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IN VITRO ANTIMICROBIAL AND CYTOTOXIC EFFECTS OF *Talaromyces islandicus* T101 FROM A POLLUTED ENVIRONMENT

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Abstract: *Talaromyces islandicus* TI01 was isolated from a marine-influenced environment that has been suffering for decades from anthropogenic actions in its body of water. Broth microdilution technique was performed to analyze the antimicrobial activity. For analysis of the cytotoxic activity, the MTT [3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide] assay was conducted. The chemical analysis of the extract was performed by LC/MS (liquid chromatography coupled to mass spectrometry). The minimum bactericidal concentration (MBC) of *T. islandicus* for *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 was 1000 µg/ml. The minimum inhibitory concentration (MIC) for *E. coli* was 250 µg/mL and for *S. aureus* 500 µg/mL, respectively, whereas for *C. tropicalis* ATCC 1369 was 62.5 µg/mL. IC50 for breast cancer cell line (MCF-7) was 45.43 ± 1.657 µg / mL. The major compounds present in the extract were: Luteoskyrin **(1)** and N-GABA-PP-V (6-[(Z)-2-Carboxyvinyl]-N-GABA-PP-V) **(2)**. The results show that *T. islandicus* TI01 has a prominent antibacterial activity against *E. coli* and *S. aureus,* making this fungi for the development of new food preservatives.
Keywords: *Talaromyces islandicus*; antibacterial; cytotoxicity; natural product.

INTRODUCTION

Filamentous fungi are known to produce a variety of secondary metabolites that are related to the resistance of these organisms to various adverse environmental factors, such as pollution, exposure to extreme temperatures, irradiation and photo-oxidation, or in ecological interactions with other organisms such as sponges, corals or other microbial communities (O'BRIEN; WRIGHT, 2011).

Pigments are among these secondary metabolites and these natural compounds are potential candidates to replace synthetic dyes, which exhibit disadvantages including toxicity to health or the environment, and mutagenic and carcinogenic properties (LOPES et al., 2013). Furthermore, many pigments have important biological properties, such as antibacterial, antifungal, antitumoral and anticholesterolemic activities, which has been leading to an increasing attention by the pharmaceutical industry (ARUMUGAM et al., 2015; CHAKRAVARTI; SAHAI, 2004; EVIDENTE et al., 2014; (GEWEELY, 2011).

The *Talaromyces* genus (*Eurotiomycetes*, *Trichocomaceae*) was initially created to comprise the teleomorphs of biverticillate *Penicillium* species. However, according to the principle "one fungus – one name" affirmed in fungal taxonomy, which adopts a single holomorphic denomination for species presenting two different stages in their life cycle(TAYLOR, 2011), *Talaromyces* sp. now includes all species in the *Penicillium* subgenus *Biverticillium*(SAMSON et al., 2011), while *Penicillium* sp. comprises the *sensu stricto* species belonging to the subgenera *Aspergilloides*, *Furcatum*, and *Penicillium*, their associated *Eupenicillium* teleomorphs, and species classified in a few related genera(HOUBRAKEN; SAMSON, 2011).

Talaromyces islandicus is one of the most destructive and harmful fungi that affect rice in storage, causing the yellowing of rice (AYAKO et al., 2005; SAITO et al., 1971) and is also able to produce mycotoxins such as cyclochlorotine, islanditoxin, erythroskyrine and luteosyrin, which are hepatotoxic and carcinogenic agents (PENG et al., 2015). Luteoskyrin is in the IARC Group 3 carcinogen and yield of this substance is the highest among the metabolites of *T. islandicus* (SIEMIATYCKI et al., 2004).

Due to the increasing resistance acquired by microrganisms to antibiotic and antifungal

drugs commonly used in the clinical setting, antimicrobial agents of natural origin are being used to treat microbial infections, thus saving millions of lives(QUAIYUM et al., 2019). Moreover, cancer is a major cause of morbidity and mortality in developing and developed countries, and it is well documented the resistance of many cancerous cells to antitumoral drugs, which consists in an obstacle for cancer eradication, mainly due to a failure of the damaged cells to activate apoptotic pathways. It is also reported a protective effect of autophagy of cancer cells, making it harder to develop an effective strategy to counteract cancer cell growth and improving the response to therapy(GIANSANTI et al., 2011).

In an attempt to discover new molecules with antimicrobial and antitumoral activities, we investigated, in the present study, the antifungal, antibacterial and cytotoxic activities *Talaromyces islandicus* TI01 isolated from a soil influenced by a marine environment (State Park of Jansen's Lagoon located in São Luís, Maranhão, Brazil) that has been suffering for decades from the anthropogenic actions of the nearby houses and commercial buildings, against *Candida albicans* ATCC 10231, *Candida tropicalis* ATCC 1369, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 as well as its cytotoxic activity against breast cancer cell line (MCF-7). We also tried to characterize the chemical compounds present in *T. islandicus* extract, comparing them to substances already described in the scientific literature.

RESULTS

YIELD OF *T. ISLANDICUS*' TI01

From a total of 1.5 liters of Potato-Dextrose Broth where the fungus was cultivated, it was obtained a total of 6.660 grams of fungal biomass and 0.857 grams of intracellular pigment, which was contained in the mycelium of the fungus (Figs 1 and 2)

CHEMICAL CHARACTERIZATION OF *T. ISLANDICUS* TI01

Figure 3 shows the chemical profile of the extract from a chromatographic run with a duration of 68 minutes at a wavelength of 254 nm, obtaining eight peaks where most of the compounds present in the extract were concentrated. The m/z, retention times and area in percentage (%) are illustrated in table 1. The spectra of each compound corresponding to the obtained peaks are illustrated in the Supplementary Material.

