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Article

Botrytis cinerea: acetylcholinesterase inhibition, cytotoxicity, antimicrobial, larvicidal activity and metabolite isolated from fungal extract

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This study highlights the importance of fungi, specifically *Botrytis cinerea* Pers., in the search for bioactive compounds with therapeutic potential. Extraction approaches using Soxhlet, and maceration methods were applied of the fungus to explore secondary metabolites production. The compound mannitol was separated from the crude extract through nuclear magnetic resonance. The results indicated a positive effect on the inhibitory action of the acetylcholinesterase enzyme for ethyl acetate fractions obtained from the broth. Additionally, significant cytotoxic effects were observed in neoplastic cell lines, with IC_{50} values of 3.5 µg/mL, 5.6 µg/mL, and 8.5 µg/mL for colon cancer cells, monocytes, and human glioma, respectively. Antimicrobial activity was also evident in *B. cinerea* extracts and fractions, particularly in the ethyl acetate fractions from the broth. Larvicidal activity was observed in the chloroform fractions of the broth, with CL_{50} values of 20,824 µg/mL and 83,401 µg/mL. Furthermore, morphological changes in larvae were observed when exposed to the fungus's extracts and fractions. The results suggest that *B. cinerea* extracts and fractions have the potential to identify substances with applications in biological activities, such as cytotoxic, antimicrobial, and larvicidal actions. Continued research is recommended to investigate compounds responsible for these activities and explore their potential applications.

Keywords: Acetylcholinesterase. Cytotoxicity. Antimicrobial. Larvicidal. *Botrytis cinerea*.

INTRODUCTION

A wide variety of organisms, especially fungi, produce compounds that are considered sources of bioactive substances. Popularly known as "gray mold," *Botrytis cinerea* Pers. is a phytopathogenic fungus that attacks more than 200 types of crops and can cause post-harvest losses of up to 40% of cultivable plants. Therefore, infection by this pathogen is extremely commercially harmful (Escobar-Niño *et al*., 2019; Wang *et al*., 2022; Ripardo-Filho *et al*., 2023).

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Several processes are involved in the mechanism of *B. cinerea* infection, including the production of enzymes. Through these enzymes, the pathogen can trigger the death of the host cell (Morževska, Bankina, Kaneps, 2019). Another process involves the contribution of the fungus to the formation of reactive oxygen species during the host-pathogen interaction, a condition called oxidative explosion. For this, the fungus has an antioxidant system and enzymes that help eliminate reactive oxygen species (Elad, Filinger, 2016). Lastly, the secondary production of metabolites includes substances and phytotoxins produced by fungi, which successfully contribute to carrying out an infection (Collado, Aleu, Hernández-Galán, 2000)

In this sense, the action of these toxic metabolites on biological activities is of great interest, as phytopathogenic fungi have been investigated for their ability to produce compounds with a wide variety of activities, including antifungal, antibiotic, and biocontrol activities. Furthermore, several studies have examined the production of metabolites by fungi and provided good overall results in this aspect (Salvatore, Andolfi, 2021).

A series of factors justify the number of studies carried out with *B. cinerea*. Existing research focuses on the fungus as a pathogen, secreter of numerous enzymes, and producer of low molecular weight compounds and other substances of interest (Daoubi *et al.*, 2006).

Numerous metabolites have been identified in the in vitro mycelium of *B. cinerea*, predominantly two phytotoxins: botrydial and botcinic acid. Whole-genome sequencing and annotation revealed genes dedicated to terpenes, non-ribosomal peptides, and alkaloids. This indicates a vast potential for the discovery of substances that have not yet been described (Collado, Viaud, 2015). Therefore, the purpose of this work is to identify a substance and explore still little-known biological activities of the fungus *B. cinerea*, as well as possible applications of its extracts and fractions.

MATERIAL AND METHODS

The Cultivation of *B. cinerea*

The *B. cinerea* isolate was obtained from the Forest Fungus Collection of Embrapa Forestry. Initially isolated from *Pinus taeda* seedlings, it was submitted for sequencing of the ITS regions of the ribosomal DNA for identification at the species level through comparison with sequences deposited in GenBank. The pathogen *B. cinerea* (*Botryotinia fuckeliana* [de Bary] Whetzel), deposited under the code KJ476441, was identified.

The *B. cinerea* was harvested and cultivated in 9 cm Petri dishes containing agar (BDA; KASVI®) potato dextrose medium. It was maintained in a Biological Organism Development (BOD) chamber at 22 °C in the dark (Alfenas, Mafia, 2007) until the complete production of hyphae, mycelia, and conidia was achieved.

Extracts

Cultivation of the fungus was carried out in liquid potato-dextrose broth (39 g of potato-dextrose extract and 1000 mL of ultrapurified water q.s.p.) to evaluate biomass growth. After 30 days of fungus growth in the liquid medium, the culture broth was filtered in a vacuum filtration system using a sintered funnel to separate the mycelium. It was then dried at 40 °C for 12 hours in a vacuum oven. To obtain the crude extract (CE) of *B. cinerea*, a Soxhlet apparatus was used with 96° G.L. ethanol for 8 hours. The crude extracts were partitioned on a modified Soxhlet. The resulting fractions were the hexane fraction (HF), the chloroform fraction (CLF), the ethyl acetate fraction (EAF) and the residual fractions (RF). The same samples were obtained through 24-hour ethanolic maceration. Extracts and crude fractions were concentrated to protect free solvents.

Identification of metabolites

Nuclear magnetic resonance was carried out using $CDCl_3$ at 294 K on a Bruker® DPX 200 MHz NMR spectrometer at 4.7 Tesla, observing ^{1}H and ^{13}C at 200.12 and 50.56 MHz, respectively. The chemical shifts (ppm) were determined with respect to an internal reference (TMS: 0.00 ppm) and coupling constants (J) were measured in Hz. The metabolites were identified by Attenuated Total Reflectance–Fourier Transform Infrared spectroscopy (ATR–FTIR; FT-IR Bruker®).

Acetylcholinesterase Activity

To evaluate acetylcholinesterase activity, the protocol from Silva *et al*. (2016) was adopted. *Dugesia tigrina* individuals collected from freshwater rivers were subjected to the administration of the extracts and fractions for 12 hours at concentrations of 200 µg/mL, in triplicates with 15 individuals each. Then, the individuals were manually homogenized in a porcelain mortar with 1.0 mL of 0.05 M Tris/HCl encourager (pH 8.0) and centrifuged at 17,000 rpm for 20 minutes at 4 ºC. The supernatants were collected, and an additional 1.0 mL of Tris/HCl preparatory was added. The concentration of

proteins present was determined using Lowry *et al.* (1951) method, utilizing bovine serum albumin (31.25–500 µg/mL) as a standard. For the acetylcholinesterase evaluation, 100 µL of *D. tigrina* homogenates were incubated with 20 µL of NADH, 20 µL of acetylthiocholine, and 0.062 µL of 0.25 mM Ellman's reagent for 3 minutes at 25 ºC (Ellman *et al*., 1961). The increase in absorbance was measured at 405 nm (ε, 13.6 mM^{-1} cm^{-1}).

Cytotoxicity

For the cytotoxicity assay, the cell lines 87MG (human glioma), HT29 (colon cancer), U937 (monocytic), Thp1 (Human monocytic), K562 (human leukemia), and H460 (lung) were provided by the Multidisciplinary Center for Chemical, Biological, and Agricultural Research in Campinas, São Paulo.

The cells were stored in bottles of culture medium by the São Paulo-based company SPS Logística, following the guidelines of the Epidemiological and Sanitary Surveillance Manual (Silva, 2015). All cell handling was performed according to Freshney (2010).

