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RESEARCH

Biological activities and metabolic profile of crude extracts from two *Nigrospora* sp. isolates

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Abstract
Secondary metabolites derived from microorganisms have attracted considerable attention due to their diverse bioactivities relevant to the pharmaceutical industry, including antimicrobial, antioxidant, and antitumor activities. However, fungal properties remain insufficiently explored due to their extensive biodiversity. Therefore, this study aimed to characterize the metabolite profile and to evaluate the antimicrobial, antitumoral, antioxidant, antihyperglycemic, and antilipidemic activities of two fungi belonging to the genus *Nigrospora*, isolated from slaughterhouse effluent and an endophyte of *Myrciaria glazioviana*. The fungi were grown in Potato Dextrose Broth (PDB) for 15 days at 28 °C. Ethyl acetate extracts of the fungal isolates were obtained and concentrated using a rotary evaporator. Subsequently, they were subjected to metabolomic profiling and to analyses of antimicrobial, antitumoral, and antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+}$) assays. UHPLC putatively annotated metabolites–MS/MS analysis. Both isolates showed significant results for all tested bioactivities. Among the evaluated fungi, the *Nigrospora* sp. Mg17 isolate stood out by exhibiting bactericidal activity against *Staphylococcus aureus* ATCC 25,923 and an IC_{50} of 479.6 µg/mL against *Streptococcus agalactiae* ATCC 1381. Both extracts demonstrated notable antilipidemic potential, with enzymatic inhibition reaching up to 90% for *Nigrospora* sp. F4, as well as the antihyperglycemic activity of up to 51% (Mg17 extract). In the antioxidant analysis, the extract from the *Nigrospora* sp. Mg17 isolate demonstrated high efficiency, achieving 94% inhibition of the DPPH radical at 1 mg/mL. Therefore, the results reinforce the potential of bioprospecting microorganisms from diverse environments as a promising strategy for the discovery of new bioactive fungal metabolites with pharmacological and biotechnological applications, thereby contributing to the development of more sustainable alternatives.

Keywords Biological activities · slaughterhouse effluent · *Nigrospora* · secondary metabolites · *Myrciaria glazioviana*

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Introduction

Metabolic syndromes represent major challenges to global public health due to their high prevalence and their association with cardiovascular diseases and type 2 diabetes (Hanna 2023). According to the World Health Organization (WHO), the prevalence of type 2 diabetes has increased dramatically worldwide over the past three decades. However, single-target medications frequently fail to adequately control blood glucose levels (Artasensi et al. 2020).

Another issue considered a global health concern is the rapid growth of bacterial resistance, which complicates the treatment of infections and creates a high demand for the discovery of new alternatives to combat resistant bacteria (Abrantes et al. 2022; Tiwari et al. 2024). This growing demand for new bioactive compounds has driven bioprospecting for novel metabolites from diverse natural sources, including fungi (Cadamuro 2022).

Microorganisms exhibit a remarkable capacity to adapt to diverse environments, including industrial effluents and plant tissues (Lorková et al. 2025). In addition, these organisms represent promising sources of bioactive compounds whose molecules are unique and structurally complex, performing multiple functions that extend beyond ecological roles to industrial and medical applications (Wang 2023).

Endophytic fungi are microorganisms that inhabit the internal tissues of plants and, when associated with vegetation, are considered excellent producers of novel bioactive natural products with diverse industrial applications (Abba et al. 2025; Correia et al. 2021). The production of secondary metabolites by these fungi is regulated by genetic mechanisms that have likely coevolved with their host plant symbiosis. In some cases, plants and their endophytes produce the same bioactive compounds (Alam et al. 2021; Rutkowska et al. 2023). Fungi belonging to the phylum Ascomycota possess numerous chemical structures and diverse biological activities that are important in medicine. Likewise, fungi of the genus *Nigrospora* produce compounds with a broad range of biological activities, including antimicrobial and antitumoral properties, and represent a potential source of natural antioxidants (Abba et al. 2025; Gautam et al. 2022; Xu et al. 2022).

Myrciaria glazioviana is a plant native to Brazil, belonging to the family Myrtaceae, and is traditionally used in folk medicine as an analgesic and anti-inflammatory agent. In addition, several studies have reported various biological activities associated with this species, including antimicrobial, antinociceptive, antioxidant, and anti-inflammatory properties (Pereira et al. 2020, 2022; Rezzadori 2021). Despite the biological functions already described for this plant, little is known about the endophytes associated with *M. glazioviana*, suggesting an opportunity to discover new bioactive metabolites.

In the search for new bioactive compounds, natural product molecules have been widely investigated. One source explored is microorganisms from extreme environments, which often exhibit complex and diverse chemical structures (Vieira 2022). Under such conditions, these organisms may develop physiological and biochemical adaptations that activate alternative metabolic pathways, promoting the production of secondary metabolites with unique chemical structures and potential applications in several fields (Bernal 2020; Vieira 2022). Among the sources explored for the discovery of new bioactive compounds, microorganisms isolated from industrial effluents stand out. Microorganisms originating from these environments often exhibit high tolerance and metabolic mechanisms capable of synthesizing a variety of enzymes, as well as bioactivity and the production of secondary metabolites of potential interest to different industrial sectors (Bernal et al. 2021; Lira 2020; Omeike et al. 2019).

Therefore, the bioprospecting of endophytic fungi associated with native plants, such as *Myrciaria glazioviana*, as well as microorganisms from industrial environments such as slaughterhouse effluents, represents a promising strategy for identifying microorganisms capable of producing novel secondary metabolites with biological potential. Previous studies have demonstrated that *Nigrospora* species produce metabolites with antimicrobial, antioxidant, antitumor, and enzyme-inhibitory activities (Xu et al. 2022; Abba et al. 2025). However, most of these investigations have focused on isolates obtained from medicinal plants or other ecological niches, while little is known about the biological potential of *Nigrospora* isolated from slaughterhouse effluents or from the endophytic microbiota of *Myrciaria glazioviana*. Thus, the present study aimed to evaluate the production of metabolites with antimicrobial, antitumoral, antioxidant, and enzyme inhibitory activities using *Nigrospora* isolates derived from slaughterhouse effluent and *Myrciaria glazioviana*.

Materials and methods

Fungal isolation and maintenance

The fungi used to produce secondary metabolites were *Nigrospora* sp. Mg17 and F4. They were obtained from the mycotheca of the Endophytic Microorganisms Laboratory – B009 at Universidade Católica Dom Bosco (UCDB). *Nigrospora* sp. Mg17 was isolated from *Myrciaria glazioviana* (unpublished data), whereas *Nigrospora* sp. F4 was isolated from slaughterhouse effluent (Ferrandin et al. 2023).

For colony maintenance, subculturing was performed by transferring a mycelial inoculum of the fungi onto Petri dishes containing Potato Dextrose Agar (PDA) medium. The plates were then incubated in a Biochemical Oxygen Demand (BOD) incubator at 28 °C for 7 days. After mycelial growth, the isolates were preserved in mineral oil.

Bacterial strains

The bacterial strains used in this study, *Escherichia coli* ATCC 25,922, *Staphylococcus epidermidis* ATCC 0128, *Pseudomonas aeruginosa* ATCC 27,853, *Acinetobacter baumannii* ATCC 19,906, *Streptococcus agalactiae* ATCC 1381 and *Staphylococcus aureus* ATCC 25,923, are part of the scientific collection of the Microbiology Laboratory of Universidade Católica Dom Bosco. For bacterial growth, 5 mL of Luria–Bertani (LB) culture medium was used for each strain. The flasks were incubated for 24 h at 37 °C under static conditions. After growth, each colony was streaked onto Petri dishes containing Mueller–Hinton agar (MHA) medium.