Peak 1 was detected at 8.4 minutes, peak 2 at 12.3 minutes, peak 3 at 20.1 minutes, peak 4 at 45.5 minutes, and peak 5 at 50.3 minutes, peak 6 detected at 59.2 minutes, peak 7 to 60.1 minutes, peak 8 to 61.5 minutes.

MOLECULAR IDENTIFICATION OF THE FUNGAL STRAIN

The size of the small subunit ribosomal RNA gene, internal transcribed spacer 1 (ITS 1) and 5.8 S ribosomal RNA gene region amplified in this work was 328 base pairs (bp) (Figure 4). The nucleotide BLAST search result showed that the amplified sequence was most similar (98.43%) to the sequence of *Talaromyces islandicus* strain CBS 178.68 and the e-value was of $3e^{-161}$. The strain is deposited on the Collection of Fungi of the Laboratory of Mycology, Nucleus for Basic and Applied Immunology, Center for Biological and Health Sciences, Federal University of Maranhão.

BIOLOGICAL ACTIVITIES OF *T. ISLANDICUS*

T. islandicus is effective against *E. coli*

T. islandicus TI01 was bactericidal against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 just at 1.000 mg/mL. MBC against both

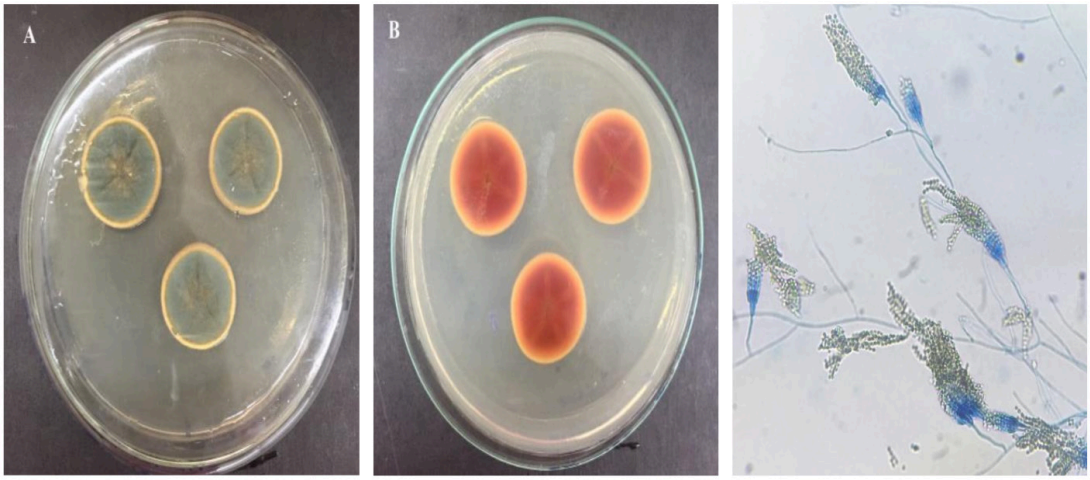

Figure 1. Macroscopy and microscopy of *Talaromyces islandicus*. 1A: Obverse of *Talaromyces islandicus* colonies isolated on Sabouraud-Dextrose Agar. 1B: Reverse of *T. islandicus* colonies isolated on Sabouraud-Dextrose Agar. 1C: Microscopy of T. *islandicus*

Figure 2. Red pigmented extract obtained from *T. islandicus* TI01.

Peak	m/z	RT	Probable Compounds	Area %	Chemical classes	References
1	481,19	8.4	HHDP-glucose	0,185709	Hydrolyzable Tannin	ROBBINS et al (2014).
2	302,97	12.3	Ganoderic acid C2	0,326711	Triterpenoid	CAO et al (2017).
3	649,04	20.1	Galloyl-HHDP-gluconate (lagerstannin C) isomer	2,992329	Tannin	MENA et al. (2012)
4	589,09	45.5	Rhamnosyl-hexosyl-acyl-quercetin	0,660952	Flavonoid	SAID et al. (2017)
5	573,05	50.3	Luteoskyrin	74,84014	Naphtalene	PENG et al. (2015)
6	595,01	59.2	Pelargonidin-3,5-diglucoside	1,458194	Anthocyanidin	BARNES; SCHUG (2011)
7	931,46	60.1	Notoginsenoside-R1	0,86856	Ginsenoside	SUN et al (2016)
8	454,20	61.5	N-GABA-PP-V (6-[(Z)-2-Carboxyvinyl]-N-GABA-PP-V)	18,66741	Azaphilone polyketide	VENKATACHALAM et al. (2018)

Table 1. Probable compounds present in *T. islandicus*' TI01extract.