Upon receiving them, the cells were transferred to new sterile culture bottles containing Dulbecco's medium, 10% foetal bovine serum, 100 U/mL of penicillin, 0.1 mg/mL of streptomycin and 0.25 µg/mL of amphotericin (complete medium). They were kept at 27 ºC in an incubator with a humid atmosphere containing 5% CO_2, for 24 hours. Since these cells were adherent, it was necessary to remove them with trypsin solution (0.25% + 1 mM acetic acid) in phosphate-buffered saline (pH 7.4). Following this, they were transferred to conical tubes containing a complete culture medium. After centrifugation at low speed, the medium and trypsin were discarded, and the cells were resuspended in a small volume of complete culture medium. For the Assay, cells from different lineages were washed and resuspended at 1 x 10^6 cells/mL in complete Dulbecco's medium. Expanded cells were resuspended at 2 x 10^6 cells/mL in complete Dulbecco's medium and three serial dilutions (3-fold) were performed. Aliquots of 100 µL of each serial dilution of cells containing $2x10^5$, $1x10^5$, and $0.5x10^5$ cells were added per well into a 96-well U-bottom plate, in triplicates.

The cytotoxic potential was evaluated using the MTT assay (Denizot, Lang, 1986; Riss *et al*., 2013). The cells (103 cells/well) were seeded in Roswell Park Memorial Institute 1640 medium (Sigma Chemical Co.), supplemented with 10% fetal bovine serum, in 96 well plates. They were incubated in a humid atmosphere with 5% CO_2 at 37 °C for 20 hours until complete adhesion to the surface (80% confluence). Then, the culture medium was replaced with a new medium supplemented with varying concentrations of the extracts and fractions (0.1–1000 µg/mL). The cells were then incubated at 37 °C with the extract's concentrations for 48 hours. After incubation, approximately 10 µL of a stock solution containing 5 mg/mL of MTT in phosphate-buffered saline was added to each well containing the cells. This mixture was incubated again for 1 hour.

Following this incubation, the culture medium containing MTT and devoid of cells was aspirated from each well, and 100 µL of dimethyl sulfoxide was added to dissolve the dark blue formazan crystals that resulted from the reduction of MTT. The medium was then homogenized on a plate shaker. The extent of MTT reduction to formazan within the cells was measured using a microplate reader at 600 nm. As a positive control, doxorubicin was employed at concentrations ranging from 0.025 µg/mL to 25 µg/mL. The concentrations for the MTT curve (metabolization versus the logarithm of the concentrations used), which inhibits 50% of cell growth (IC_{50}), were calculated in µg/mL. To represent the IC_{50}, the methodology proposed by Berridge, Herst and Tan (2005) and Nordin *et al.* (2018), was followed, where dose-response curves (percentage of cell survivability vs concentration) were generated using linear regression interpolation analysis to obtain IC_{50} (minimum concentration of extracts that giving 50% survival of cells).

Antimicrobial activity

A collection of microorganisms was used, including seven bacteria (*Staphylococcus aureus* [ATCC 6538P], *Staphylococcus epidermidis* [ATCC 12228], *Staphylococcus saprophyticus* [ATCC 15305], *Escherichia coli* [ATCC 10536], *Klebsiella pneumoniae*

[ATCC 4352], *Bacillus subtilis* [ATCC 23856], and *Shigella sonnei* [ATCC 25931]) and fungi (*Candida albicans* [ATCC 14053], *Candida tropicalis* [ATCC 28707], *Cryptococcus neoformans* [ATCC 90112].

The strains utilized in the present study were provided by the Oswaldo Cruz Foundation (Fiocruz/INCQS) Laboratory of Reference Microorganisms, following the guidelines of the Epidemiological and Sanitary Surveillance Manual (Silva, 2015).

To evaluate the antimicrobial activity, the minimum inhibitory concentration (MIC) method was employed. For the assay, solutions of the extracts and dry fractions at a concentration of 1000 µg/mL, dissolved in 0.1% dimethyl sulfoxide, were prepared (Salvat *et al*., 2004). The MIC was determined in 96-well microplates using the microdilution method following the protocols outlined by Clinical and Laboratory Standards Institute (CLSI 2008a, 2008b). Inoculum were prepared in test medium and adjusted to 0.5 MacFarland. Bacterial samples were prepared with a microorganism suspension of $5x10^5$ UFC/mL and for fungi, a suspension of $2.5x10^3$ UFC/mL.

To determine the minimum lethal concentration (MLC) were determined by subculture of Mueller–Hinton agar microplates (applies to bacteria, incubated for 24 h at 37°C) and Sabouraud agar plates (applies to fungi, incubated by 48 h at 35°C). *C. neoformans* was incubated for 72 h at 35 °C (Cantón, Espinel-Ingroff, Pemán, 2009; Benkova, Soukup, Marek, 2020). Control and test samples were concurrently tested in triplicate (Hammer, Carson, Riley, 1999). The standards used were bacitracin as an antibiotic and Polymyxin B as an antifungal (Yousfi *et al.,* 2019).

Larvicidal activity

The determination of the larvicidal activity of *B. cinerea* extracts and fractions against *Aedes aegypti* was carried out in accordance with the World Health Organization's (WHO, 1981a) guidelines with modifications. The eggs of *A. aegypti* from the Rockefeller lineage were supplied by the Oswaldo Cruz Foundation, Rio de Janeiro. For hatching, the eggs were placed in a plastic tray, and 500 mL of dechlorinated water was added. They were then transferred to a BOD oven (Novatecnica, model NT 704) with a temperature of 27 ºC ± 2 and a relative humidity of 80% ± 5. The larvae's diet consisted of fish chow (Aldon Basic, MEP 200 Complex) from the hatching period to the third larval instar. For the test, concentrated solutions of the extracts and fractions at a concentration of 1000 µg/mL were prepared and solubilized in 0.5% dimethyl sulfoxide. These solutions were then diluted with chlorine-free water to achieve different concentrations: 1000 µg/mL, 100 µg/mL, and 10 µg/mL. Samples containing 15 larvae in the third instar were placed in plastic cups, and the volume was adjusted to 1 mL. For each concentration, 10 larvae were used in triplicate. A 0.5% dimethyl sulfoxide aqueous solution was used in triplicate as a negative control.

The insecticide used as a positive control was technical grade temephos 90% (batch 005/2011, manufactured by Fersol in Mairinque, São Paulo). It was calibrated according to the protocol recommended by WHO (1981a, b) and Lima *et al*. (2003). This involved using 0.060 mg/mL as the diagnostic concentration, which is twice the lethal concentration that causes 99% mortality in a susceptible strain, as defined by WHO (1981a, b). The protocol comprises the mortality response following exposure to the diagnostic concentration as well as exposure to a concentration gradient (multiple concentrations). The larvicidal activity was evaluated after 24 hours by counting the number of dead larvae in each sample. Moribund larvae, those unable to reach the water surface when touched, were considered dead (WHO, 1981a). The lethal concentration values (LC_{50}) in µg/mL were determined using the probit analysis method (Finney, 1971).

For the evaluation of the internal and external morphology of the larvae, individuals in the fourth instar were selected due to their more developed tissues. The collected larvae were immediately fixed in a solution of 2% glutaraldehyde, 2% paraformaldehyde, and 3% sucrose in 0.1 M sodium cacodylate buffer (pH 7.2). These fixed larvae were then stored at 25 ºC until the analysis stage (Arruda, Oliveira, Silva, 2003). Slides containing the larvae were prepared and photographed using a digital video camera (Leica) connected to a Zeiss inverted microscope (500 µm).

Statistical analysis

The data were statistically analyzed using the one-way ANOVA test, followed by Tukey's multiple comparison tests. The software employed for these analyses was GraphPad Prism 9.5.1. For the antimicrobial activity data, the results were presented in terms of the minimum inhibitory concentrations and bactericidal concentrations.