Production of fungal secondary metabolites

Secondary metabolites were produced in Potato Dextrose Broth (PDB) medium following the protocol described by Almeida et al. (2017). Briefly, a mycelial inoculum was transferred to Erlenmeyer flasks containing 500 mL of culture medium and incubated at 28 °C for 15 days.

After incubation, the mycelial fraction was removed by filtration using filter paper and gauze and subsequently discarded. For liquid–liquid partitioning, the supernatant was transferred to graduated cylinders containing ethyl acetate at a 1:1 ratio, then to a separatory funnel for phase separation. The ethyl acetate phase was collected, and the extraction process was repeated four times to ensure efficient metabolite recovery. The collected solvent was subsequently evaporated in a rotary evaporator to obtain the crude extract.

Minimum inhibitory concentration (MIC)

For stock solution preparation, fungal extracts were solubilized to obtain a concentration of 5 mg/mL. The *Nigrospora* sp. F4 extract was directly dissolved in 1,000 µL of sterile ultrapure water and therefore did not require DMSO. In contrast, the *Nigrospora* sp. Mg17 extract was not readily soluble in water and was first dissolved in 80 µL of 100% DMSO. Subsequently, 920 µL of sterile ultrapure water was added, resulting in a stock solution containing 8% DMSO.

For the preparation of the working solutions (2 mg/mL), 380 µL of the F4 stock solution was mixed with 570 µL of the corresponding culture medium. For the Mg17 extract, 400 µL of the stock solution was mixed with 600 µL of culture medium, resulting in a working solution containing 4% DMSO. After dilution in the broth microdilution assay, the final DMSO concentration in the assay wells was 2%. Thus, DMSO was used exclusively for the Mg17 extract, whereas the F4 extract was prepared entirely in sterile ultrapure water.

The pre-inoculum was obtained from bacterial colonies grown on agar plates. Using a sterile loop, colonies were transferred to tubes containing liquid Mueller–Hinton or Todd–Hewitt medium (for *Streptococcus agalactiae* ATCC 1381). Cultures were incubated at 37 °C with shaking for 18–20 h.

Subsequently, bacterial suspensions were adjusted by spectrophotometry to 1×10^6 CFU/mL, and after extract addition to 96-well plates, the final concentration was 5×10^5 CFU/mL. Serial dilutions were performed in 96-well plates using 50 µL of bacterial suspension and 50 µL of extract with culture medium, resulting in final extract concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.625, and 7.81 µg/mL. The final DMSO concentration for the Mg17 extract was 2%, whereas no DMSO was used for the F4 extract, which was directly dissolved in sterile ultrapure water. A vehicle control containing 2% DMSO (without fungal extract) was included to evaluate any potential inhibitory effect of the solvent on bacterial growth, and no significant effect was observed under the experimental conditions.

After plate preparation, the plates were incubated for 18–20 h at 37 °C, and absorbance was measured at 600 nm using a spectrophotometer. Gentamicin was used as a positive control for *Acinetobacter baumannii*, and ciprofloxacin was used for the other bacterial strains. Antibiotics were diluted at the same concentrations as the extracts. For the negative control, 50 µL of culture medium and 50 µL of bacterial suspension were used. All experiments were performed in triplicate.

The percentage of bacterial growth inhibition was calculated using the following equation:

$$\%\, Inhibition = \frac{Control - Tratament}{Control} \times 100$$

Dose–response curves were generated from the percentage of bacterial growth inhibition, and the inhibitory concentrations required to reduce bacterial growth by 50% (IC_{50}) and 80% (IC_{80}) were estimated by linear interpolation of the inhibition curves.

Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration depended on the MIC results. For MBC determination, 15 µL of each

concentration was plated onto Mueller–Hinton agar for *E. coli* ATCC 25,922, *S. aureus* ATCC 25,923, *P. aeruginosa* ATCC 27,853, *S. epidermidis* ATCC 0128, and *A. baumannii* ATCC 19,906, and onto blood agar for *S. agalactiae* ATCC 1381. Plates were incubated at 37 °C for 24 h. After incubation, the plates were visually inspected to determine the MBC. All experiments were conducted in triplicate.

Antioxidant activity

Samples were evaluated for their 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity according to Ahmad and Ahmad (2018) and for 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) radical scavenging capacity using the methodology described by Rufino et al. (2007), with adaptations.

For the antioxidant activity assay, crude extract samples were dissolved in ethanol prior to analysis. Therefore, ethanol was used as the solvent in the DPPH assay. Initially, 2.5 mL of the ethanolic sample solution and 1 mL of the DPPH solution were added to the test tubes. The mixture was incubated at room temperature in the dark for 40 min. Absorbance was measured at 518 nm using a spectrophotometer (Cary 50 Scan UV–Vis).

For the negative control, 2.5 mL of deionized water, 1.25 mL of ethanol, and 1 mL of DPPH were used. Trolox was used as the reference compound. Analyses were performed in duplicate.

For ABTS+ radical scavenging activity, the $ABTS^+$ radical cation was generated by mixing a 7 mM ABTS solution with a 2.45 mM potassium persulfate solution. The reaction mixture was kept in the dark at room temperature for 12–16 h to allow radical formation. Before the assay, the $ABTS^+$ solution was diluted with absolute ethanol to obtain an absorbance of 0.70 ± 0.02 at 731 nm.

For the assay, 30 µL of sample solution at different concentrations and 3 mL of $ABTS^+$ solution were vortexed. The samples were then kept in the dark for 6 min. Absorbance was measured at 734 nm using absolute ethanol as the blank. Trolox was used as the standard. Analyses were performed in duplicate.

Antihyperglycemic activity

Inhibition of the enzyme α-amylase was evaluated according to the method of Apostolidis et al. (2007), with adaptations. The α-amylase activity was evaluated using soluble starch as the substrate at concentrations ranging from 2 to 20 mg/mL. The use of this concentration range enabled the assessment of enzymatic activity as a function of substrate availability and the investigation of potential inhibitory effects of the extracts at different concentrations. Initially, 500 µL of starch solution (2–20 mg/mL) as the enzymatic substrate and 250 µL of extract were vortexed and incubated at 37 °C for 5 min. Subsequently, 250 µL of α-amylase solution (2 U/mL) was added without removing the tubes from the water bath, and the tubes were manually shaken.

After 10 min of incubation, 250 µL of DNS reagent containing 1% (w/v) 3,5-dinitrosalicylic acid was added, and the tubes were boiled for 5 min. The tubes were cooled to room temperature, and their contents were diluted with 2.5 mL of deionized water and vortexed. Absorbance was measured at 540 nm with a spectrophotometer, and enzyme kinetics were determined from a linear maltose standard curve ($R^2 = 0.9977$). Concentrations ranged from 0.5 µg/mL to 0.05 µg/mL.

For the control, starch as substrate, buffer, enzyme, DNS, and deionized water were used. For the blank, the same composition was used except for the absence of starch. In both cases, the same experimental procedure was followed. α-Amylase inhibitory activity was expressed as percentage inhibition. Analyses were performed in duplicate.

Antilipidemic activity

Lipase inhibition was evaluated following the methodology described by Oliveira et al. (2015). The reaction mixture consisted of 0.5 mL of substrate 4-nitrophenyl palmitate (1 mM), 1.8 mL of 100 mM Tris–HCl buffer, and 0.1 mL of the extract sample at concentrations ranging from 200 µg/mL to 10 µg/mL.

Samples were incubated for 5 min at 37 °C. Subsequently, 0.1 mL of enzyme was added, and the mixture was incubated for an additional 10 min at 37 °C. The solutions were then boiled for 5 min. Finally, samples were centrifuged at 1200 rpm for 10 min, and the supernatant was immediately read in a spectrophotometer at 410 nm.

Control and blank assays were performed using the same experimental procedure. For the control, p-nitrophenyl palmitate was used as the enzyme substrate. In the blank, neither substrate nor sample was added. Results were expressed as percentage inhibition. Analyses were performed in duplicate.