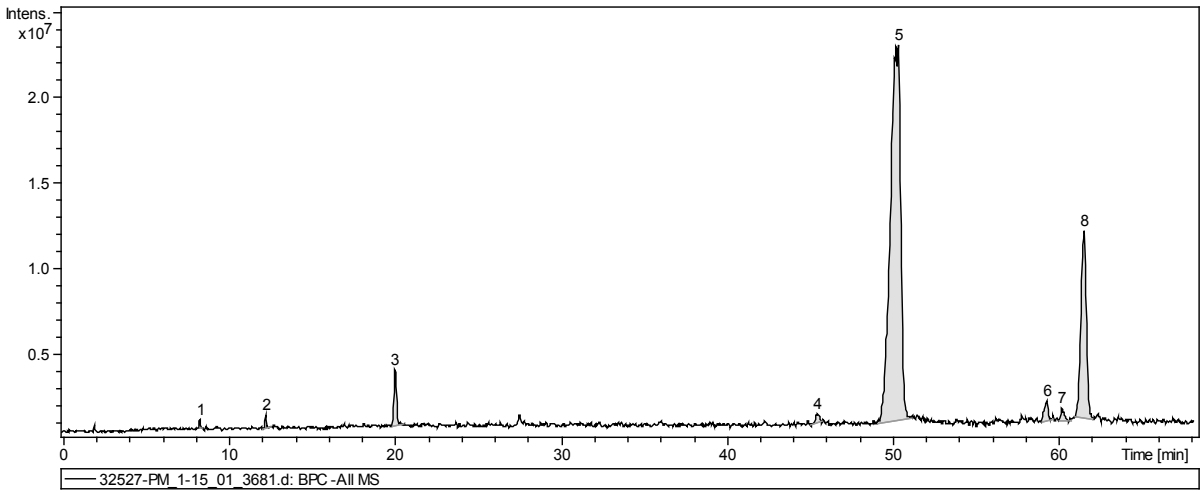

Figure 3. Chromatographic profile of *T. islandicus*' extract (wavelength of 254 nm).

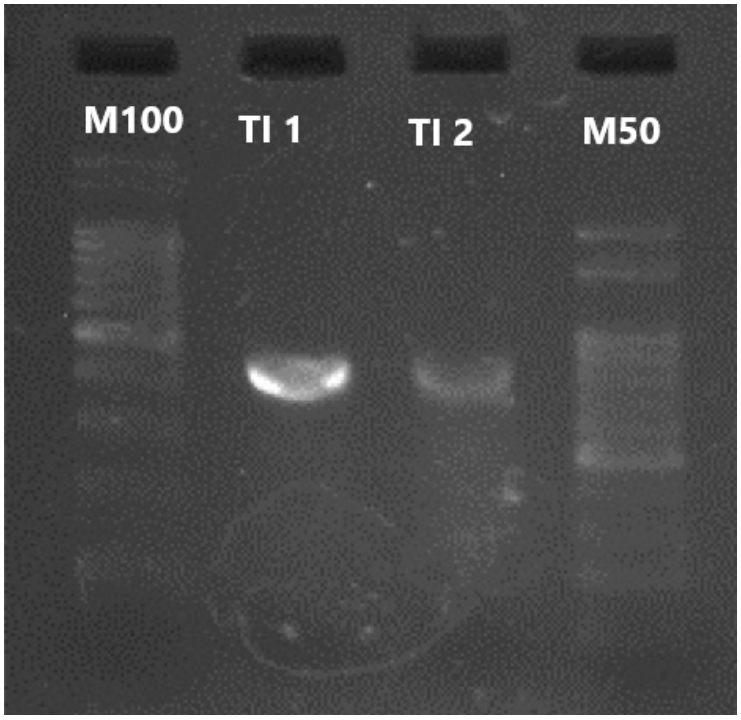

Figure 4. PCR amplification of DNA from *T. islandicus* with specific primers TM1 and TM2. M100: Size marker of 100-bp-ladder standard DNA. M50: size marker of 50-bp-ladder standard DNA

bacteria was 3x the MIC. MIC (Minimum Inhibitory Concentration) against *E. coli* was 250 µg/mL ($p = 0.0005$) and 500 µg/mL against *S. aureus* ($p = 0.0078$) since the log of colony forming units (CFUs) showed a significant statistical difference compared to the negative control (Figure 5). There was no growth of colonies in the positive controls (4 µg/mL gentamycin for *E. coli)* and 8 µg/mL vancomycin for *S. aureus*).

MIC value for *C. tropicalis* ATCC 1369 was 62.5 µg/mL ($p < 0.0001$) (Figure 5). It is noteworthy that at the concentration of 125 µg/mL, the log of CFUs (2.71) was lower compared to the other concentrations that also showed a significant statistical difference in relation to the negative control (500-62.5 µg/mL), showing that this concentration presented a greater fungistatic activity compared to the others. *T. islandicus* was not active against *C. albicans* ATCC 10231. There was no growth of colonies in the positive controls (AMB at 16 µg/mL).