RESULTS AND DISCUSSION

Metabolite identification

The yellow-white amorphous powder that precipitated from the crude extract was identified as mannitol (C6H14O6, 182.17 g.mol^{-1}; see Figure 1) using nuclear magnetic resonance, as outlined by Elias (2003), Branco *et al*. (2010), and Ferreira Alves (2014). The experimental ^{1}H chemical shifts (δ ppm) were as follows: 71.25 s, 69.64 s, 63.75 s. The experimental ^{13}C chemical shifts (δ ppm) were: 3.51 (C-1), 3.53 (C-2), 3.58 (C-3), 4.12 (C-4'), 4.16 (C-5'), 4.33 (C-6'), 4.40 (C-7), 4.42 (C-8). Mannitol, a polyol with a molecular weight of 182.17 g.mol^{-1}, finds diverse applications in the industry (Kiviharju, Nyyssölä, 2008). Its uses span formulations, resin production, surfactants, and the manufacturing of dry electrolytic capacitors (Saha, Racine, 2011; Tomaszewska, Rywinska, Gładkowski, 2012). The production of bioethanol from mannitol has been the subject of multiple studies (Ota *et al*., 2013; Wang *et al*., 2013; Fasahati, Woo, Liu, 2015). From a clinical perspective, mannitol serves as a diuretic and hypertonic agent. Research indicates its role in enhancing drug transport through the blood-brain barrier, reducing intracranial pressure, lowering intraocular pressure, preventing or treating acute renal failure, and aiding in the excretion of toxins from the body (Tenny, Patel, Thorell, 2022). A substantial body of evidence suggests that mannitol acts as an antioxidant, protecting against oxidative stress (Patel, Williamson, 2016).

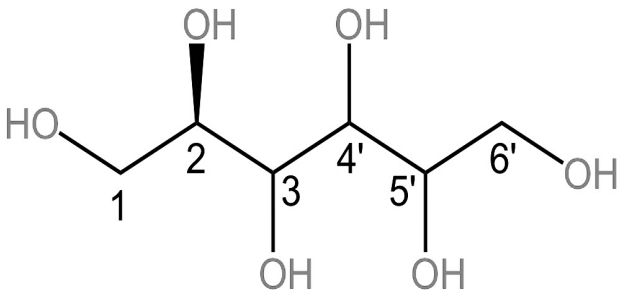

FIGURE 1 – Molecular structure of *Mannitol*.

Acetylcholinesterase activity

Acetylcholinesterase serves several applications, with one of the most important being for Alzheimer's disease. Acetylcholinesterase inhibitors are used as a therapeutic strategy in the treatment of Alzheimer's because they prevent neurotransmitter inhibition by increasing the brain's acetylcholine level, by enhancing deficient brain cholinergic neurotransmission (Asaduzzaman *et al*., 2014; Marucci *et al*., 2021).

Accordingly, the extracts and fractions obtained from *B. cinerea* were tested for acetylcholinesterase. The extracts and fractions of the growth broth presented the most expressive results. The broth samples obtained by maceration obtained the following results: CE (0.01 μmol/min^{-1}), CLF (0.03 μmol/min^{-1}), and EAF (0.01 μmol/min^{-1}). The potential activity was also observed in the broth samples obtained by Soxhlet: HF (0.02 μmol/min^{-1}), CLF (0.02 μmol/min^{-1}), and EAF (0.01 μmol/min^{-1}). The values obtained from the mycelium fractions following both maceration and Soxhlet were EAF (0.02 μmol/min^{-1}) and CLF (0.03 μmol/min^{-1}), respectively (Figure 2).

Thus, the effects demonstrated by the sample are indicative of the skillful potential of *B. cinerea* extracts and fractions as an acetylcholinesterase inhibitor agent since, in some cases, the manifestations demonstrated a capacity similar to that of tacrine (0.03 μmol/min^{-1}), a drug used as a reversible acetylcholinesterase inhibitor.

Data for potential acetylcholinesterase inhibitory activity have previously been reported for endophytic fungi (Singh *et al*., 2012; Yu *et al*., 2016; Popli *et al*., 2018). A larger study conducted by Wang *et al*. (2016) selected a total of 247 endophytic fungi isolated from the plant *Huperzia serrata* to perform the acetylcholinesterase

inhibitory activity test. The results of this study revealed that among these 221 fungi belonging to 41 distinct genera and 31 unidentified strains exhibited robust acetylcholinesterase inhibitory activity.

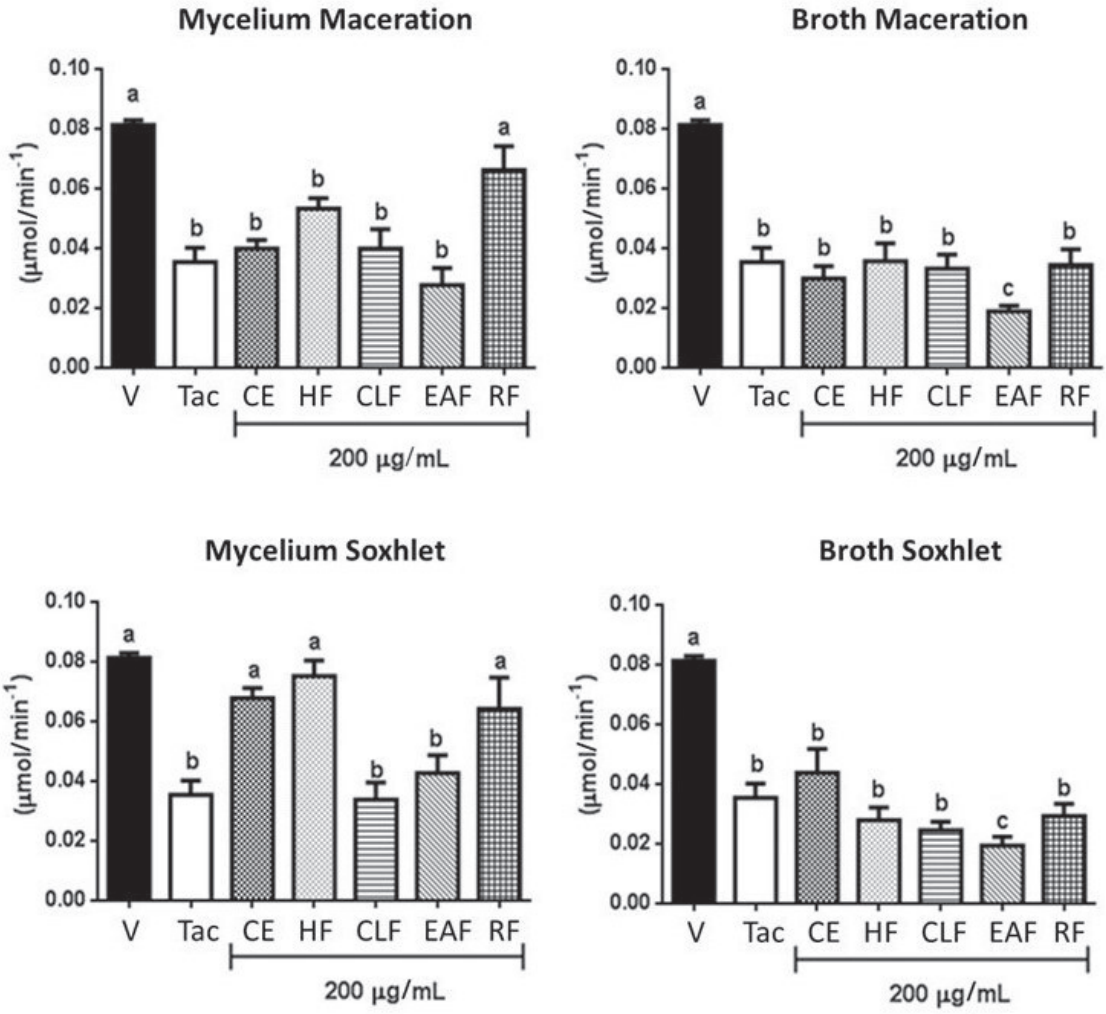

FIGURE 2 – Acetylcholinesterase activity of *Botrytis cinerea.* Crude Extract (CE), Chloroform Fraction (CLF), Hexane Fraction (HF), Ethyl Acetate Fraction (EAF), Remaining Fraction (RF), Tacrine (Tac), Vehicle (V). *The means followed by the same letter do not differ according to Tukey's test ($p < 0.05$).

Cytotoxicity

Table I illustrates the cytotoxic activity of the crude extract and fractions of *B. cinerea,* which were evaluated by the MTT assay in neoplastic cell lines. The results show that there is variability due to the extract, fractions, and cell lines tested.