Cytotoxicity and antitumoral evaluation

Cell viability was determined using the MTT assay (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2 H-tetrazolium bromide) (Mosmann 1983). To evaluate cytotoxicity, RAW 264.7 macrophage cells were used. For the evaluation of antitumoral activity, MDA-MB-231 (breast cancer – double positive), MCF-7 (breast cancer), and B16-F10 (melanoma) cell lines were used.

For the experiment, cells in the exponential growth phase, cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, were seeded at 1×10^6 cells mL^{-1} in sterile 96-well plates. Plates were incubated for 24 h at 37 °C and 5% CO_2 to allow cell adhesion and monolayer formation.

For treatments, serial dilutions of the extracts ranging from 1000 µg/mL to 7.81 µg/mL were prepared and added to the wells containing the monolayer. Plates were then incubated for 24 h under the same culture conditions. Subsequently, 10 µL of MTT solution (5 mg mL^{-1}) was added to each well, and the plates were incubated for 2 h at 37 °C in the dark.

After incubation, 60 µL of a solubilizing solution containing isopropyl alcohol and HCl was added to dissolve the formazan crystals formed in each well. After homogenization, absorbance was measured at 570 nm using a spectrophotometer (ELISA reader Power Wave XS).

For the negative control, viable cells plus 2.5% Triton were used, and for the positive control, only viable cells were used. All experiments were performed in triplicate.

Cell viability was calculated according to the following equation:

$$Cell\ viability\ (\%) = \frac{Optical\ density\ of\ treated\ cells}{Optical\ density\ of\ untreated\ cells} \times 100$$

Targeted MS analysis of fungal extracts

Crude extracts obtained from *Nigrospora* sp. (F4 and Mg17) were diluted in water and subjected to mass spectrometric analysis by direct infusion at a flow rate of 10 µL/min. Prior to the analysis, a literature survey was conducted to compile the *m/z* values of metabolites previously reported for fungi of the genus *Nigrospora*. Based on this information, a list of target ions was established and used for targeted (on-target) screening of the extracts. The selected ions corresponded to metabolites previously described in the literature (Chen et al. 2024; Fanele et al. 2023; Huang et al. 2016; Kumar et al. 2021; Metwaly et al. 2014; Supaphon et al. 2019; Safwan et al. 2021; Yang et al. 2012; Zhang et al. 2009).

Mass spectrometry parameters were acquired using both negative and positive electrospray ionization (ESI) modes. The ESI source block temperature was set to 130 °C, and the desolvation temperature to 550 °C. The capillary voltage was maintained at 3 kV.

Nitrogen was used as the desolvation and nebulization gas at flow rates of 700 and 50 L/min, respectively. Collision-induced dissociation was performed using argon at 3×10^{-3} mbar.

Due to the use of low-resolution mass spectrometry and the absence of authentic standards, the proposed compounds should be considered tentative assignments.

Statistical analysis

For statistical analysis, the mean and standard deviation were calculated for all experimental variables. For each cell line and extract, differences among the tested concentrations were evaluated by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. For the microbiological assays, Dunnett's post hoc test was used to compare each treatment group with the corresponding control. A significance level of $\alpha = 0.05$ was adopted for all statistical analyses.

To visually represent antimicrobial activity, a heatmap was generated in Python (version 3.12.12) that illustrates bacterial responses to different extract concentrations. All statistical analyses and graphical representations were performed using R software (version 4.4.1).

Results

Antimicrobial activity of *Nigrospora* fungal extracts

The Mg17 extract showed statistically significant inhibitory activity against *Staphylococcus epidermidis* ATCC 0128, *Acinetobacter baumannii* ATCC 19,906, *Streptococcus agalactiae* ATCC 1381, and *Staphylococcus aureus* ATCC 25,923. In contrast, the F4 extract exhibited only weak inhibitory activity against these strains, with inhibition remaining below 50% throughout the tested concentration range. No significant antimicrobial activity was observed against *Escherichia coli* ATCC 25,922 or *Pseudomonas aeruginosa* ATCC 27,853 (Figs. 1 and 2).

The Mg17 (*Nigrospora*) extract exhibited inhibitory activity against all susceptible strains, with *Staphylococcus aureus* ATCC 25,923 showing the highest sensitivity, as indicated by the lowest IC_{50} and IC_{80} values (118.9 and 336 µg/mL, respectively). A bactericidal effect was also observed against this strain at 1000 µg/mL. In contrast, *Streptococcus agalactiae* ATCC 1381 showed lower susceptibility, allowing only the estimation of the IC_{50}. Furthermore, *Acinetobacter baumannii* ATCC 19,906 and *Staphylococcus epidermidis* ATCC 0128 showed high IC_{80} values (878.2 and 942.5 µg/mL, respectively), indicating low susceptibility to the extract and consequently weak inhibitory activity (Table 1). For *Streptococcus agalactiae* ATCC 1381, unlike the other bacteria, only the IC_{50} could be estimated (479.6 µg/mL), as inhibition was insufficient to estimate the IC_{80}. This result indicates that, although the Mg17 extract

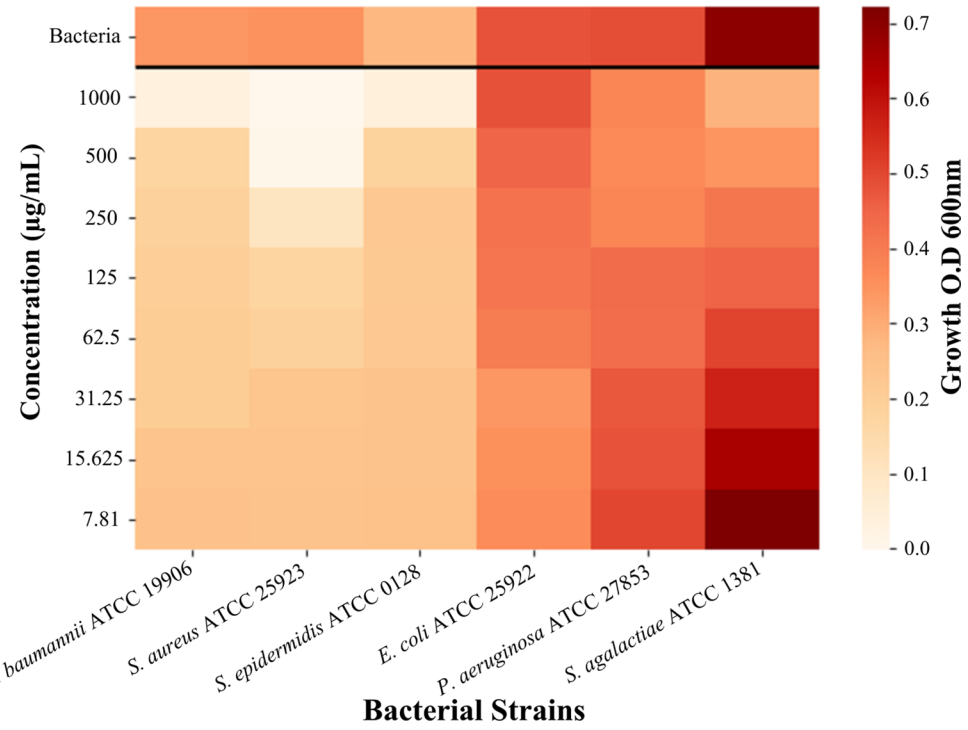


Fig. 1 Heatmap representing bacterial growth (OD_{600} nm) at different concentrations of the *Nigrospora* sp. extract (Mg17) tested