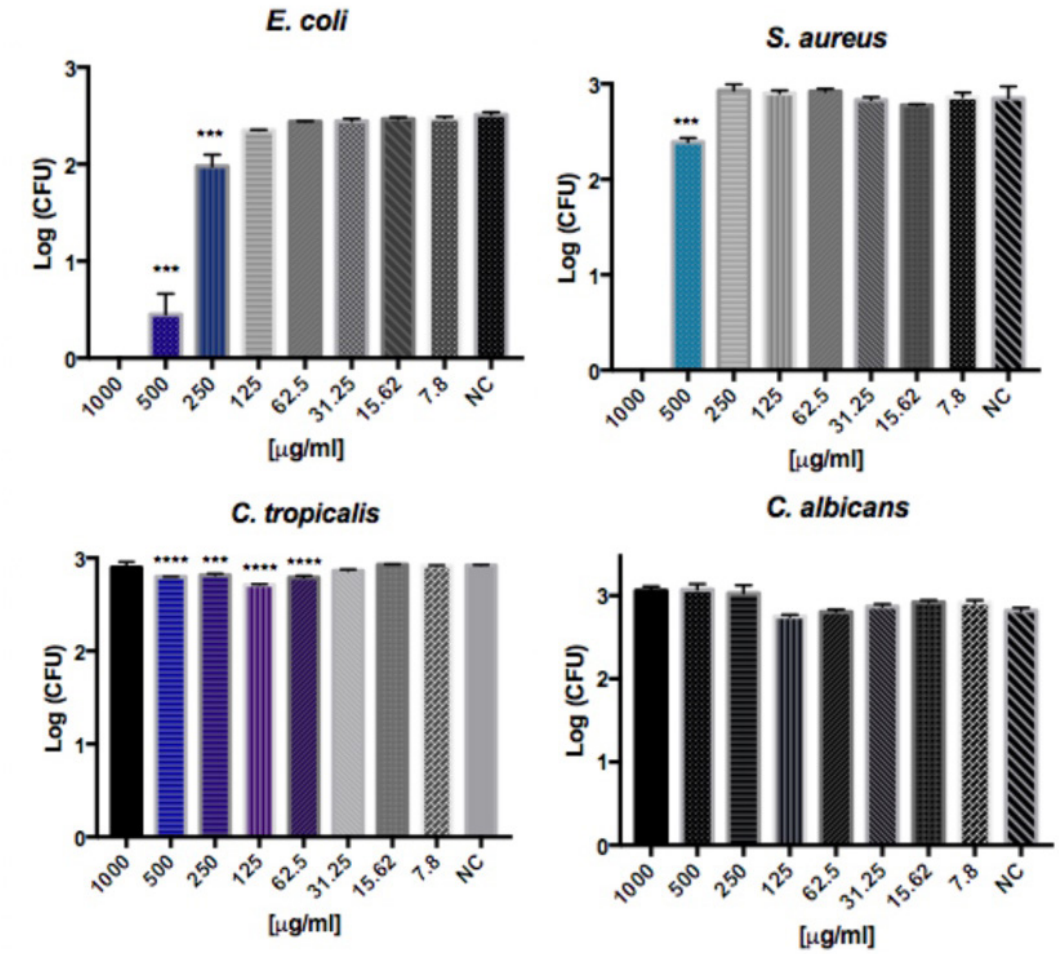

Figure 5. Logarithm of the number of Colony Forming Units (CFUs) of the tested microorganisms in the presence of decreasing concentrations of *T. islandicus* TI01 extract. From the top left to the bottom right: *E. coli* ATCC 25922; *S. aureus* ATCC 25923; *C. tropicalis* ATCC 1369 and *C. albicans* ATCC 10231. Error bars represent standard deviation. Statistical p values (represented by *** or ****) indicates concentrations that are significantly different from control. *** $p < 0.05$; **** $p < 0.001$.

T. ISLANDICUS EXTRACT INDUCES BREAST CANCER CELL DEATH

The intracellular red pigmented extract (IRPE) from *T. islandicus* TI01 promoted breast cancer cell's viability reduction after 72 hours of treatment at the lowest concentration tested: 0.25 µg/mL, compared to the other treatments, with a mean cellular viability of 63.13%, although it showed no statistical difference compared to the negative control (p=0.0713) (Figure 6). The mean IC50 value was 45.43 ± 1.657 µg/mL.

T. ISLANDICUS EXTRACT HAS ANTIOXIDANT ACTIVITY

Antioxidant activity (%) increased proportionally with extract concentration, producing an EC_{50} (concentration required to achieve 50% antioxidant activity) of 84,3201µg/mL (Figure 7).

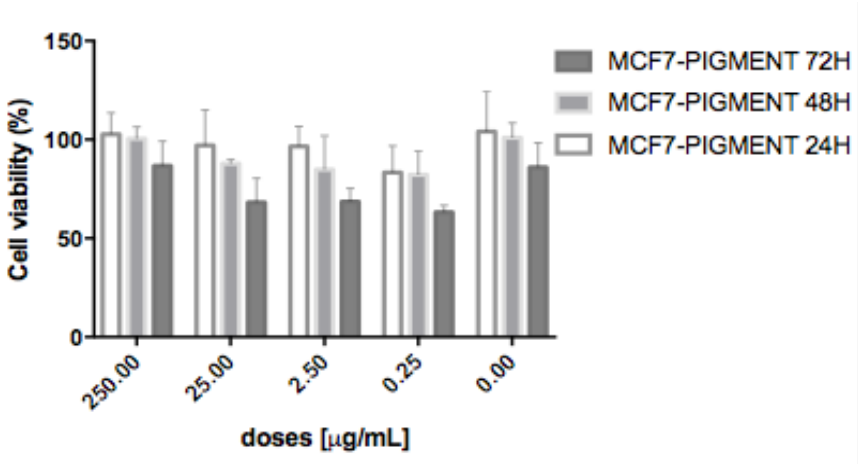

Figure 6. Reduction of MCF-7 cells' viability in all treatments, with a greater cell viability reduction after 72 hours of treatment at the dose of 0.25 µg/mL. 0.00: negative control.