TABLE I – Cytoxicity Activity (IC_{50}) µg/mL of *Botrytis cinerea*

SAMPLE	FRACTION	CYTOXICITY (IC_{50}) µg/mL						
		87MG*	HT29*	U937*	Thp1*	K562*	H460*	RAW* 264.7
Broth Maceration	CE	12,5	8,5	21,3	22,3	44,3	17,5	33,2
	HF	35,4	54,3	43,2	45,3	43,2	67,4	25,6
	CLF	28,4	32,1	32,1	66,8	32,4	44,3	32,1
	EAF	8,5	3,5	5,6	14,5	10,3	12,4	12,3
	RF	21,5	12,2	23,1	17,5	8,4	16,4	34,7
Mycelium Maceration	CE	46,5	86,4	125,4	86,5	68,4	65,4	178,2
	HF	67,8	123,2	144,8	123,4	72,1	53,2	155
	CLF	64,3	145,5	132,2	94,3	93,2	44,7	123,2
	EAF	45,3	71,4	117,1	67,4	75,5	36,4	131,8
	RF	78,9	65,2	137,5	93,2	94,3	65,4	177,3
Broth Soxhlet	CE	22,4	12,3	33,7	38,3	55,4	23,3	22,4
	HF	43,2	78,4	56,3	65,2	68,7	72,8	43,2
	CLF	31,2	46,5	44,2	84,9	43,6	55,1	31,2
	EAF	16,5	12,5	18,9	23,1	32,1	38,4	16,5
	RF	34,7	24,7	53,8	35,6	25,4	27,5	34,7
Mycelium Soxhlet	CE	125	170	180,3	174,2	200	177,3	236,7
	HF	256,5	221,3	250	175	150,5	68,7	328,1
	CLF	87,4	228,1	165,5	123,2	221	75,5	451
	EAF	68,3	103,7	221,5	69,3	105	58,3	327,3
	RF	125,5	84,5	170	125,5	154	77,4	258,1
DOXO	-	0,055	0,12	0,087	0,21	0,041	< 0.031	1,12

NOTE: Crude Extract (CE), Chloroform Fraction (CLF), Hexane Fraction (HF), Ethyl Acetate Fraction (EAF), Remaining Fraction (RF). 87MG* (human glioma); HT29* (colon cancer cell line); U937* (monocytic lineage); Thp1* (human monocytic lineage); K562* (human leukemia) and H460* (lung). DOXO: doxorubicin.

The cytotoxic effect of *B. cinerea* extracts and fractions was evaluated as described by Nordin *et al.* (2018). Where the results are based on the minimum concentration of extract that considers at least 50% of the survival capacity of IC_{50} cancer cells. the results were interpreted according to four categories: very active ($IC_{50} \leq 20$ µg/mL), moderately active ($IC_{50} > 20$–100 µg/mL), weakly active ($IC_{50} > 100$–1000 µg/mL) and inactive ($IC_{50} > 1000$ µg/mL).

The samples obtained from the growth broth showed greater activity than in relation to the mycelium. The highlight is the broth obtained by EAF maceration, which was very active for all cell lines and appears to be more cytotoxic with an IC_{50} of 3.5 µg/mL for HT29* (colon cancer strain), 5.6 µg /mL for U937* (monocytic strain) and 8.5 µg/mL for 87MG* (human glioma strain). Similar effects were also observed for CE and RF from

the same extraction. Some fractions of the broth obtained by Soxhlet are also very active, however they seem to be influenced by the type of extraction. The samples obtained from the mycelium appear to be moderately active for the CE and EAF fractions from the maceration. The others are weak or inactive for the cell lines tested.

The cytotoxic potential of other fungal species has been addressed in the literature. In a study by Katoch *et al*. (2014), it was found that the extracts of different fungi isolated from *Bacopa monnieri* exhibited cytotoxic activity, with an IC_{50} value equal to 5 μg/mL for the extract of *Phomopsis* sp. against the cell line HCT-116 (colorectal carcinoma) and 6 μg/mL for the extract of *Fusarium oxysporum* for the A549 strain (lung cancer).

The crude extracts of the marine fungus species *Xylaria psidii* and *Mycelium sterillium* were tested for their cytotoxicity against the human bladder carcinoma cell line 5637 (ATCC HTB-9). The strains showed IC_{50} values of 4 μg/mL and 1.5 μg/mL, respectively. When these extracts are obtained through culture with seawater from the collection sites, the activity of the extracts is lower, with IC_{50} values of 15 μg/mL and 14 μg/mL, respectively (Tarman *et al*., 2011).

Previous studies have already demonstrated the potential of fungal extracts. Hazalin *et al.* (2009) demonstrated the cytotoxicity of the extracts of 40 species of fungi isolated from plants from Pahang National Park, Malaysia against P388 (murine leukemia) and K562 (chronic human leukemia) cells, showing that almost half (47.6%) of the extracts exhibited $IC_{50} < 10$ μg/mL against the P388 strain compared to 25% active fungi against the K562 strain with $IC_{50} <1$ μg/mL.

Thus, the potential use of fungal extracts as candidates for the isolation and identification of substances that may demonstrate cytotoxic activity was demonstrated. The values found in the current study are compatible with the National Cancer Institute's criteria for the potential cytotoxicity ($IC_{50} < 20$ μg/mL) of both plant extracts and other microorganisms (Lee, Houghton, 2005).

Antimicrobial activity

Phytopathogenic fungi can produce secondary metabolites with known antimicrobial activity, as is the case for the fungus *Fusarium* sp. (Xu *et al.,* 2023) and *Colletotrichum gloeosporioides* (Nurunnabi *et al.,* 2018). In this way, the antimicrobial activity of different types of extracts obtained from *B. cinerea* was tested, which showed good antimicrobial potential against the different strains tested.

Antimicrobial activity was evaluated according to the criteria of Morales *et al*. (2008). Therefore, extracts with an MIC lower than 100 μg/mL had good antimicrobial activity; from 100 μg/mL to 500 μg/mL the antimicrobial activity was moderate; from 500 μg/mL to 1000 μg/mL the antimicrobial activity was weak; above 1000 μg/mL the extract was considered inactive.

Broth extracts exhibited a greater antibacterial effect when compared to mycelium extracts, with the most effective results found for the broth extract obtained by Soxhlet extraction, where the EC and EAF exhibited MIC values equal to 25 μg/mL for the strains of *S. aureus*, *S. epidermidis* and *S. saprophyticus*. EAF was still effective at the same MIC for *E. coli* (Table II). The other extracts also exhibited activities between 50 and 500 μg/mL, with relevant MIC values being observed for the extracts and mycelium fractions.

TABLE II – Inhibitory concentration of *Botrytis cinerea* against bacterial strains

SAMPLE	FRACTION	MICRORGANISM- INHIBITION/ CONCENTRATION (µg/mL)				
		S. aureus (ATCC 6538P)	*S. epidermidis* (ATCC 12228)	*S. saprophyticus* (ATCC 15305)	*E. coli* (ATCC 10536)	*K. pneumoniae* (ATCC 4352)
Broth Maceration	CE	50	50	50	50	75
	HF	75	75	75	250	250
	CLF	75	125	75	125	125
	EAF	50	50	50	50	50
	RF	125	125	125	125	125
Mycelium Maceration	CE	125	125	125	250	500
	HF	250	250	250	500	500
	CLF	125	125	125	125	250
	EAF	75	75	75	75	75
	RF	250	125	125	125	250
Broth Soxhlet	CE	25	25	25	75	50
	HF	50	50	50	75	50
	CLF	75	75	75	75	100
	EAF	25	25	25	25	50
	RF	100	125	125	75	50
Mycelium Soxhlet	CE	75	75	75	125	250
	HF	125	125	125	250	250
	CLF	125	125	125	125	500
	EAF	50	50	50	75	75
	RF	250	250	250	125	250
Bacitracin	-	1,26	1,12	1,23	0,45	1,33

NOTE: Crude Extract (CE), Chloroform Fraction (CLF), Hexane Fraction (HF), Ethyl Acetate Fraction (EAF), Remaining Fraction (RF).