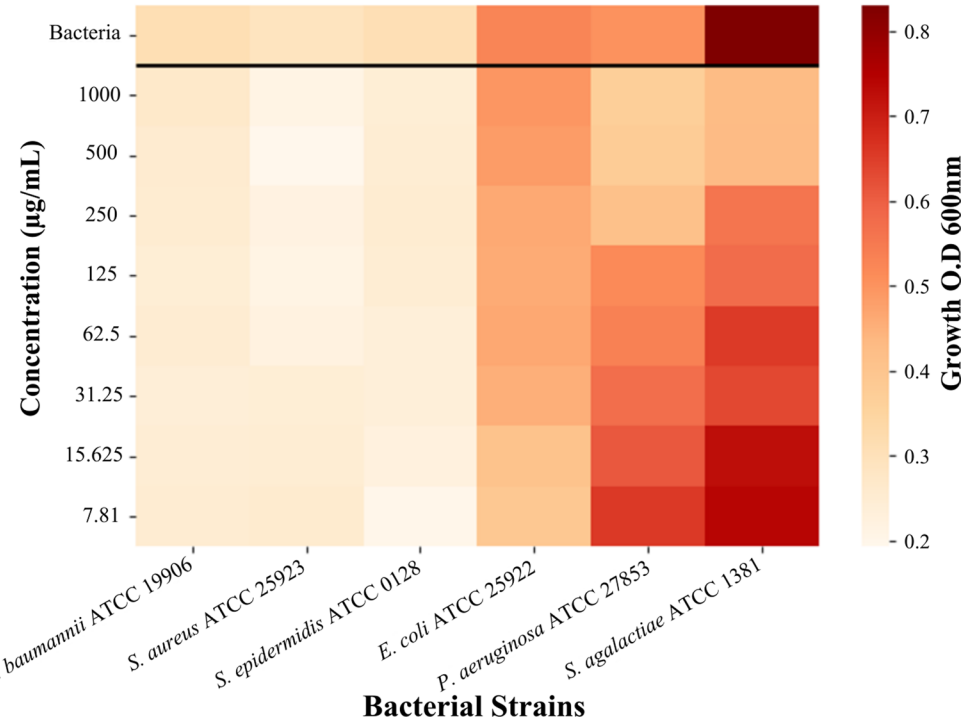


Fig. 2 Heatmap representing bacterial growth (OD_{600} nm) at different concentrations of the *Nigrospora* sp. extract (F4) tested

exhibited inhibitory activity against *S. agalactiae*, its antibacterial effect was weaker than that observed for *S. aureus*, as inhibition did not reach the level required for IC_{80} estimation. In contrast, although the F4 (*Nigrospora*) extract showed weak inhibitory activity at the highest tested concentrations, the inhibition remained below 50% for all evaluated bacterial strains. Therefore, IC_{50} and IC_{80} values could not be determined within the tested concentration range.

The positive controls (ciprofloxacin and gentamicin) exhibited complete or near-complete inhibition of bacterial growth against the susceptible strains, confirming the reliability of the antimicrobial assay.

Table 1 Antimicrobial activity parameters (IC_{50}, IC_{80}, and Minimum Bactericidal Concentration (MBC)) of the *Nigrospora* sp. Mg17 extract against tested bacterial species

Bacterial species	Extract Mg17		
	IC_{50} (µg/mL)	IC_{80} (µg/mL)	MBC (µg/mL)
Acinetobacter baumanni	520.8	878.2	-
Staphylococcus aureus	118.9	336	1000
Staphylococcus epidermidis	655.1	942.5	-
Streptococcus agalactiae	479.6	-	-

Antioxidant evaluation

In the antioxidant evaluation, both extracts showed moderate activity, as indicated by increasing absorbance at higher concentrations (Fig. 3).

Antioxidant assays revealed that, in the DPPH radical scavenging assay, only the Mg17 extract showed significantly higher radical scavenging activity than the negative control (Dunnett's test, $p<0.01$). In the ABTS assay, both Nigrospora extracts also showed significantly higher antioxidant activity than the negative control (Dunnett's test, $p<0.01$).

In the $ABTS^{+}$ radical inhibition assay, decreasing activity was observed as concentrations decreased for both extracts. The Mg17 extract inhibited by 31% at 1 mg/mL, decreasing to 1.5% at the lowest concentration (0.1 mg/mL). The F4 extract showed 47% inhibition at the highest concentration (1 mg/mL), while no antioxidant activity was observed at the lowest tested concentration (0.1 mg/mL) (Fig. 3).

In the DPPH assay, the Mg17 extract showed strong activity at concentrations of 1 and 2 mg/mL, with inhibition percentages above 90%, while at the lowest concentration (0.25 mg/mL), inhibition reached 28%. In contrast, the

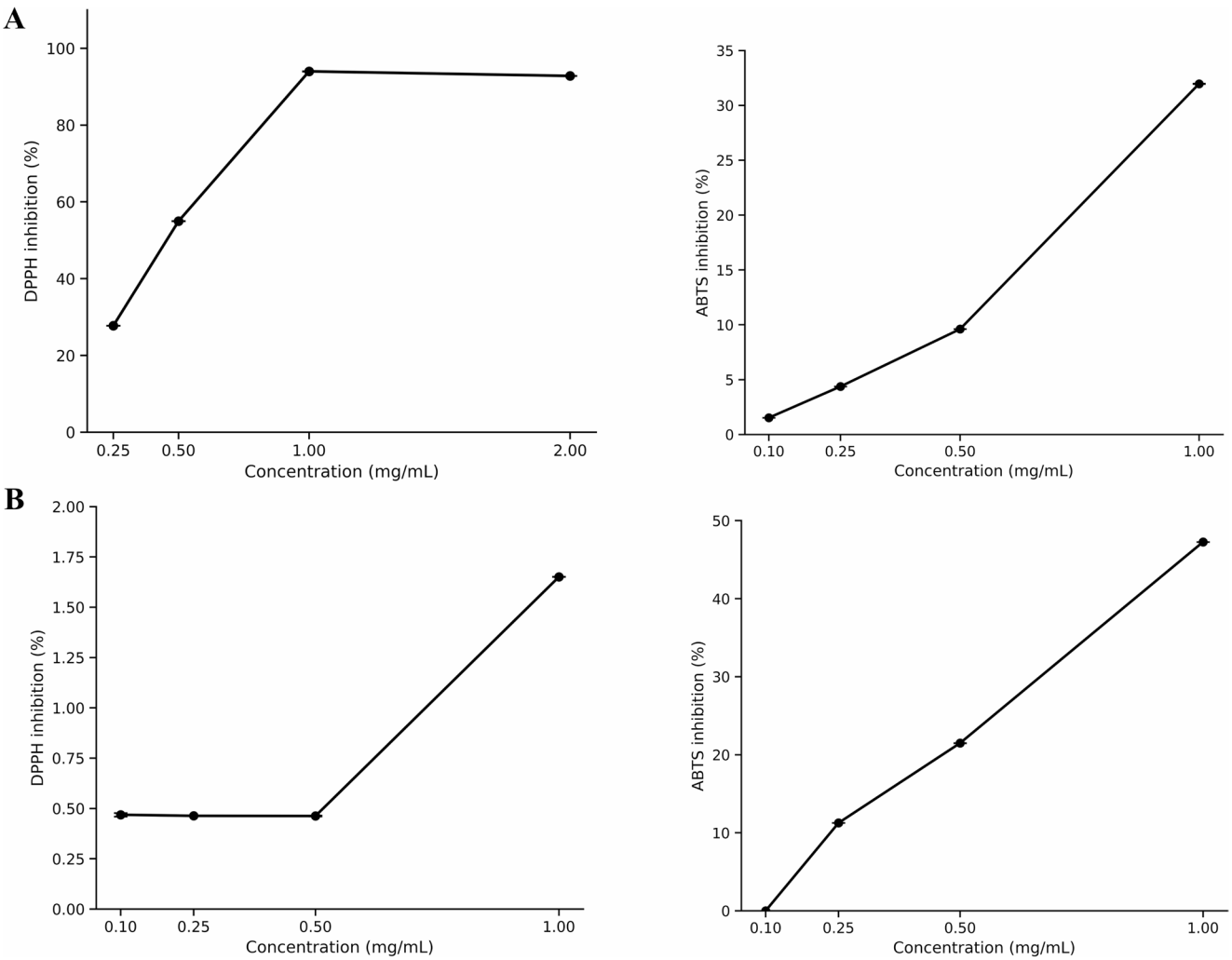


Fig. 3 Antioxidant activity (% inhibition) of *Nigrospora* sp. extracts evaluated by the DPPH and ABTS assays at different concentrations (mg/mL). **(A)** Extract obtained from isolate Mg17. **(B)** Extract obtained from isolate F4

F4 extract did not show significant activity, inhibiting only 1.6% of DPPH at the highest concentration (1 mg/mL) and showing no antioxidant activity at the other tested concentrations (Fig. 3).