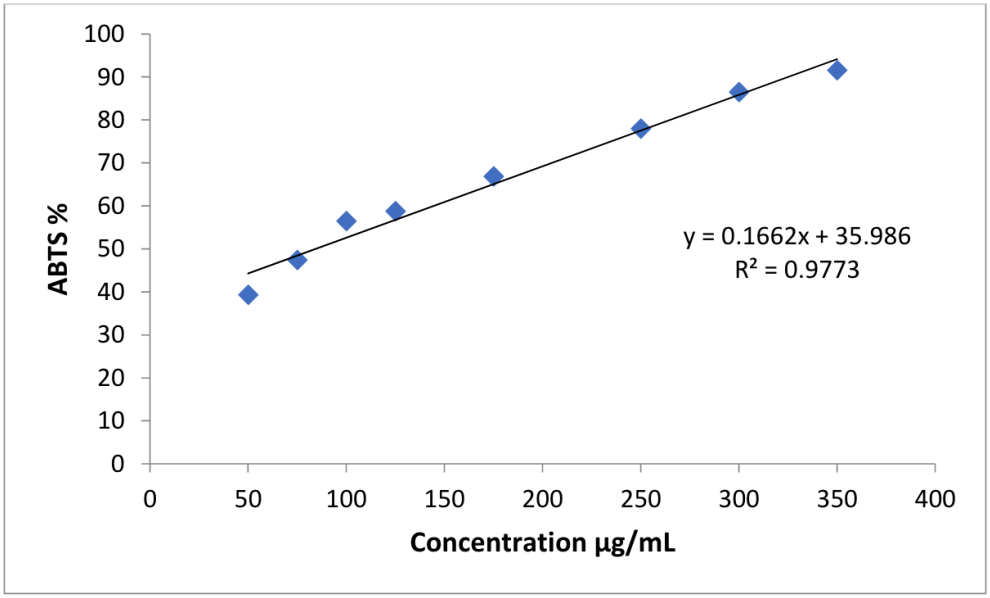

Figure 7. Antioxidant activity of the red pigmented extract of *T. islandicus* by ABTS method.

DISCUSSION

In this study, we used ethyl acetate as single solvent to extract *T. islandicus*' TI01. According to Venkatachalam et al. (2018), this is the best solvent to be used to extract pigments from fungi belonging to the *Talaromyces* genus followed by ethanol because it is able to extract the major pigmented compounds from these fungi.

Yield of luteoskyrin is the highest among metabolites of *T. islandicus*, which corroborates the finding of this study, where we tentatively identified that luteoskyrin was the majoritarian compound, with an area of 74.84%. In addition, scientific literature reports that this substance inhibits replication, transcription, and DNA repair in bacteria, yeast, and animal cells and forms chelates with nucleic acids(MAGAN; OLSEN, 2004), which may be responsible for the bactericidal acitivity of *T. islandicus*' TI01 at the highest concentration tested.

Talaromyces species are known to produce intra and extracelullar yellow, orange and red pigments. Azaphilone polyketide pigments like mitorubrins (mitorubrin, mitorubrinol, mitorubrinol acetate and mitorubrinic acid) and the *Monascus* red pigments (N-glutaryl monascorubramin, N-glutarylrubropunctamin, monascorubramine, monascin, PP-R and others)(MAPARI et al., 2009) are responsible for the red pigments and are produced in different ratios and amounts between different isolates and species. N-GABA-PP-V (6-[(Z)-2-Carboxyvinyl]-N-GABA-PP-V) is an azaphilone polyketide and it was tentatively identified as the second majoritarian compound present in *T. islandicus*' TI01, which corroborates these data found in the literature.

Furthermore, literature affirms that phenolic compounds and flavonoids possess anti-bacterial activity (BOUARAB-CHIBANE et al., 2019; FARHADI et al., 2019; HOU et al., 2000; TALEB-CONTINI et al., 2003). Galloyl-HHDP-gluconate (lagerstannin C) isomer is a phenolic compound and it was tentatively identified as the third majoritarian compound present in *T. islandicus*' TI01. Rhamnosyl-hexosyl-acyl-quercetin is a flavonoid, and it it was tentatively identified in the present extract. It is also known that the presence of gallic or galloyl moiteties to chemical compounds induces damage to the bacterial membrane(IKIGAI et al., 1993), while flavonoids inhibit nucleic acid synthesis, cytoplasmic membrane function, energy metabolism, attachment and biofilm formation, porin on the cell membrane, alters the membrane permeability and attenuates the pathogenicity(XIE et al., 2014). Therefore, these substances may have also contributed to the relevant anti-bacterial activity exhibited by the present extract.

Soumya et al(SOUMYA et al., 2018) found an IC50 value of a solid red pigment of oily nature produced by *Fusarium chlamydosporum*, also a filamentous fungus, of 62 μg/mL against MCF-7. The tested substance in this work showed a lower IC50 value (45.43 μg/mL) against the same tumor cell line, revealing its cytostatic activity in MCF-7 cels.

T. islandicus TI01 showed bactericidal activity against two human pathogenic bacteria: *S. aureus* and *E. coli* at the highest concentration tested. Furthermore, *T.islandicus* extract exhibited a weak cytotoxic activity on MCF-7 cell line. In view of the results found, future *in vivo* studies should be performed to verify if *T. islandicus* does not exert any cytotoxic effect on an animal model. It is important to highlight that these pathogens are related to food contamination making this pigment a potential candidate to be used as a food dye and conservative agent.

MATERIAL AND METHODS

COLLECTION SITE

T. islandicus TI01 was isolated from the soil of a polluted environment in São Luís, Maranhão, Brazil, named Jansen Lagoon State Park, whose geographic coordinates are: 2º 30' 13" S 44º 17' 53" W.

The samples were obtained at a depth of up to 20 cm, with the aid of a sterile spoon according to (SILVA et al., 2011)with modifications. Then, they were placed in a zipped plastic bag and transported to the Laboratory of Mycology (Nucleus for Basic and Applied Immunology/Department of Pathology/Center of Biological and Health Sciences/ Federal University of Maranhão), where they were processed. The fungal strain is deposited in the Collection of Fungi of the Federal University of Maranhão.

