance in the patient with cancer: escalating challenges and paths forward. CA Cancer J Clin. 2021;71:488–504.
9. Chaughule RS, Barve RS. Role of herbal medicines in the treatment of infectious diseases. Vegetos. 2024;37:41–51.
10. Mudau TE, Olowoyo JO, Amoo SO. Ethnobotanical assessment of medicinal plants used traditionally for treating diabetes in Vhembe district, Limpopo Province, South Africa. S Afr J Bot. 2022;146:304–24.
11. Asigbaase M, Adusu D, Anaba L, Abugre S, Kang-Milung S, Acheamfour SA, et al. Conservation and economic benefits of medicinal plants: insights from forest-fringe communities of Southwestern Ghana. Trees People. 2023;14:100462.
12. Ganie AH, Tali BA, Khuroo AA, Reshi ZA, Nawchoo IA. Impact assessment of anthropogenic threats to high-valued medicinal plants of Kashmir Himalaya, India. J Nat Conserv. 2019;50:125715.
13. Mishra S, Sahu PK, Agarwal V, Singh N. Exploiting endophytic microbes as micro-factories for plant secondary metabolite production. Appl Microbiol Biotechnol. 2021;105:6579–96.
14. Sopalun K, Laosripaiboon W, Wachirachaikarn A, Iamtham S. Biological potential and chemical composition of bioactive compounds from endophytic fungi associated with Thai Mangrove plants. S Afr J Bot. 2021;141:66–76.
15. Sułkowska-Ziaja K, Trepa M, Olechowska-Jarząb A, Nowak P, Ziaja M, Kała K, et al. Natural compounds of fungal origin with antimicrobial activity—Potential cosmetics applications. Pharmaceuticals. 2023;16:1200.
16. Wen J, Okyere SK, Wang S, Wang J, Xie L, Ran Y, et al. Endophytic fungi: an effective alternative source of plant-derived bioactive compounds for Pharmacological studies. J Fungi. 2022;8:205.
17. Ding CH, Wang QB, Guo S, Wang ZY. The improvement of bioactive secondary metabolites accumulation in *Rumex gmelini* Turcz through co-culture with endophytic fungi. Braz J Microbiol. 2018;49:362–9.
18. Stierle A, Strobel G, Stierle D. Taxol and Taxane Production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science (1979) 1993;260:214–6.
19. Tiwari P, Bae H. Endophytic fungi: key insights, emerging prospects, and challenges in natural product drug discovery. Microorganisms. 2022;10:360.
20. Khadka G, Annamalai T, Shetty KG, Tse-Dinh YC, Jayachandran K. Isolation and characterization of fungal endophytes from *Petiveria alliacea* and their antimicrobial activities in South Florida. Microbiol Res. 2023;14:1470–82.
21. Prajapati J, Goswami D, Rawal RM. Endophytic fungi: A treasure trove of novel anticancer compounds. Curr Res Pharmacol Drug Discov. 2021;2:100050.
22. Zhang D, Sun W, Xu W, Ji C, Zhou Y, Sun J, et al. Antimicrobial and cytotoxic activity of endophytic fungi from *Lagopsis Supina*. J Microbiol Biotechnol. 2023;33:543–51.
23. Mohamed IE, Alati TAA. Antimicrobial activity and cytotoxicity of endophytic fungi associated with four medicinal plants from Sudan. J Phytopharmacol. 2020;9:348–55.
24. Abdalla MA, McGaw LJ. Bioprospecting of South African plants as a unique resource for bioactive endophytic microbes. Front Pharmacol. 2018;9:456.

25. Maroyi A. *Kirkia acuminata* Oliv.: A review of its ethnobotany and Pharmacology. Afr J Tradit Complement Altern Med. 2017;14:217–26.
26. Mongalo NI, Makhafola TJ. Ethnobotanical knowledge of the Lay people of Blouberg area (Pedi tribe), Limpopo Province, South Africa. J Ethnobiol Ethnomed. 2018;14:1–23.
27. Shan Z, Zhang Q, Qi Y, Ye J, Hao D, Xiao P, et al. Production regionalization of commonly used medicinal plants in China based on botanical big data. Ind Crops Prod. 2023;202:117024.
28. Abdalla MA, Aro AO, Gado D, Passari AK, Mishra VK, Singh BP, et al. Isolation of endophytic fungi from South African plants, and screening for their antimicrobial and extracellular enzymatic activities and presence of type I polyketide synthases. S Afr J Bot. 2020;134:336–42.