For comparison, the reference antioxidant Trolox showed IC_{50} values of 51.8 µg/mL in the DPPH assay and 0.22 µg/mL in the ABTS assay, serving as a positive control and confirming the sensitivity of both assays.

Antihyperglycemic activity

Both extracts showed significant antihyperglycemic activity (Fig. 4). The two extracts exhibited higher antihyperglycemic activity at lower concentrations (0.05 and 0.1 mg/mL). The Mg17 isolate inhibited 51% of amylase activity at 0.05 mg/mL. This inhibitory capacity decreased at 0.1 and 0.2 mg/mL and slightly recovered at 0.3 and 0.4 mg/mL to approximately 30%, then further decreased to 23% at the highest tested concentration.

The F4 extract inhibited 29% of the enzyme at the lowest concentration (0.05 mg/mL). At concentrations of 0.1, 0.2, and 0.3 mg/mL, the inhibitory capacity of amylase stabilized at approximately 24%. At the highest concentration (0.4 mg/mL), antihyperglycemic activity decreased to 17%.

Antilipidemic activity

The *Nigrospora* sp. Mg17 and F4 extracts showed significant inhibitory activity against lipase (Fig. 5). The Mg17 extract showed high antilipidemic activity across all tested concentrations, with the lowest inhibitory capacity of 78% at 25 mg/mL and the highest at 90% at 50 mg/mL.

While Mg17 showed 90% inhibition at 50 mg/mL, the F4 extract reached the same level of inhibition at the lowest evaluated concentration (10 mg/mL). At concentrations of 25, 50, and 75 mg/mL, inhibitory activity decreased to 86%, 84%, and 83%, respectively, and stabilized at 100 and 200 mg/mL, with 67% inhibition.

Cytotoxic activity

For antitumoral activity, both *Nigrospora* extracts showed significant results ($p < 0.05$) at higher concentrations for MDA-MB-231 and B16-F10 cells (Table 2). The Mg17 extract significantly reduced the viability of the MDA cell line by 46%, 30%, and 26% at concentrations of 1000, 500, and 250 µg/mL, respectively. The F4 extract reduced cell viability by approximately 40% at 1000 µg/mL and 30% at 500 µg/mL.

The F4 extract reduced B16-F10 cell viability by 36% at 1000 µg/mL, while no activity was observed at the other tested concentrations. In contrast, the Mg17 extract reduced B16 cell viability by 19% and 29% at the two highest tested concentrations (1000 and 500 µg/mL). For the MCF cell line, the F4 extract did not show a reduction in cell viability, whereas the Mg17 extract showed significant results only at the highest concentration (1000 µg/mL).

The Mg17 extract showed high cytotoxicity toward RAW 264.7 macrophage cells at the highest concentrations (1000, 500, and 250 µg/mL), with values ranging from 20 to 40%. In contrast, no cytotoxic activity was observed at the remaining concentrations. In contrast, the F4 extract showed low cytotoxicity only at 1000 µg/mL (15%), with no effect at the other concentrations.

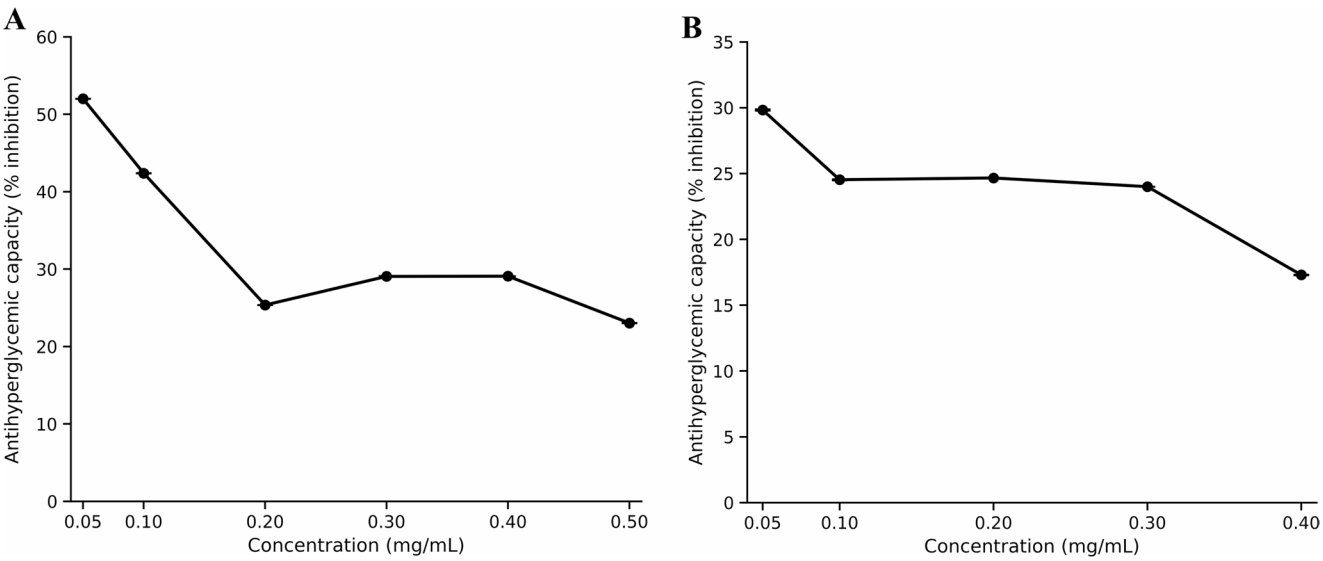


Fig. 4 Antihyperglycemic capacity (% inhibition) of *Nigrospora* sp. extracts at different concentrations (mg/mL). **(A)** Extract obtained from isolate Mg17. **(B)** Extract obtained from isolate F4