FUNGUS IDENTIFICATION

The isolate was identified by analysis of its morphological characteristics and ITS (internal transcribed spacer) gene sequence, which has been submitted to GenBank (accession number: MN831880). DNA was extracted using DNEasy Plant Mini Kit from Qiagen. The pair of primers designed was: 5' CGT AAC AAG GTT TCC GTA GGT 3' (forward) and 5'GTG CTT GAG GGC AGA AAT GA 3' (reverse). A BLAST search result indicated that the sequence is almost the same (98.48%) to the sequence of *Talaromyces islandicus* strain CBS 178.68.

The pair of primers cited above was subjected to automatic sequencing using the ABI PRISMTM 310 Big Dye Terminator v3.1 Matrix Standards kit (Applied Biosystems). With the help from a Phred/Phrap/Consed pack (GORDON, David; GREEN, 2013) (EWING; GREEN, 1998; GORDON, D.; ABAJIAN; GREEN, 1998; GORDON, D.; DESMARAIS; GREEN, 2001) it was generated a consensus sequence that was used for the molecular identification of the fungal strain.

NATURAL PRODUCT EXTRACTION

The fermentation was carried out statically, in the dark, in Potato-Dextrose Broth (PDB) (Kasvi, pH 5.1 ± 0.2 at 25° C) in 1,000 mL Erlenmeyers flasks containing 500 mL of the liquid medium in triplicate. The flasks were incubated for 21 days at 25° C. Thereafter, 300 ml of Ethyl Acetate was added to 500 ml of the fermented broth and left overnight for decantation. The mycelium was then separated from the liquid medium by filtration on Whatman paper no. 4. Then, 300 ml of Ethyl Acetate was added to the fungal biomass to provide the intracellular pigment. The solutions were placed in a separation funnel to obtain purer solutions. They were then concentrated in a rotaevaporator and stored at 4°C for further analysis of the biological activities *in vitro*.

CHEMICAL CHARACTERIZATION OF *T. ISLANDICUS*' TI01

After solubilization, the extract was analyzed by high performance liquid chromatography (HPLC) using a Shimadzu® chromatograph (ShimadzuCorp. Kyoto, Japan), consisting of a solvent injection module with a LC-20ADShimadzu detector UV-Vis pump (SPDA-20A) - Shimadzu. The column used was SupelcoAscentisC-18 (250x4.6mm - 5um). The elution solvents used were A (water) and B (methanol).

The established elution gradient started from 5% B for 1 minute, 30% B for 14 minutes, 60% B for 15 minutes, 60% B for 30 minutes, 70% B for 30 min, 70 % B for 7 minutes, 5% B for 1 minute rebalancing the column, using a flow rate of 1.0 mL / min, using oven temperature of 40 ° C. The sample injection volume was 20 µL. The data were collected and processed using the LCSolution

software (Shimadzu). Under the conditions employed, a baseline separation was obtained for the main components of the sample in a chromatographic run of 68 minutes with a wavelength of 254nm.

ANTIMICROBIAL ASSAY

Antimicrobial activity evaluation against four human pathogens (*E. coli*, *S. aureus*, *C. albicans,* and *C. tropicalis*) was carried out by microplate assay in triplicate, according to the NCCLS (Clinical and Laboratory Standards Institute (2015).To determine MBCs and MICs against these microorganisms, each well containing the pigment extract was plated on Petri dishes and the log of Colony Forming Units was calculated and compared to the negative and positive controls. Gentamycin at a concentration of 10 µg/ml was used for *E. coli* ATCC 25922 and vancomycin at 8 µg/ml was used for *S. aureus* ATCC 25923 (NCCLS - Clinical and Laboratory Standards Institute (2015). Amphotericin at 16 µg/ml was used for both yeasts (*C. albicans* ATCC 10231 and *C. tropicalis* ATCC 1369), as positive controls (NATIONAL COMITEE ON CLINICAL LABORATORY STANDARDS, 2002). Negative controls consisted of *T. islandicus*' TI01 added to the culture medium (Mueller-Hinton Broth for bacteria and BHI Broth for yeasts) at concentrations that ranged from 1.000 to 7.8 µg/ml and microorganisms' inocula standardized at 0.5 MacFarland scale.

CELL VIABILITY ASSAY

Antitumoral activity was evaluated by the MTT assay [3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide]. Briefly, MCF-7 cells (2.0 x 10^4 per well plus DMEM medium) were seeded onto a sterile 96-well plate. After a 24 hr incubation, the medium was replaced with 100 mL of fresh FBS-free medium which contained IRPE at concentrations of 250, 25, 2.5 and 0.25 mg/mL. Negative control, containing only medium with 10% SFB and tumoral cells. The plate was incubated for 24, 48 and 72 h at 37 C in 5% CO_2 and then the media were discarded. Afterwards, the cells were stained with 100 mL of MTT solution (0.5 mg/ml) at 37° C for 4 h. Thereafter, the supernatant was aspirated, and 100 mL of ethyl alcohol was added to dissolve the formazan. The optical density at 540 nm (OD540) was determined (FANG et al., 2012). The experiment was performed in triplicate.

The viability of the cells was quantified in percentage, using the following equation:

Cell viability (%) = (ODtest/ODcontrol) x 100 where ODcontrol is OD570 in the negative control wells (cells incubated with media only) and ODtest is the OD570 of the cells exposed to IRPE.