29. Toghueo RMK, Zabalgogeazcoa I, Vázquez de Aldana BR, Boyom FF. Enzymatic activity of endophytic fungi from the medicinal plants *Terminalia catappa*, *Terminalia mantaly* and *Cananga odorata*. S Afr J Bot. 2017;109:146–53.
30. White TJ, Bruns TD, Lee SB, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: A guide to methods and applications. San Diego: Academic; 1990;18:315–22.
31. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. 1997;25:3389–402.
32. Rashid TS. Bioactive metabolites from tomato endophytic fungi with antibacterial activity against tomato bacterial spot disease. Rhizosphere. 2021;17:100292.
33. dos Santos GD, Gomes RR, Gonçalves R, Fornari G, Maia BHLNS, Schmidt-Dannert C, et al. Molecular identification and antimicrobial activity of foliar endophytic fungi on the Brazilian pepper tree (*Schinus terebinthifolius*) reveal new species of *Diaporthe*. Curr Microbiol. 2021;78:3218–29.
34. Zouirech O, Alyousef AA, El Barnossi A, El Moussaoui A, Bourhia M, Salamatullah AM, et al. Phytochemical analysis and antioxidant, antibacterial, and antifungal effects of essential oil of black Caraway (*Nigella sativa* L.) seeds against drug-resistant clinically pathogenic microorganisms. Biomed Res Int. 2022;2022:5218950.
35. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 1998;64:711–3.
36. Makgoo L, Laka K, Mbita Z. Downregulation of variant 1 during arsenic trioxide-mediated cell cycle arrest and curcumin-induced apoptosis in MCF-7 breast cancer cells. Future Sci OA. 2019;5:409.
37. Hamid R, Rotshteyn Y, Rabadi L, Parikh R, Bullock P. Comparison of Alamar blue and MTT assays for high through-put screening. Toxicol Vitro. 2004;18:703–10.
38. Sharma N, Arya G, Mankamna Kumari R, Gupta N, Nimesh S. Evaluation of anticancer activity of silver nanoparticles on the A549 human lung carcinoma cell lines through Alamar blue assay. Bio Protoc. 2019;9:3131–3131.
39. Matotoka MM, Mashabela GT, Masoko P. Phytochemical content, antibacterial activity, and antioxidant, anti-inflammatory, and cytotoxic effects of traditional medicinal plants against respiratory tract bacterial pathogens. Evid Based Complement Alternat Med. 2023;2023:1243438.
40. Manganyi MC, Ateba CN. Untapped potentials of endophytic fungi: A review of novel bioactive compounds with biological applications. Microorganisms. 2020;8:1–25.
41. Jain R, Bhardwaj P, Pandey SS, Kumar S. *Arnebia euchroma*, a plant species of cold desert in the Himalayas, harbors beneficial cultivable endophytes in roots and leaves. Front Microbiol. 2021;12:696667.
42. Rashmi M, Kushveer JS, Sarma VV. A worldwide list of endophytic fungi with notes on ecology and diversity. Mycosphere. 2019;10:798–1079.
43. Dong Z, Manawasinghe IS, Huang Y, Shu Y, Phillips AJL, Dissanayake AJ, et al. Endophytic *Diaporthe* associated with *Citrus grandis* Cv. Tomentosa in China. Front Microbiol. 2021;11:609387.
44. Manawasinghe IS, Dissanayake AJ, Li X, Liu M, Wanasinghe DN, Xu J, et al. High genetic diversity and species complexity of *Diaporthe* associated with grapevine dieback in China. Front Microbiol. 2019;10:1936.
45. Niaz SI, Khan D, Naz R, Safdar K, Abidin SZU, Khan IU, et al. Antimicrobial and antioxidant chlorinated Azaphilones from Mangrove *Diaporthe perseae* Sp. isolated from the stem of Chinese Mangrove *Pongamia pinnata*. J Asian Nat Prod Res. 2021;23:1077–84.
46. Abdelaziz AM, Hashem AH, Abd-Elsalam KA, Attia MS. Biodiversity of fungal endophytes. Fungal endophytes volume I. Biodiversity and Bioactive Materials, Springer; 2025. pp. 43–61.