Antifungal activity was similar for broth extracts obtained by Soxhlet extraction, with MIC values equal to 25 µg/mL for *C. albicans*, *C. tropicalis* and *C. neoformans*. Equal MIC values were observed for EC against *C. neoformans* strains. The FEA obtained from the broth obtained by maceration also presented the same MIC value for *S. cerevisiae*. The other extracts and fractions presented MIC values ranging from 50 to 500 µg/mL (Table III).

TABLE III – Inhibitory concentration of *Botrytis cinerea* against fungal strains

SAMPLE	FRACTION	MICRORGANISM- INHIBITION/ CONCENTRATION (µg/mL)		
		C. *albicans* **(ATCC 14053)**	*C. tropicalis* **(ATCC 28707)**	*C. neoformans* **(ATCC 90112)**
Broth Maceration	CE	75	75	125
	HF	250	250	250
	CLF	250	250	250
	EAF	75	75	250
	RF	250	250	250
Mycelium Maceration	CE	125	125	250
	HF	250	250	500
	CLF	250	250	500
	EAF	125	125	500
	RF	250	250	500
Broth Soxhlet	CE	75	75	50
	HF	50	50	25
	CLF	75	125	50
	EAF	25	25	25
	RF	25	25	50
Mycelium Soxhlet	CE	250	250	125
	HF	125	125	125
	CLF	500	250	250
	EAF	125	125	125
	RF	250	250	250
Polymycin B Sulfate	-	0,76	0,34	1,12

NOTE: Crude Extract (CE), Chloroform Fraction (CLF), Hexane Fraction (HF), Ethyl Acetate Fraction (EAF), Remaining Fraction (RF).

Rani *et al.* (2017) reported the use of extracts from 20 different species of fungi. Among these, *Aspergillus nidulans*, *Curvularia hawaiiensis*, *Chaetomium arcuatum* and *Chaetomium atrobrunneum* were the most effective in controlling the bacteria *E. coli* and *S. aureus*, whose MIC values varied between 15.6 µg/mL and 250 µg/mL, respectively. In studies carried out with fungi isolated from *Ophiopogon japonicus*, *Gibberella* sp. presented MIC values equal to 20 µg/mL and 80 µg/mL for strains of *S. aureus* and *C. neoformans*, respectively, demonstrating that this strain can be a source of bioactive antibacterial agents (Liang *et al.,* 2012).

Fungi have been presented as prototypes of antimicrobial substances, mainly as an alternative in cases of resistance that create a serious problem for health services. This scenario has required the exploration of new

niches and habitats, which directs attention to fungi as they present a diversity of microbial captures that evolved in unique and unusual environments (Santos, 2015).

It is also important to correlate the results observed in the antimicrobial assay with the cytotoxicity assay for the EAF and CE samples obtained from the maceration broth. Compounds that have both activities are extensively studied, since substances that have antineoplastic and antimicrobial effects are important in the clinic of cancer patients who are more susceptible to infections. Studies focus on these substances and the possibility of interaction as antineoplastics and antibiotics or research focuses on the prolonged use of these substances and the occurrence of bacterial resistance in these patients (Henriksson, Holm, Littbrand, 1990; Majchrzak-Stiller *et al.,* 2023).

Larvicidal activity

The determination of the larvicidal activity of the extracts and fractions of *B. cinerea* on *A. aegypti* was conducted. The chloroform fraction obtained from the broth extract obtained through maceration was the sample in which the bioinsecticide activity showed the greatest potential (CL_{50} of 20.824 µg/mL), followed by the chloroform fraction obtained from the broth extract obtained by Soxhlet extraction (CL_{50} of 83.401 µg/mL), which exhibited a toxic effect against the larvae. Detailed outcomes are expressed in Table IV. It is worth noting that some fungi report larvicidal activity against *A. aegypti*, as is the case with *Trichophyton mentagrophytes* (Murugesan *et al*., 2009), *Trichoderma harzianum* (Sundaravadivelan, Padmanabhan, 2014), the endophyte *Pestalotiopsis virgatula* and the saprophyte *Pycnoporus sanguineus* (Bücker *et al*., 2013), *Aspergillus terreus* (Ragavendran, Natarajan, 2015) and *Hyalodendriella* sp. (Mao *et al*., 2017). Furthermore, phytotoxins obtained from *Seiridium cupressi*, *Diplodia cupressi,* and *Ascochyta agropyrina* also demonstrated bioactivity (Cimmino *et al*., 2013).

TABLE IV – Larvicidal Activity (LC_{50}) µg/mL of *Botrytis cinerea*

SAMPLE	FRACTION	MORTALITY (%) / CONCENTRATION (µg/mL)			LC_{50} (µg/mL) (LCL - UCL)	LC_{90} (µg/mL) (LCL - UCL)	x^2	DF
		10	100	1000				
Broth Maceration	CE	0.0 ± 0.00	0.0 ± 0.00	6.7 ± 0.00	> 1000	> 1000	0.012	1*
	HF	0.0 ± 0.00	6.7 ± 3.33	13.3 ± 3.33	> 1000	> 1000	0.682	1*
	CLF	33.3 ± 3.33	83.3 ± 6.67	90.0 ± 3.33	20.824 (6.077 – 44.587)	512.862 (203.216 – 3,500.054)	2.637	1*
	EAF	0.0 ± 0.00	10 ± 0.00	53.3 ± 6.67	854.896 (477.257 – 2312.978)	> 1000	0.118	1*
	RF	0.0 ± 0.00	3.3 ± 0.00	13.3 ± 0.00	> 1000	> 1000	0.143	1*
Mycelium Maceration	CE	0.0 ± 0.00	0.0 ± 0.00	10.0 ± 0.00	> 1000	> 1000	0.012	1*
	HF	0.0 ± 0.00	3.3 ± 0.00	26.7 ± 10.00	> 1000	> 1000	0.034	1*
	CLF	2.2 ± 1.93	3.3 ± 3.33	16.7 ± 6.67	> 1000	> 1000	0.722	1*
	EAF	3.3 ± 0.00	13.3 ± 6.67	26.7 ± 3.33	> 1000	> 1000	0.095	1*
	RF	0.0 ± 0.00	0.0 ± 0.00	6.7 ± 3.33	> 1000	> 1000	0.012	1*

TABLE IV – Larvicidal Activity (LC_{50}) µg/mL of *Botrytis cinerea*

SAMPLE	FRACTION	MORTALITY (%) / CONCENTRATION (µg/mL)			LC_{50} (µg/mL) (LCL - UCL)	LC_{90} (µg/mL) (LCL - UCL)	x^2	DF
		10	100	1000				
Broth Soxhlet	CE	0.0 ± 0.00	3.3 ± 0.00	3.3 ± 0.00	> 1000	> 1000	0.662	1*
	HF	3.3 ± 0.00	3.3 ± 0.00	30.0 ± 6.67	> 1000	> 1000	1.793	1*
	CLF	3.3 ± 3.33	63.3 ± 6.67	93.3 ± 3.33	83.401 (49.459 – 137.301)	500.364 (275.242 – 1,352.426)	1.982	1*
	EAF	0.0 ± 0.00	0.0 ± 0.00	90.0 ± 6.67	492.373 (110.850 – 710.697)	985.099 (668.049 – 1,703.303)	0.056	1*
	RF	0.0 ± 0.00	3.3 ± 3.33	3.3 ± 3.33	> 1000	> 1000	0.662	1*
Mycelium Soxhlet	CE	3.3 ± 0.00	3.3 ± 0.00	70.0 ± 3.33	566.143 (-)	> 1000	9.399	1
	HF	0.0 ± 0.00	3.3 ± 3.33	73.3 ± 10.00	557.801 (359.786 – 852.044)	> 1000	0.000	1*
	CLF	0.0 ± 0.00	0.0 ± 0.00	23.3 ± 10.00	> 1000	> 1000	0.027	1*
	EAF	0.0 ± 0.00	0.0 ± 0.00	13.3 ± 10.00	> 1000	> 1000	0.014	1*
	RF	0.0 ± 0.00	0.0 ± 0.00	6.7 ± 0.00	> 1000	> 1000	0.012	1*

NOTE: Crude Extract (CE), Chloroform Fraction (CLF), Hexane Fraction (HF), Ethyl Acetate Fraction (EAF), Remaining Fraction (RF); No mortality was observed in the control; LC_{50} = lethal concentration that kills 50% of the exposed organisms; LC_{90} = lethal concentration that kills 90% of the exposed organisms; UCL = 95% upper confidence limit; LCL = 95% lower confidence limit; x^2 = chi squared; DF = degrees of freedom; *$p > 0.05$.