ANTIOXIDANT ACTIVITY BY 2,2'-AZINOBIS-[3-ETHYLBENZTHIAZOLINE-6-SULFONIC ACID] DECOLORIZATION ASSAY (ABTS)

The antioxidant activity of the extracts was evaluated using 1,2'-azinobis-[3-ethylbenzthiazoline-6-sulfonic acid (ABTS), according to methods described by (Re et al. (1999). For a range of extract concentrations (50 a 350 µg/mL), reaction mixtures with ABTS were prepared.

The ABTS • + radical was prepared by reacting 5.0 mL of a 3840 µg / mL ABTS solution with 88 µL of the 37,840 µg / mL of potassium persulfate solution. The mixture was left in a dark environment for 16 hours. After radical formation, the mixture was diluted in ethanol (approximately 1:30 v/v) until an absorbance of 0.7 to 734 nm was obtained.

From the concentrations of the extracts (50 to 350 µg / mL) the reaction mixture was prepared with the cation radical ABTS. In a dark environment, an aliquot of 30 µL

of each concentration of the extracts was transferred in test tubes containing 3.0 mL of the radical ABTS cation and homogenized in a tube shaker. After 6 minutes, the absorbance of the reaction mixture was read on a spectrophotometer. in length of 734 nm.

The analyzes were performed in triplicate and the capture of the free radical was expressed as a percentage of inhibition (% I) of the ABTS radical cation according to Babili et al. (2011).

$$I(\%) = \left(\frac{Abs_B - Abs_A}{Abs_B}\right) * 100$$

Where: I%: Percentage of inhibition of the ABTS radical
AbsA: absorbance of the sample (extract) after 6 min
AbsB: absorbance at 734 nm of the ABTS radical solution

From the data obtained, the efficient concentration or EC50% was calculated, defined as the concentration of the sample required to sequester 50% of the ABTS radicals. The extract is considered active when it has an EC50% <500 μg / mL (CAMPOS et al., 2003).

STATISTICAL ANALYSIS

Results were analyzed using one-way analysis of variance (ANOVA) and applying Dunnett's multiple comparisons post-test, using GraphPad Prism 6 (Graphpad Software, CA, USA).

DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request.

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A preprint has previously been published in bioRxiv, available at https://www.biorxiv.org/content/10.1101/2020.06.11.145821v1 (RODRIGUES et al. (2020).

FUNDING STATEMENT

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AUTHOR CONTRIBUTIONS

I.V.P.R. prepared the manuscript and performed the experiments for the extraction and antimicrobial evaluation; K.R.A.B and R.N.S.A helped in the antimicrobial evaluation; N.C. helped in the extraction of the red pigmented extract from *T. islandicus* TI01; A.M.T. helped in the chemical analysis; M.S.A., J.D.P. JCR and A.S.K. helped in the molecular identification of the fungal strain; A.M.V, MACNS and S.J.A.G helped in the antitumoral evaluation and M.D.S.B.N. and G.F.B.B. supervised the research work and revised the manuscript.

CONFLICT OF INTEREST

All authors revised the manuscript and there is no conflict of interest.

REFERENCES

ARUMUGAM, G. K. et al. Production and characterization of bioactive metabolites from piezotolerant deep sea fungus Nigrospora sp. in submerged fermentation. **Journal of Applied Microbiology**, v. 118, n. 1, p. 99–111, 2015.

AYAKO, S. et al. Mycological Examination of Domestic Unpolished Rice and Mycotoxin Production by Isolated Penicillium islandicum. n. October, p. 205–212, 2005.

BABILI, F. el et al. Oregano: Chemical analysis and evaluation of its antimalarial, antioxidant, and cytotoxic activities. **Journal of Food Science**, v. 76, n. 3, p. C512–C518, 2011.

BARNES, J. S.; SCHUG, K. A. Structural characterization of cyanidin-3,5-diglucoside and pelargonidin-3,5-diglucoside anthocyanins: Multi-dimensional fragmentation pathways using high performance liquid chromatography-electrospray ionization-ion trap-time of flight mass spectrometry. **International Journal of Mass Spectrometry**, v. 308, n. 1, p. 71–80, 2011. Disponível em: <http://dx.doi.org/10.1016/j.ijms.2011.07.026>.

BOUARAB-CHIBANE, L. et al. Antibacterial properties of polyphenols: Characterization and QSAR (Quantitative structure-activity relationship) models. **Frontiers in Microbiology**, v. 10, n. APR, 2019.

CAMPOS, M. G. et al. Age-induced diminution of free radical scavenging capacity in bee pollens and the contribution of constituent flavonoids. **Journal of Agricultural and Food Chemistry**, v. 51, n. 3, p. 742–745, 29 jan. 2003.

CAO, F. R. et al. Ganoderic acid A metabolites and their metabolic kinetics. **Frontiers in Pharmacology**, v. 8, n. MAR, 2017.

CHAKRAVARTI, R.; SAHAI, V. Compactin - A review. **Applied Microbiology and Biotechnology**, v. 64, n. 5, p. 618–624, 2004.

EVIDENTE, A. et al. Fungal metabolites with anticancer activity. **Natural Product Reports**, v. 31, n. 5, p. 617–627, 2014.

EWING, B.; GREEN, P. Basecalling of automated sequencer traces using phred. II. Error probabilities. **Genome Research**, v. 8, n. 63, p. 186–194, 1998.

FANG, S.-M. et al. Cyclohexenone Derivatives Produced by a Mutant Obtained by Diethyl Sulfate Mutagenesis of a Marine-Derived. **Marine Drugs**, v. 10, p. 1266–1287, 2012.

FARHADI, F. et al. Antibacterial activity of flavonoids and their structure–activity relationship: An update review. **Phytotherapy Research**, v. 33, n. 1, p. 13–40, 2019.