47. Garcia JF, Lawrence DP, Morales-Cruz A, Travadon R, Minio A, Hernandez-Martinez R, et al. Phylogenomics of plant-associated *Botryosphaeriaceae* species. Front Microbiol. 2021;12:652802.
48. Li G, Wu W, Lu L, Chen B, Chen S. Characterization of *Pseudofusicoccum* species from diseased plantation-grown *Acacia mangium*, *Eucalyptus* spp., and *Pinus massoniana* in Southern China. Pathogens. 2023;12:574.
49. de Almeida AB, Concas J, Campos MD, Materatski P, Varanda C, Patanita M, et al. Endophytic fungi as potential biological control agents against grapevine trunk diseases in Alentejo region. Biology (Basel). 2020;9:1–23.
50. Sessa L, Abreo E, Lupo S. Diversity of fungal latent pathogens and true endophytes associated with fruit trees in Uruguay. J Phytopathol. 2018;166:633–47.
51. Slippers B, Wingfield MJ. *Botryosphaeriaceae* as endophytes and latent pathogens of Woody plants: diversity, ecology and impact. Fungal Biol Rev. 2007;21:90–106.
52. Osorio JA, Crous CJ, de Beer ZW, Wingfield MJ, Roux J, Endophytic. *Botryosphaeriaceae*, including five new species, associated with mangrove trees in South Africa. Fungal Biol 2017;121:361–93.
53. Jami F, Marincowitz S, Slippers B, Wingfield MJ. New *Botryosphaeriales* on native red Milkwood (*Mimusops caffra*). Australas Plant Pathol. 2018;47:475–84.
54. Pirtskhalava M, Amstrong AA, Grigolava M, Chubinidze M, Alimbarashvili E, Vishnepolsky B, et al. DBAASP v3: database of antimicrobial/cytotoxic activity and structure of peptides as a resource for development of new therapeutics. Nucleic Acids Res. 2021;49:288–97.
55. Nurunnabi TR, Sabrin F, Sharif DI, Nahar L, Sohrab MH, Sarker SD, et al. Antimicrobial activity of endophytic fungi isolated from the Mangrove plant *Sonneratia Apetala* (Buch.-Ham) from the sundarbans Mangrove forest. Adv Tradit Med. 2020;20:419–25.
56. Gurgel RS, de Melo Pereira DÍ, Garcia AVF, Fernandes de Souza AT, Mendes da Silva T, de Andrade CP, et al. Antimicrobial and antioxidant activities of endophytic fungi associated with *Arrabidaea chica* (*Bignoniaceae*). J Fungi (Basel). 2023;9:864.
57. Mabadahanye K, Bhembe NL, Green E. Crude extracts activity of three selected medicinal plants from the Venda region against some pathogenic organisms. Afr Health Sci. 2022;22:717–27.
58. Alegun O, Pandeya A, Cui J, Ojo I, Wei Y. Donnan potential across the outer membrane of Gram-negative bacteria and its effect on the permeability of antibiotics. Antibiotics. 2021;10:701.
59. Breijyeh Z, Jubeh B, Karaman R. Resistance of Gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules. 2020;25:1340.
60. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev. 2003;67:593–656.
61. Li H, Yang J, Zhang X, Xu X, Song F, Li H. Biocontrol of *Candida albicans* by antagonistic microorganisms and bioactive compounds. Antibiotics. 2022;11:1238.
62. Zhou J, Yang C, Lei W, Yang Z, Chen J, Lin H, et al. Exploration of the correlation between intestinal flora and *Escherichia coli* peritoneal dialysis-related peritonitis. BMC Nephrol. 2022;23:76.
63. Varshan GSA, Namasivayam SKR. A critical review on sustainable formulation of anti-quorum sensing compounds using nanotechnology principles against *Candida albicans*. Bionanoscience. 2025;15:161.
64. Mishra A, Gond SK, Kumar A, Sharma VK, Verma SK, Kharwar RN, et al. Season and tissue type affect fungal endophyte communities of the Indian medicinal plant *Tinospora cordifolia* more strongly than geographic location. Microb Ecol. 2012;64:388–98.
65. Souza JADM, Gubiani Juliana R, de Siqueira KA, de Camargo MJ, Garcez WS, De Sousa PT, et al. Antimicrobial metabolites produced by endophytic fungi associated with the leaves of *Vochysia divergens*. Nat Prod Res. 2024;38:978–85.