The analysis of the external morphology of the larvae highlighted some alterations. In this study, larvae treated with the extracts and fractions obtained from the fungus *B. cinerea* exhibited significant morphological changes that indicate the fungus's contribution to their death, as shown in Figure 3.

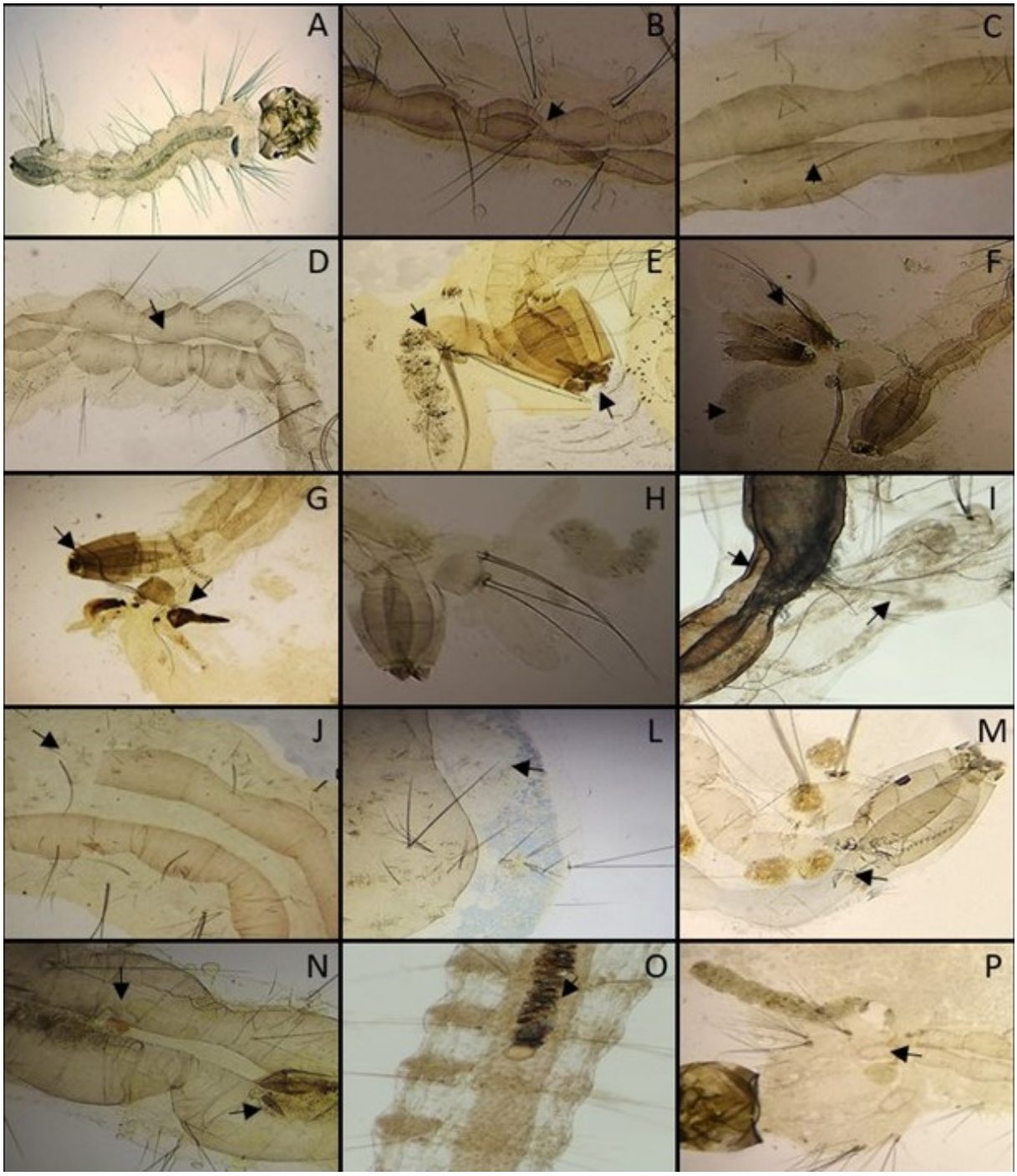

FIGURE 3 – Morphological changes in *Aedes aegypti* larvae submitted to the extract and fractions of *Botrytis cinerea*. A = control; B, C, D = narrowing of the midgut (arrows); E, F, G = inhibition of anal papillae (arrows); F, G, H, I = bowel leakage (arrows); I = cuticle darkening (arrows); J = disruption between the fat layer and the muscle epithelium (arrows); L = decrease in body fat (arrows); M, N, O, P = displacement of parts of the abdomen (arrows).

According to the literature, substances that are toxic to the insect act directly on the intestine receptors, causing abnormal development of the larva. As a defense mechanism, the larva extrudes from the intestine to the external environment to expel the contaminating agent and thus, reduce tissue damage (Valotto *et al*., 2011; Revathi *et al*., 2013). This is why the closure of the intestine, particularly in the middle region of the midgut, occurs.

The *B. cinerea* extracts also altered the body fat layer. This occurred because the feeding mechanism of the larvae was inhibited by intoxication. When the body fat is not supplemented, a place considered reserve storage is recruited. This reorganization is mediated by hormones for the maintenance of larval metamorphosis. Moreover, the intoxication provoked a rupture between the fat layer and the muscular epithelium of the segment. This can occur due to the degeneration of the epidermis and thickening of the cuticle (Conte, 1994; Cruz-Landim, Cruz-Hofling, 2000).

As claimed by Yu *et al.* (2015), extensive damage to the intestinal epithelium and peritrophic matrix decreases the survival capacity of the insect, as the midgut plays a key role in the secretion of digestive enzymes and absorption of nutrients. Therefore, damage to the midgut cells leads to larva death. This study also demonstrates the darkening of the cuticle and suppression of the anal papillae, which occurs due to the disruption of the larva's ion regulation, causing an imbalance in homeostasis. Additionally, the destruction and rupture of the larval stigmal plate are caused by the destruction of the hydrophobic surface, leading to water from the medium entering the tracheal trunk, which impairs the breathing system of the larva. Suppression of anal papillae was also reported in Chaithong *et al.* (2006) study on the larvicidal activity of plants of the *Piperaceae* family on *A. aegypti*. Although intoxication did not cause visible morphological alterations, the anal papillae were found to be abnormal; ultrastructural analyses demonstrated that in these cases, the cuticle of the anal papillae is destroyed.

Another study by Grzybowski *et al*. (2013) demonstrated that the *Annona squamosa* extract tested against *A. aegypti* larvae was able to inhibit the production of NADH (ubiquinone oxidoreductase), which prevented the transport of electrons in mitochondrial complex I. The electrons in mitochondrial complex I prevented the production of adenosine triphosphate and caused the death of the insects, affecting the cellular muscles and contributing to the decrease of body fat.

CONCLUSION

In conclusion, the isolation of substances revealed the presence of mannitol, a compound recognized for its role in fungal metabolism and documented in the literature for various clinical and industrial applications. When the inhibitory action of the acetylcholinesterase enzyme was evaluated, it revealed inhibition power, with emphasis on the chloroform, ethyl acetate and hexane fractions, which may contain compounds that act against degenerative diseases. In the analysis of cytotoxicity, extracts and fractions exhibited significant toxic activity for certain cell lines, suggesting potential path for drug investigation in cancer treatment. Additionally, good antimicrobial activity was observed against bacterial and fungal strains, with particular emphasis on the ethyl acetate fractions obtained through maceration. The larvicidal activity study underscored the significant potential of chloroform fractions obtained by maceration. It is important to note that the extraction method employed influenced the observed biological activities. Furthermore, the cultivation time of the fungus in the growth broth was identified as a limitation for research. Considering the provided information, this study indicates that extracts and fractions of *B. cinerea* have potential application in several areas. However, more research is needed to detail the behaviour of these substances and their potential for use.