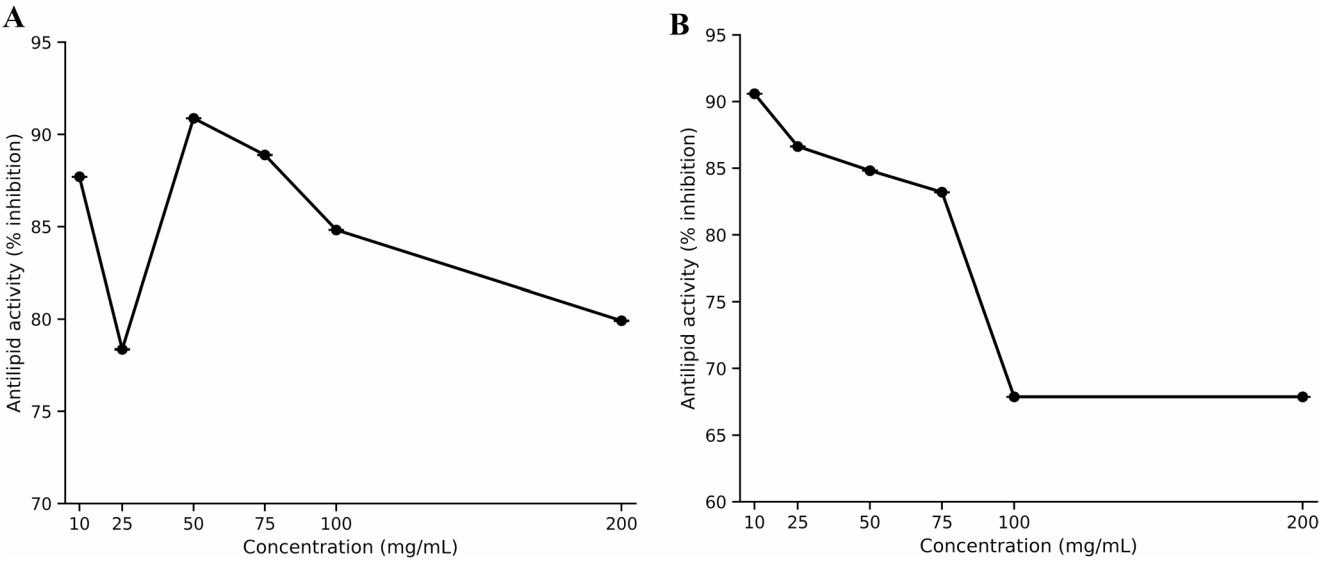


Fig. 5 Antilipid activity (% inhibition) of *Nigrospora* sp. Mg17 and F4 extracts at different concentrations (mg/mL). **(A)** Extract obtained from isolate Mg17. **(B)** Extract obtained from isolate F4

Table 2 Viability (%) of tested cells after treatment with different concentrations of *Nigrospora* sp. Mg17 and F4 extracts

Concentration (µg/mL)	Cell viability (%) extract Mg17				Cell viability (%) extract F4			
	MDA-MB-231	B16-F10	MCF-7	RAW 246.7	MDA-MB-231	B16-F10	MCF-7	RAW 246.7
1000	54 ± 1^{c}	81 ± 2.65^{a}	89.7 ± 5.69^{b}	20 ± 7^{c}	60 ± 4.36^{b}	64 ± 3.61^{b}	100 ± 0^{a}	85 ± 5.29^{b}
500	70 ± 5^{b}	71.3 ± 11.5^{a}	96.7 ± 5.77^{ab}	39.3 ± 8.33^{b}	70 ± 13.7^{b}	98.7 ± 2.31^{a}	100 ± 0^{a}	100 ± 0^{a}
250	74 ± 9.54^{b}	86 ± 9.54^{a}	99 ± 1.73^{a}	20.7 ± 4.73^{c}	92 ± 4.58^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
125	76.3 ± 7.64^{b}	74.3 ± 16.9^{a}	100 ± 0^{a}	92.7 ± 6.43^{a}	95.7 ± 7.51^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
62,5	98.7 ± 2.31^{a}	73.3 ± 27.4^{a}	100 ± 0^{a}	100 ± 0^{a}	95 ± 3.61^{a}	100 ± 0^{a}	99 ± 1.73^{a}	100 ± 0^{a}
31,25	99 ± 1.73^{a}	97.3 ± 4.62^{a}	100 ± 0^{a}	100 ± 0^{a}	95 ± 4.36^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
15,62	98.7 ± 2.31^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
7,81	98.7 ± 2.31^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	99.7 ± 0.57^{a}	100 ± 0^{a}

Values are presented as mean ± standard deviation (SD) of three independent experiments ($n = 3$). Statistical analysis was performed using one-way ANOVA, followed by Tukey's post-hoc test for multiple comparisons, considering $p < 0.05$ as statistically significant. Triton 2.5% was used as the positive control for cytotoxicity, and untreated cells were used as the negative control

Chemical characterization by UHPLC–MS/MS

Chemical characterization by UHPLC-MS/MS allowed the detection of 65 chromatographic features in the Mg17 extract and 64 features in the F4 extract.The UHPLC–MS/MS chromatograms of both extracts are provided in the Supplementary Material (Figures S1 and S2). Based on mass spectral data and database comparison, several metabolites were putatively annotated in both extracts (Table 3). The overall metabolic profiles were largely similar, except that a signal consistent with 10-deoxybostrycin was detected only in the Mg17 extract.

Overall, the results indicate that the extracts share a largely similar metabolic profile. However, differences in the relative abundance of certain putatively annotated metabolites were observed, which may contribute to variations in their biological activities.

Discussion

Metabolites associated with the genus *Nigrospora* have been widely linked to diverse biological activities, including anti-inflammatory, immunoregulatory, antihyperlipidemic, antitumoral, neuroprotective, antioxidant, antibacterial, antiviral, antifungal, antiparasitic, analgesic, antidiabetic, antiproliferative, and cardioprotective effects (Ahmed et al. 2024; Mihaylova et al. 2024; Wang et al. 2021; Zhao et al. 2023). In this context, the present study prioritized the functional evaluation of the extracts, enabling correlations between the suggested metabolic profile and the observed bioactivities.

Regarding antimicrobial activity, the *Nigrospora* Mg17 extract showed the highest efficacy against *Staphylococcus aureus*, with IC_{50} and IC_{80} values of 118.9 µg/mL and 336 µg/mL, respectively. The extract also inhibited *Acinetobacter baumannii*, *Staphylococcus epidermidis*, and *Streptococcus*

Table 3 Metabolites putatively detected in extracts of *Nigrospora* sp. based on targeted mass spectrometry analysis

Putative compound	Reference	m/z	Ion mode	MG17 (detection)	F4 (detection)
4a-epi-9α-Methoxydihydrodeoxybostrycin	Yang et al. (2012)	338	+	+	+
10-Deoxybostrycin (2)	Yang et al. (2012)	321.1	+	+	-
3-Acetoxy-4-deoxybostrycin	Yang et al. (2012)	361.0	-	+	+
3-Acetoxybostrycin	Yang et al. (2012)	378	+	+	+
8-Acetoxy-3,5-dihydroxy-7-methoxy-2-methylanthracene9,10-dione	Yang et al. (2012)	342	+	+	+
5-Acetoxy-3,8-dihydroxy-7-methoxy-2-methylanthracene9,10-dione	Yang et al. (2012)	342	+	-	-
3-Acetoxy-5,8-dihydroxy-7-methoxy-2-methylanthracene9,10-dione	Yang et al. (2012)	342	+	-	-
5,8-Diacetoxy-3-hydroxy-7-methoxy-2-methylanthracene9,10-dione	Yang et al. (2012)	384	+	+	+
3,8-Diacetoxy-5-hydroxy-7-methoxy-2-methylanthracene9,10-dione	Yang et al. (2012)	384	+	-	-
3,5-Diacetoxy-8-hydroxy-7-methoxy-2-methylanthracene9,10-dione	Yang et al. (2012)	384	+	-	-
8-Acetoxyaustrocortirubin	Yang et al. (2012)	326	+	+	+
1,2,8-Trihydroxyanthraquinone	Huang et al. (2016)	257	+	+	+
1,3,8-Trihydroxyanthraquinone	Huang et al. (2016)	257	+	-	-
Rheoemodin	Huang et al. (2016)	295	+	+	+
1,3,6-Trihydroxy-8-methylanthraquinone	Huang et al. (2016)	293	+	+	+
Aloesaponarin II	Huang et al. (2016)	255	+	+	+
Isozyganein	Huang et al. (2016)	277	+	+	+
1-Acetyl-4,5-dihydroxyanthraquinone	Huang et al. (2016)	283	+	+	+
cis-4-Hydroxyscytalone	Huang et al. (2016)	211	+	+	+
Cerebroside B	Huang et al. (2016)	726	-	+	+
3-(3,4-Dihydroxyphenyl)-4,6,8-trihydroxy-1 H-isochromen-1-one-6-O-β-Dglucopyranoside	Metwaly et al. (2014)	465.0990	+	+	+
1-(5-Oxotetrahydrofuran-2-yl) ethyl 2-phenylacetate	Zhang et al. (2009)	249.1123	+	+	+
3-Hydroxybutan-2-yl 2-hydroxy-3- phenylpropanoate	Zhang et al. (2009)	239.1279	+	+	+
Acetic acid, pentyl ester	Kumar et al. (2022)	130	+	+	+
Heptane, 2,4,6-trimethyl-	Kumar et al. (2022)	142	+	+	+
1-Hexanol, 2-ethyl-	Kumar et al. (2022)	130	+	+	+
Ethyl 3-acetoxybutyrate	Kumar et al. (2022)	174	+	+	+
Maltol	Kumar et al. (2022)	126	+	+	+
Phenylethyl Alcohol	Kumar et al. (2022)	122	+	+	+