66. Nagarajan K, Tong WY, Leong CR, Tan WN. Potential of endophytic *Diaporthe* Sp. as a new source of bioactive compounds. J Microbiol Biotechnol. 2021;31:493–500.
67. Zhang SQ, Wang JP, Zhang FM, Yao LL, Li BX, Li YN et al. Investigations of specialised metabolites of endophyte *Diaporthe destruens* hosted in *Illigera orbiculata* C. Y. Wu. Phytochemistry. 2022;203:113357.
68. Duan X, Xu F, Qin D, Gao T, Shen W, Zuo S, et al. Diversity and bioactivities of fungal endophytes from *Distylium chinense*, a rare waterlogging tolerant plant endemic to the three Gorges reservoir. BMC Microbiol. 2019;19:1–14.
69. Vu THN, Pham NS, Le PC, Pham QA, Quach NT, Nguyen VT, et al. Distribution, cytotoxicity, and antioxidant activity of fungal endophytes isolated from

Tsuga chinensis (Franch.) Pritz. In Ha Giang Province, Vietnam. Ann Microbiol. 2022;72:36.
70. de Carvalho CR, Maia MQ, Sobral M, Pereira GMD, da Silva K, Vital MJS, et al., et al. Diversity and antimicrobial activity of culturable endophytic fungi associated with the Neotropical ethnomedicinal plants Copaifera langsdorffii and Copaifera pubiflora. S Afr J Bot. 2021;142:305–15.
71. Cadamuro RD, Bastos IMA, da S, de Freitas ACO, Rosa MdaS, Costa GdeO, IT daS, et al. Bioactivity screening and chemical characterization of biocompound from endophytic neofusicoccum parvum and Buergenerula spartinae isolated from Mangrove ecosystem. Microorganisms. 2023;11:1599.
72. Mishra A, Gond SK, Sharma VK, Verma SK, Kumar J, Singh DK, et al. Characterization of *Pseudofusicoccum adansoniae*, an endophytic fungus residing in photosynthetic root of *Tinospora cordifolia*, a medicinal plant. Proc Natl Acad Sci India Sect B Biol Sci. 2019;89:1319–26.
73. Fadiji AE, Babalola OO. Exploring the potentialities of beneficial endophytes for improved plant growth. Saudi J Biol Sci. 2020;27:3622–33.
74. Janardhanan S, Mahendra J, Mahendra L, Devarajan N. Cytotoxic effects of mangosteen pericarp extracts on oral cancer and cervical cancer cells. Asian Pac J Cancer Prev. 2020;21:2577–83.
75. Kundishora A, Sithole S, Mukanganyama S. Determination of the cytotoxic effect of different leaf extracts from *Parinari curatellifolia* (*Chrysobalanaceae*). J Toxicol. 2020;2020:1–11.
76. Kumari M, Taritla S, Sharma A, Jayabaskaran C. Antiproliferative and antioxidative bioactive compounds in extracts of marine-derived endophytic fungus *Talaromyces purpureogenus*. Front Microbiol. 2018;9:1777.
77. Sathiyaseelan A, Saravanakumar K, Mariadoss AVA, Kim KM, Wang MH. Antibacterial activity of Ethyl acetate extract of endophytic fungus (*Paraconiothyrium brasiliense*) through targeting dihydropteroate synthase (DHPS). Process Biochem. 2021;111:27–35.
78. Naveen KV, Saravanakumar K, Sathiyaseelan A, Wang MH. Comparative analysis of the antioxidant, antidiabetic, antibacterial, cytoprotective potential and metabolite profile of two endophytic *Penicillium* spp. Antioxid (Basel). 2023;12:248.
79. Moreira CC, Genoveva LFL, Bianca S, Rafael C, Andréa CB, de Cristina P. Leishmanicidal, cytotoxic, antimicrobial and enzymatic activities of *Diaporthe* species, a mangrove-isolated endophytic fungus. Afr J Microbiol Res. 2020;14:516–24.
80. Rai N, Gupta P, Keshri PK, Verma A, Mishra P, Kumar D, et al. Fungal endophytes: an accessible source of bioactive compounds with potential anticancer activity. Appl Biochem Biotechnol. 2022;194:3296–319.
81. Sonowal S, Gogoi U, Buragohain K, Nath R. Endophytic fungi as a potential source of anti-cancer drug. Arch Microbiol. 2024;206:112.