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DECLARATION OF INTEREST STATEMENT

The author(s) have not declared any conflict of interests.

REFERENCES

Alfenas AC, Mafia RG. Métodos em fitopatologia. 2007.

Arruda W, Oliveira GMC, Silva IG. Alterações morfológicas observadas em larvas de Aedes aegypti (Linnaeus, 1762) submetidas à ação do extrato bruto etanólico da casca do caule da Magonia pubenscens. Entomol Vect. 2003;10:47-60.

Asaduzzaman M, Uddin J, Kader MA, Alam AHMK, Rahman AA, Rashid M, et al. In vitro acetylcholinesterase inhibitory activity and the antioxidant properties of Aegle marmelos leaf extract: implications for the treatment of Alzheimer's disease. Psychogeriatrics. 2014;14(1):1-10.

Branco A, Santos JDG, Pimentel MMAM, Osuna JTA, Lima LS, David JM. D-mannitol from Agave sisalana biomass waste. Ind Crops Prod. 2010;32(3).

Benkova M, Soukup O, Marek J. Antimicrobial susceptibility testing: currently used methods and devices and the near future in clinical practice. J Appl Microbiol. 2020;129:806-822.

Berridge MV, Herst PM, Tan AS. Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction. Biotechnol Annu Rev. 2005;11:127–152.

Bücker A, Bücker NCF, Souza AQL, Gama AM, Rodrigues-Filho E, Costa FM, et al. Larvicidal effects of endophytic and basidiomycete fungus extracts on Aedes and Anopheles larvae (Diptera: Culicidae). Rev Soc Bras Med Trop. 2013;46(4):411-419.

Cantón E, Espinel-Ingroff A, Pemán J. Trends in antifungal susceptibility testing using CLSI reference and commercial methods. Expert Rev Anti Infect Ther. 2009;7(1):107-119.

Chaithong UW, Choocote K, Kamsuk A, Jitpakdi P, Tippawngkosol D, Chaivasit D, et al. Larvicidal effect of pepper plants on Aedes aegypti (L.) (Diptera: Culicidae). J Vector Ecol. 2006;31(1):138-144.

Cimmino A, Andolfi A, Avolio F, Ali A, Tabanca N, Khan IA, et al. Cyclopaldic acid, seiridin, and sphaeropsidin A as fungal phytotoxins, and larvicidal and biting deterrents against Aedes aegypti (Diptera: Culicidae): structure-activity relationships. Chem Biodivers. 2013;10(7):1239-1251.

Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically (Document M07-A8). Wayne, PA, USA: CLSI. 2008a.

Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi (Approved standard Document M38-A2). Wayne, P.A.: CLSI. 2008b.

Collado IG, Aleu J, Hernández-Galán R. Botrytis species: an intriguing source of metabolites with a wide range of biological activities. Structure, chemistry and bioactivity of metabolites isolated from Botrytis species. Curr Org Chem. 2000;4:1261-1286.

Collado IG, Viaud M. Secondary Metabolism in Botrytis cinerea: Combining Genomic and Metabolomic Approaches. In: Botrytis – the Fungus, the Pathogen and Its Management in Agricultural Systems. 2015;291–313.

Conte H. Morfologia do corpo gorduroso em larvas de Diatrea saccharlis (Lepidóptera: Pyralidae) não parasitadas e parasitas pelo Cotesia flavipes (Hymenoptera: Braconidae). Rio Claro: Universidade Estadual Paulista; 1994.

Cruz-Landim C, Cruz-Hofling MA. Ultrastructure of ovarian follicular epithelium of the Amazonian fish Pseudotylosurus microps (Gunther) (Teleostei, Belonidae): I. the follicular cells cycle of development. Rev Bras Zool. 2000;18(1):99-109.

Daoubi M, Duran-Patron R, Hernandez-Galan R, Benharref A, Hansonc JR, Collado IJ. The role of botrydienediol in the biodegradation of the sesquiterpenoid phytotoxin botrydial by Botrytis cinerea. Tetrahedron. 2006;62(35):8256-61.

Denizot F, Lang R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods. 1986;89(2):271-7.

Elad Y, Filinger Y. Botrytis – the fungus, the pathogen and its management in agricultural systems. Switzerland: Springer International Publishing. 2016.

Elias BC. Chemical study and biological activities of crude extracts obtained from different cultures of Penicillium verrucosum Dierck. Ribeirão Preto: Faculdade de Ciências Farmacêuticas de Ribeirão Preto; 2003.

Ellman GL, Courtney KD, Andres JRV, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7(2):88-95.

Escobar-Niño A, Liñeiro E, Amil F, Carrasco R, Chiva C, Fuentes C, et al. Proteomic study of the membrane components of signalling cascades of Botrytis cinerea controlled by phosphorylation. Sci Rep. 2019;9(9860).

Fasahati P, Woo HC, Liu JJ. Industrial-scale bioethanol production from brown algae: Effects of pretreatment processes on plant economics. Appl Energy. 2015;139(1):175-187.

Ferreira Alves, JS. Estudo químico e biológico de Genipa americana L. (Jenipapo). [Master's dissertation]. Natal: Universidade Federal do Rio Grande do Norte; 2014.

Finney DJ. Probit analysis. Cambridge: Cambridge University Press; 1971.

Freshney R. Culture of animal cells: a manual of basic technique and specialized applications. 6th ed. Scotland: Wiley; 2010.

Grzybowski A, Tiboni M, Silva MA, Chitolina RF, Passos M, Fontana JD. Synergistic larvicidal effect and morphological alterations induced by ethanolic extracts of Annona muricata

and Piper nigrum against the dengue fever vector Aedes aegypti. Pest Manag Sci. 2013;69(5):589-601.

Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol. 1999;86(6):985.

Hazalin, MAMN, Ramasamy K, Lim SM, Wahab IA, Cole ALJ, Majeed ABA. Cytotoxic and antibacterial activities of endophytic fungi isolated from plants at the National Park, Pahang, Malaysia. BMC Complement Altern Med. 2009;9(46).

Henriksson R, Holm S, Littbrand B. Interactions Between Antibiotics and Antineoplastic Drugs on Antibacterial Activity in Vitro. Acta Oncologica. 1990;29(1):43-6.

Katoch M, Singh G, Sharma S, Gupta N, Sangwan PL, Saxena AK. Cytotoxic and antimicrobial activities of endophytic fungi isolated from Bacopa monnieri (L.) (Pennell Scrophulariaceae). BMC Complement Altern Med. 2014;14(52):52.

Kiviharju K, Nyyssölä A. Contributions of biotechnology to the production of mannitol. Recent Pat Biotechnol. 2008;2(2):73-8.

Lee CC, Houghton P. Cytotoxicity of plants from Malaysia and Thailand used traditionally to treat cancer. J Ethnopharmacol. 2005;100(3):237-43.

Liang H, Xing Y, Chen J, Zhang D, Guo S, Wang C. Antimicrobial activities of endophytic fungi isolated from Ophiopogon japonicus (Liliaceae). BMC Complement Altern Med. 2012;12(238):238.

Lima JB, Pereira Da Cunha M, Carneiro Da Silva R, Galardo AKR, Soares SS, Braga IA, et al. Resistance of Aedes aegypti to organophosphates in several municipalities in the state of Rio de Janeiro and Espirito Santo, Brazil. Am J Trop Med Hyg. 2003;68:329-33.

Lowry OH, Rosebrough NJ, Farr AL, Randall R. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-275.

Majchrzak-Stiller B, Buchholz M, Peters I, et al. Oxathiazinane derivatives display both antineoplastic and antibacterial activity: a structure activity study. J Cancer Res Clin Oncol. 2023;149:9071-9083.