Table 3 (continued)

Putative compound	Reference	m/z	Ion mode	MG17 (detection)	F4 (detection)
Creosol	Kumar et al. (2022)	138	+	+	+
1 H-Indene, 1-methylene-	Kumar et al. (2022)	128	+	+	+
Dodecane	Kumar et al. (2022)	170	+	+	+
1-Phenoxypropan-2-ol	Kumar et al. (2022)	152	+	+	+
2,4-Dimethyldodecane	Kumar et al. (2022)	198	+	+	+
Tetradecane, 4-methyl-	Kumar et al. (2022)	212	+	+	+
Cetene	Kumar et al. (2022)	224	+	+	+
Tetradecane	Kumar et al. (2022)	198	+	+	+
(-)-Mellein	Kumar et al. (2022)	178	+	+	+
9 Oxabicyclo [3.3.1] nonane-2,6- diol	Kumar et al. (2022)	158	+	+	+
6-Tetradecanesulfonic acid, butyl ester	Kumar et al. (2022)	334	+	+	+
Diethyl Phthalate	Kumar et al. (2022)	222	+	+	+
E-14-Hexadecenal	Kumar et al. (2022)	238	+	+	+
Hexadecane	Kumar et al. (2022)	226	+	+	+
1,2,5-Oxadiazole-3,4-dicarboxamide	Kumar et al. (2022)	156	+	+	+
Nonadecane, 9-methyl-	Kumar et al. (2022)	282	+	+	+
Tetradecane, 4-ethyl-	Kumar et al. (2022)	226	+	+	+
Heptadecane, 3-methyl-	Kumar et al. (2022)	254	+	+	+
3-Eicosene, (E)-	Kumar et al. (2022)	280	+	+	+
Octadecane	Kumar et al. (2022)	254	+	+	+
Cyclohexadecane	Kumar et al. (2022)	224	+	+	+
(E)1-Allyl-2 methylcyclohexanol	Kumar et al. (2022)	154	+	+	+
n-Pentadecanol	Kumar et al. (2022)	228	+	+	+
Hexadecanoic acid, methyl ester	Kumar et al. (2022)	270	+	+	+
Benzoic acid, 2-(dimethylamino)ethyl ester	Kumar et al. (2022)	193	+	+	+
Nonadecane, 3-methyl-	Kumar et al. (2022)	282	+	+	+
5-Eicosene, (E)-	Kumar et al. (2022)	280	+	+	+
Dodecane, 2-methyl-	Kumar et al. (2022)	184	+	+	+

Table 3 (continued)

Putative compound	Reference	m/z	Ion mode	MG17 (detection)	F4 (detection)
1-Hexadecanol	Kumar et al. (2022)	242	+	+	+
Hexadecen-1-ol, trans-9-	Kumar et al. (2022)	240	+	+	+
Nonane, 3-methyl-5-propyl-	Kumar et al. (2022)	184	+	+	+
Cyclohexane, eicosyl-	Kumar et al. (2022)	364	+	+	+
Tridecanol, 2-ethyl-2-methyl-	Kumar et al. (2022)	242	+	+	+
Phthalic acid, di(hept-2-yl) ester	Kumar et al. (2022)	390	+	+	+
1-heptanol, 2,4-dimetil	Fanele et al. (2023)	144	+	+	+
Nigrosporine A	Chen et al. (2024)	256.0945	+	+	+
Nigrosporine B	Chen et al. (2024)	256.0947	+	-	-
Nigrosporine C	Chen et al. (2024)	254.0791	+	+	+
Nigaurdiol	Safwan et al. (2021)	183.1381	+	+	+
5-Pentyldihydrofuran-2(3 H)-one	Supaphon et al. (2019)	156	+	+	+
(Z)-Methyl 4-(isobutyryloxy)but-3-enoate	Supaphon et al. (2019)	200	+	+	+
2-Phenylacetic acid	Supaphon et al. (2019)	136	+	+	+

agalactiae. In contrast, although the F4 extract inhibited the evaluated strains, the inhibition was insufficient to estimate IC_{50} and IC_{80} values.

The Mg17 extract showed greater antimicrobial activity against Gram-positive bacteria, particularly *Staphylococcus aureus* and *Staphylococcus epidermidis*. Structural differences between bacterial groups may explain this difference. Gram-negative bacteria possess an additional outer membrane that acts as an impermeable barrier, limiting the entry of harmful compounds. This barrier comprises components such as lipopolysaccharides, which play a crucial role in antimicrobial susceptibility and hinder bacterial inhibition (Ebbensgaard et al. 2018; Nikaido 2003). Similar results have previously been reported for *Nigrospora* fungal extracts against different pathogens, reinforcing the antimicrobial potential of this genus (Fanele and Ndlovu 2023; Ramesha et al. 2020). Although the Mg17 extract exhibited promising antibacterial activity against *Staphylococcus aureus*, the cytotoxicity assay showed that reductions in RAW 264.7 macrophage viability were observed at concentrations of 250 µg/mL and above. Considering that the IC_{50} against *S. aureus* was 118.9 µg/mL, these findings suggest a relatively narrow therapeutic window. Therefore, additional studies aimed at isolating the active compounds and evaluating their selectivity are necessary to improve the therapeutic potential of the extract.

A limitation of the present study is that a DMSO-only vehicle control was not included in the antimicrobial assay performed with the *Nigrospora* sp. Mg17 extract, in which the final DMSO concentration was 2%. Because solvent tolerance may vary across bacterial species and susceptibility testing methods (Koeth et al. 2015; Summer et al. 2022), future studies should include an appropriate vehicle control to formally exclude any solvent contribution to the observed antimicrobial activity.

In addition to their antimicrobial effects, compounds with antioxidant activity can neutralize reactive oxygen and nitrogen species, thereby preventing the development of several degenerative diseases, including cancer, autoimmune disorders, cardiovascular diseases, and neurodegenerative conditions. These compounds also have applications in various fields, including food preservation, healthcare, and cosmetics (Vitale et al. 2020).

The *Nigrospora* extracts evaluated in the present study demonstrated considerable antioxidant potential, particularly the Mg17 extract, which inhibited up to 90% of free radicals in the DPPH assay. In comparison, the F4 extract showed 47% inhibition of the $ABTS^+$ radical at 1 mg/mL. These results indicate that the metabolites present in the extracts can scavenge free radicals, acting as electron donors or, in some cases, as electron acceptors (Martínez 2021).