82. Liu S, He Q, Shao Y, Meng J, Song F, Liu W, et al. Anti-bacterial and anti-cervical cancer tumor properties of secondary metabolites of endophytic fungi from *Ginkgo biloba*. Int J Clin Exp Med. 2019;12:12236–42.
83. Pedra NS, Canuto KM, de Queiroz Souza AS, Ribeiro PRV, Bona NP, Ramos-Sobrinho R, et al. Endophytic fungus of *Achyrocline satureioides*: molecular identification, chemical characterization, and cytotoxic evaluation of its metabolites in human melanoma cell line. Appl Biochem Biotechnol. 2023;195:4011–35.
84. Lo YH, Kolahi KS, Du Y, Chang CY, Krokhotin A, Nair A, et al. A CRISPR/Cas9-engineered ARID1A-deficient human gastric cancer organoid model reveals essential and nonessential modes of oncogenic transformation. Cancer Discov. 2021;11:1562–81.
85. Hoque N, Hasan CM, Rana S, Varsha A, Sohrab H, Rahman KM. Fusaproliferin, a fungal Mycotoxin, shows cytotoxicity against pancreatic cancer cell lines. Molecules. 2018;23:3288.
86. Rees P, Summers HD, Filby A, Carpenter AE, Doan M. Imaging flow cytometry. Nat Reviews Methods Primers. 2022;2:86.
87. Hasan AEZ, Julistiono H, Bermawie N, Riyanti EI, Arifni FR. Soursop leaves (*Annona muricata* L.) endophytic fungi anticancer activity against HeLa cells. Saudi J Biol Sci. 2022;29:103354.
88. Minarni, Artika IM, Julistiono H, Bermawie N, Riyanti EI, Hasim, et al. Anticancer activity test of Ethyl acetate extract of endophytic fungi isolated from soursop leaf (*Annona muricata* L). Asian Pac J Trop Med. 2017;10:566–71.
89. Singh A, Singh DK, Kharwar RN, White JF, Gond SK. Fungal endophytes as efficient sources of plant-derived bioactive compounds and their prospective applications in natural product drug discovery: insights, avenues, and challenges. Microorganisms. 2021;9:1–42.
90. Kugara J, Chinheya R, Wakandigara A, Mungondori HH. Extraction and characterisation of phytochemicals from white Seringa (*Kirkia acuminata*) bark extracts. Acta Sci Pharm Sci. 2018;2:2581–5423.
91. Abotaleb M, Liskova A, Kubatka P, Büsselberg D. Therapeutic potential of plant phenolic acids in the treatment of cancer. Biomolecules. 2020;10:221.
92. Indriaty I, Ginting B, Hasballah K, Djufri D. A comparative study of total tannin contents and antimicrobial activities in methanol extracts of *Rhizophoraceae* species. Heca J Appl Sci. 2023;1:62–70.
93. Tang Z, Qin Y, Chen W, Zhao Z, Lin W, Xiao Y, et al. Diversity, chemical constituents, and biological activities of endophytic fungi isolated from *Ligusticum Chuanxiong* hort. Front Microbiol. 2021;12:771000.
94. Tijjani H, Zangoma MH, Mohammed ZS, Obidola SM, Egbuna C, Abdulai SI. Polyphenols: classifications, biosynthesis and bioactivities. Funct Foods Nutraceuticals: Bioact Compon Formul Innov 2020:389–414.
95. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, et al. Important flavonoids and their role as a therapeutic agent. Molecules. 2020;25:5243.
96. Chua RW, Song KP, Ting ASY. Antimicrobial activities and phytochemical screening of endophytic fungi isolated from Cymbidium and Dendrobium orchids. S Afr J Bot. 2022;151:909–18.
97. Lim SM, Agatonovic-Kustrin S, Lim FT, Ramasamy K. High-performance thin layer chromatography-based phytochemical and bioactivity characterisation of anticancer endophytic fungal extracts derived from marine plants. J Pharm Biomed Anal. 2021;193:113702.
98. Parthasarathy R, Chandrika M, Yashavantha Rao HC, Kamalraj S, Jayabaskaran C, Pugazhendhi A. Molecular profiling of marine endophytic fungi from green algae: assessment of antibacterial and anticancer activities. Process Biochem. 2020;96:11–20.
99. Marmann A, Aly AH, Lin W, Wang B, Proksch P. Co-cultivation - A powerful emerging tool for enhancing the chemical diversity of microorganisms. Mar Drugs. 2014;12:1043–65.

Publisher's note