Mao Z, Lai D, Liu X, Fu X, Meng J, Wang A, et al. Dibenzo-α-pyrones: a new class of larvicidal metabolites against Aedes aegypti from the endophytic fungus Hyalodendriella sp. Ponipodef12. Pest Manag Sci. 2017;73(7):1478-85.

Marucci G, Buccioni M, Dal Bem D, Lambertucci C, Volpini R, Amenta F. Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease. Neuropharmacology. 2021;190(1):108352.

Morales G, Paredes A, Sierra P, Loyola LA. Antimicrobial activity of three baccharis species used in the traditional medicine of Northern Chile. Molecules. 2008;13:790-794.

Morževska EB, Bankina B, Kaņeps J. Botrytis genus fungi as causal agents of legume diseases: a review. Res Rural Dev. 2019;2:63-9.

Murugesan AG, Prabu CS, Selvakumar C. Biolarvicidal activity of extracellular metabolites of the keratinophilic fungus Trichophyton mentagrophytes against larvae of Aedes aegypti - a major vector for chikungunya and dengue. Folia Microbiol. 2009;54(3):213-6.

Nordin LM, Kadir AA, Zakaria ZA, Abdullah R, Abdullah MNH. In vitro investigation of cytotoxic and antioxidative activities of Ardisia crispa against breast cancer cell lines, MCF-7 and MDA-MB-231. BMC Complement Altern Med. 2018;18(1):1-10.

Nurunnabi TR, Al-Majmaie S, Nakouti I, Nahar L, Rahman SMM, Sohrab MH, et al. Antimicrobial activity of kojic acid from endophytic fungus Colletotrichum gloeosporioides isolated from Sonneratia apetala, a mangrove plant of the Sundarbans. Asian Pac J Trop Med. 2018;11(5):350-354.

Ota A, Kawai S, Oda H, Iohara K, Murata K. Production of ethanol from mannitol by the yeast strain Saccharomyces paradoxus NBRC 0259. J Biosci Bioeng. 2013;116(3):327-32.

Patel TK, Williamson JD. Mannitol in plants, fungi, and plant–fungal interactions. Trends Plant Sci. 2016;21(6):486-497.

Popli D, Anil V, Subramanyam AB, Namratha MN, Ranjitha R, Rao SN, et al. Endophyte fungi, Cladosporium species-mediated synthesis of silver nanoparticles possessing in vitro antioxidant, anti-diabetic and anti-Alzheimer activity. Artif Cells Nanomed Biotechnol. 2018;5(n):1-8.

Ragavendran C, Natarajan D. Insecticidal potency of Aspergillus terreus against larvae and pupae of three mosquito species Anopheles stephensi, Culex quinquefasciatus, and Aedes aegypti. Environ Sci Pollut Res. 2015;22(21):17224-37.

Rani R, Sharma D, Chaturvedi M, Yadav JP. Antibacterial activity of twenty different endophytic fungi isolated from Calotropis procera and time kill assay. Clin Microbiol. 2017;6(3):2-6.

Revathi K, Chandrasekaran R, Thanigaivel A, Senthil-Nathan S, Kirubakaran S, Sathish-Narayanan S. Effects of Bacillus subtilis metabolites on larval Aedes aegypti L. Pestic Biochem Physiol. 2013;107(3):369-76.

Ripardo-Filho HS, Ruíz VC, Suárez I, Moraga J, Aleu J, Collado IG. From genes to molecules, secondary metabolism in Botrytis cinerea: new insights into anamorphic and teleomorphic stages. Plants. 2023;12:553.

Riss S, Mohamed F, Dayal S, Cecil T, Stifta A, Bachleitner-Hofmann T, et al. Peritoneal metastases from colorectal cancer: patient selection for cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. Eur J Surg Oncol. 2013;39(9):931-37.

Saha BC, Racine FM. Biotechnological production of mannitol and its applications. Appl Microbiol Biotechnol. 2011;89(4):879-91.

Santos IP. Caracterização dos metabólitos produzidos pelo fungo Nigrospora sphaerica (sacc.) mason endofítico de Indigofera suffruticosa mill. E avaliação do potencial biotecnológico [PhD thesis]. Recife: Universidade Federal de Pernambuco; 2015.

Salvat A, Antonacci L, Fortunato RH, Suarez EY, Godoy HM. Antimicrobial activity in methanolic extracts of several plant species from northern Argentina. Phytomedicine. 2004;1(2–3):230-4.

Salvatore MM, Andolfi A. Phytopathogenic fungi and toxicity. Toxins. 2021;13(10):689.

Silva AK. Manual de vigilância e epidemiológica e sanitária. 2nd ed. Goiânia: AB; 2015.

Silva CB, Pott A, Elifio-Esposito S, Dalarmi L, Nascimento KF, Burci L, et al. Effect of donepezil, tacrine, galantamine and rivastigmine on acetylcholinesterase inhibition in dugesia tigrine. Molecules. 2016;21(1):53

Singh B, Thakur A, Kaur S, Chadha BS, Kaur A. Acetylcholinesterase inhibitory potential and insecticidal activity of an endophytic Alternaria sp. from Ricinus communis. Appl Biochem Biotechnol. 2012;168(5):991-1002.

Sundaravadivelan C, Padmanabhan MN. Effect of mycosynthesized silver nanoparticles from filtrate of Trichoderma harzianum against larvae and pupa of dengue vector Aedes aegypti L. Environ Sci Pollut Res. 2014;21(6):4624-33.

Tarman K, Lindequist U, Wende K, Porzel A, Arnold N, Wessjohann LA. Isolation of a new natural product and cytotoxic and antimicrobial activities of extracts from fungi of Indonesian marine habitats. Mar Drugs. 2011;9(3):294-306.

Tenny S, Patel R, Thorell W. Mannitol. In: StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470392/

Tomaszewska L, Rywinska A, Gładkowski W. Production of erythritol and mannitol by Yarrowia lipolytica yeast in media containing glycerol. J Ind Microbiol Biotechnol. 2012;39:1333-43.

Valotto CFB, Silva HHG, Cavasin G, Geris R, Filho ER, Silva IG. Alterações ultraestruturais em larvas de Aedes aegypti submetidas ao diterpeno labdano, isolado de Copaifera reticulata (Leguminosae), e à uma fração rica em taninos de magonia pubescens (Sapindaceae). Rev Soc Bras Med Trop. 2011;44(2):194-200.

Wang J, Kim YM, Rhee HS, Lee MW, Park JM. Bioethanol production from mannitol by a new isolated bacterium, Enterobacter sp. JMP3. Bioresour Technol. 2013;135(sn):199-206.

Wang Y, Lai Z, Li XX, Yan RM, Zhang ZB, Yang HL, et al. Isolation, diversity and acetylcholinesterase inhibitory activity of the culturable endophytic fungi harboured in Huperzia serrata from Jinggang Mountain. World J Microbiol Biotechnol. 2016;32(20).

Wang K, Zhang H, Zhu W, Peng J, Li X, Wang Y, et al. Preliminary study of resistance mechanism of Botrytis cinerea to SYAUP-CN-26. Molecules. 2022;27:936.

World Health Organization. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. Geneva: WHO; 1981a.

World Health Organization. Criteria and meaning of tests for determining the susceptibility or resistance of insects to insecticides. Geneva: WHO; 1981b.

Yousfi H, Ranque S, Rolain JM, Bittar F. In vitro polymyxin activity against clinical multidrug-resistant fungi. Antimicrob Resist Infect Control. 2019;8:66.

Yu FX, Li L, Chen Y, Yang YH, Li GH, Zhao PJ. Four new steroids from the endophytic fungus Chaetomium sp. M453 derived of Chinese Herbal Medicine Huperzia serrata. Fitoterapia. 2016.

Yu KX, Wong CL, Ahmad, R, Jantan I. Larvicidal activity, inhibition effect on development, histopathological alteration and morphological aberration induced by seaweed extracts in Aedes aegypti (Diptera: Culicidae). Asian Pac J Trop Med. 2015;8(12):1006-12.

Xu M, Huang Z, Zhu W, Liu Y, Bai X, Zhang H, Fusarium-Derived Secondary Metabolites with Antimicrobial Effects. Molecules. 2023;28:3424.

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