Distinct behaviors were observed between the *Nigrospora* extracts (Mg17 and F4). While the Mg17 extract showed greater antioxidant capacity against the DPPH radical, the F4 extract exhibited better performance in the $ABTS^+$ assay. This difference may be explained by the fact that each extract may act differently depending on the assay, since the DPPH and $ABTS^+$ methods involve distinct reaction mechanisms and electron transfer processes (Rumpf et al. 2023). Thus, different compounds present in each extract may respond differently to each radical, influencing the antioxidant profile. Therefore, both the assay conditions and the chemical characteristics of the sample strongly influence the reactivity of a given antioxidant, whether in pure compounds or complex mixtures (Abramovič et al. 2017). Furthermore, a difference was observed between the results obtained for the Mg17 extract in the DPPH and $ABTS^+$ assays. While DPPH radical inhibition exceeded 90% at a concentration of 1 mg/mL, $ABTS^+$ radical inhibition reached only 31% at the same concentration. This distinction may be attributed to differences in the reaction mechanisms and chemical properties of the radicals employed. DPPH is a stable radical soluble in organic solvents and is generally more sensitive to lipophilic antioxidant compounds, whereas $ABTS^+$ can react with both hydrophilic and lipophilic compounds. Therefore, the metabolites present in the Mg17 extract may exhibit greater affinity or efficiency in scavenging the DPPH radical, suggesting an antioxidant behavior that depends on the assay method used. Similar differences between DPPH and ABTS assays have frequently been reported for microbial and plant extracts, reflecting the structural diversity of bioactive compounds and their distinct antioxidant mechanisms of action (Platzer et al. 2021; Granados-Guzmán et al. 2017).Previous reports have also described *Nigrospora* extracts as potential natural antioxidants in assays such as DPPH and $ABTS^+$, as well as in other methods such as FRAP, reinforcing the consistency of the results obtained in the present study (Abba et al. 2025; Gautam et al. 2022; Kantari et al. 2023).

Furthermore, the extracts showed antilipidemic activity, inhibiting 90% of lipase activity at 50 mg/mL (Mg17) and 10 mg/mL (F4). The high lipase-inhibitory activity observed in this study suggests that the bioactive compounds in the extracts may contribute to pancreatic lipase inhibition. This assay aimed to investigate a potential beneficial effect on lipid metabolism through an in vitro lipase inhibition assay. The antilipase activity observed in this work may support further studies aimed at evaluating the potential use of these extracts as pharmacological lipase inhibitors, including comparisons with commercially available inhibitors such as Orlistat. Although this approach is suitable for preliminary screening, the absence of a reference inhibitor limits direct comparisons with established anti-obesity therapies. Future studies should evaluate the extracts alongside Orlistat under the same experimental conditions to better contextualize their inhibitory potential. In addition, further investigations using in vivo models are necessary to confirm their efficacy, safety, and potential therapeutic applications.In addition, antihyperglycemic activity was observed, particularly with Mg17, which inhibited amylase activity by more than 50% at 0.05 mg/mL, whereas the F4 extract showed 30% inhibition at the same concentration. Previous studies with *Nigrospora* extracts have reported significant antihyperglycemic activity through α-glucosidase inhibition (IC_{50} of 6.9 ± 0.5 µM) and anti-inflammatory effects (IC_{50} of 6.2 ± 0.5 µM), further supporting the multifunctional potential of these secondary metabolites (Ukwatta et al. 2019).

Both extracts (Mg17 and F4) reduced cell viability in the MDA-MB-231 and B16-F10 cell lines, whereas the MCF-7 cell line showed lower sensitivity. The differences observed among the tested cell lines may be related to tumor cell heterogeneity, as distinct cellular components can respond differently to treatment, representing one of the major challenges in cancer therapy (Shen et al. 2020; Teodossiou et al. 2019). Consistent with these observations, previous studies have demonstrated the antitumor potential of *Nigrospora* extracts against cell lines such as HCT116 (colon cancer) and MDA-MB-231 (breast cancer) (Ghanem et al. 2025; Ukwatta et al. 2019).

Regarding extract cytotoxicity, the F4 (*Nigrospora*) extract did not show cytotoxicity at concentrations of 500 µg/mL or lower. In contrast, the Mg17 extract exhibited cytotoxicity at 1000–125 µg/mL, with no cytotoxicity at lower concentrations. Despite cytotoxicity at higher concentrations, the Mg17 extract demonstrated relevant biological activities at lower concentrations, without affecting RAW 264.7 cells. Moreover, as this is a crude extract composed of a complex mixture of multiple compounds, the cytotoxic activity is not necessarily associated with the same compounds responsible for the observed bioactivities.

Although the compounds detected in the present study have previously been reported to exhibit various biological activities in the literature, it is not possible to attribute the observed bioactivities to a single specific metabolite. Considering that these are crude extracts, it is plausible that the observed effects result from the combined or synergistic action of multiple components.

Overall, both extracts evaluated in this study exhibited distinct biological activities despite belonging to the same fungal genus. This variation is expected, since the biosynthesis of natural metabolites is a complex process influenced by internal factors, such as enzymatic reactions and gene expression regulation, as well as by external environmental factors that affect fungal development (Calvo et al. 2002). Additionally, studies have shown that even within a species, fungi may exhibit significant intraspecific variation in gene clusters involved in secondary metabolite production. These structural and regulatory polymorphisms lead to different chemical profiles among isolates, influencing the observed bioactivities (Lind et al. 2017).

The distinct ecological origins of the two isolates may also have contributed to their different metabolic and biological profiles. Endophytic fungi establish long-term interactions with plant hosts and experience selective pressures related to host defense, signaling, and symbiosis, which can shape secondary metabolite production (Péllissier et al. 2023; Lu et al. 2021; Alam et al. 2021). In contrast, fungi isolated from slaughterhouse effluents are exposed to environments characterized by high organic load and diverse physicochemical stresses. Although this specific ecological niche has not been extensively investigated with respect to secondary metabolite production, studies on fungi from contaminated or stressful environments suggest that environmental conditions can modulate metabolic responses and may contribute to differences in the biological (Egas et al. 2025).

The UHPLC–MS/MS analysis allowed the detection and putative assignment of metabolites belonging to the classes of anthraquinones, esters, alcohols, and alkanes in the evaluated extracts. Among the suggested compounds are 10-deoxybostrycin, 3-acetoxybostrycin, maltol, creosol, dodecane, tetradecane, mellein, rheoemodin, hexadecane, octadecane, and 8-acetoxyaustrocortirubin. It should be noted that, as this is a low-resolution analysis, these assignments should be interpreted as indicative and represent a preliminary chemical profile of the extracts. Nevertheless, some of these metabolites have already been described in fungi of the genus *Nigrospora*, reinforcing the biological coherence of the obtained data and the recognized biosynthetic capacity of this fungal group (Kumar and Prasher 2021).

Thus, this study reinforces the potential of the genus *Nigrospora* as a source of bioactive compounds while highlighting the need for future investigations to isolate, elucidate, and evaluate the metabolites responsible for the observed activities.

Conclusion

The results demonstrate that fungal extracts of the genus *Nigrospora* exhibit metabolic diversity and bioactive potential. Both extracts (Mg17 and F4) showed promising biological activities, particularly antioxidant and antilipidemic effects, as well as enzyme inhibitory capacity associated with metabolic regulation. The endophytic extract (Mg17) stood out for presenting a broader range of bioactivities, including antioxidant, antihyperglycemic, and antimicrobial activities, with greater susceptibility observed for Gram-positive bacteria. In addition, the extracts showed antitumoral activity against the tested cell lines while maintaining low cytotoxicity at certain concentrations.

The differences observed between the isolates highlight the influence of genetic and environmental factors on the production of fungal secondary metabolites. Overall, the data reinforce the potential of the genus *Nigrospora* as a promising source of bioactive compounds with applications that could help develop natural, sustainable alternatives.

The targeted MS screening provided a preliminary overview of the extracts' metabolite profile; however, further metabolomic studies using high-resolution analytical techniques are necessary to confirm metabolite identities and better elucidate the compounds responsible for the observed biological activities.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

Generative AI and AI-assisted technologies in the writing process In preparing this manuscript, Grammarly was used only for revision and language improvement. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the published article.

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