GIANSANTI, V. et al. Killing of tumor cells: A drama in two acts. **Biochemical Pharmacology**, v. 82, n. 10, p. 1304–1310, 2011. Disponível em: <http://dx.doi.org/10.1016/j.bcp.2011.05.023>.

GORDON, D.; ABAJIAN, C.; GREEN, P. Consed: A Graphical Tool for Sequence Finishing. **Genome Research**, v. 8, p. 195–202, 1998.

GORDON, D.; DESMARAIS, C.; GREEN, P. Automated Finishing with Autofinish. **Genome Research**, v. 11, n. 4, p. 614–625, 2001.

GORDON, David; GREEN, P. Consed: A graphical editor for next-generation sequencing. **Bioinformatics**, v. 29, n. 22, p. 2936–2937, 15 nov. 2013.

HOU, A. J. et al. Hydrolyzable tannins and related polyphenols from Eucalyptus globulus. **Journal of Asian Natural Products Research**, v. 2, n. 3, p. 205–212, 2000.

HOUBRAKEN, J.; SAMSON, R. A. Phylogeny of Penicillium and the segregation of Trichocomaceae into three families. **Studies in Mycology**, v. 70, p. 1–51, 2011.

IKIGAI, H. et al. Bactericidal catechins damage the lipid bilayer. **BBA - Biomembranes**, v. 1147, n. 1, p. 132–136, 1993.

LOPES, F. C. et al. Pigment production by filamentous fungi on agro-industrial byproducts: An eco-friendly alternative. **Applied Biochemistry and Biotechnology**, v. 171, n. 3, p. 616–625, 2013.

MAGAN, N.; OLSEN, M. **Mycotoxins in Food: Detection and Control**. [S.l: s.n.], 2004.

MAPARI, S. A. et al. Identification of potentially safe promising fungal cell factories for the production of polyketide natural food colorants using chemotaxonomic rationale. **Microb. Cell Fact.**, v. 8, n. 1, p. 24, 2009.

MENA, P. et al. Rapid and comprehensive evaluation of (Poly)phenolic compounds in pomegranate (Punica granatum L.) Juice by UHPLC-MSn. **Molecules**, v. 17, n. 12, p. 14821–14840, 2012.

NATIONAL COMITEE ON CLINICAL LABORATORY STANDARDS. **Reference method for broth dilution antifungal susceptibility testing of yeasts ; approved standard — second edition**. [S.l: s.n.], 2002. v. 22.

NCCLS (CLINICAL AND LABORATORY STANDARDS INSTITUTE). **Performance Standards for Antimicrobial Susceptibility Testing**. . [S.l: s.n.], 2015.

O'BRIEN, J.; WRIGHT, G. D. An ecological perspective of microbial secondary metabolism. **Current Opinion in Biotechnology**, v. 22, n. 4, p. 552–558, 2011.

PENG, Q. et al. Determination of Luteoskyrin in Rice Wine by High-Performance Liquid Chromatography–Ion Trap Tandem Mass Spectrometry. **Analytical Letters**, v. 48, n. 1, p. 9–15, 2015.

QUAIYUM, S. et al. Drug Resistance in Pathogenic Micro-organisms Isolated from Oral Herbal Medicinal Products and a Survey on the Usage of Herbal Medicine in Bangladesh. **Journal of Antimicrobial Agents**, v. 04, n. 04, 2019.

RE, R. et al. **Original Contribution ANTIOXIDANT ACTIVITY APPLYING AN IMPROVED ABTS RADICAL CATION DECOLORIZATION ASSAY**. . [S.l: s.n.], 1999.

ROBBINS, K. S. et al. Separation and characterization of phenolic compounds from U.S. pecans by liquid chromatography-tandem mass spectrometry. **Journal of Agricultural and Food Chemistry**, v. 62, n. 19, p. 4332–4341, 2014.

SAID, R. ben et al. Tentative characterization of polyphenolic compounds in the male flowers of Phoenix dactylifera by liquid chromatography coupled with mass spectrometry and DFT. **International Journal of Molecular Sciences**, v. 18, n. 3, p. 1–18, 2017.

SAITO, M. et al. Yellowed rice toxins. Luteoskyrin and related compounds, chlorine-containing compounds, and citrinin. In: CIEGLER, A.; KADIS, S.; AJL, S. J. (Org.). . **Microbial toxins**. New York: Academic Press Inc., 1971. p. 299–308.

SAMSON, R. A. et al. Phylogeny and nomenclature of the genus Talaromyces and taxa accommodated in *Penicillium* subgenus *Biverticillium*. **Studies in Mycology**, v. 70, p. 159–183, 2011.

SIEMIATYCKI, J. et al. Listing occupational carcinogens. **Environmental Health Perspectives**, v. 112, n. 15, p. 1447–1459, 2004.

SILVA, D. C. V. da et al. Isolamento e seleção de fungos filamentosos do solo de sistemas agroflorestais do Município de Bom Jardim (PE) com base na capacidade de produção de enzimas hidrolíticas. **Brazilian Journal of Botany**, v. 34, n. 4, p. 607–610, 2011.

SOUMYA, K. et al. Characterization of a red pigment from Fusarium chlamydosporum exhibiting selective cytotoxicity against human breast cancer MCF-7 cell lines. **Journal of Applied Microbiology**, v. 125, n. 1, p. 148–158, 2018.

SUN, T. T. et al. Rapid separation and identification of 31 major saponins in shizhu ginseng by ultra-high performance liquid chromatography–electron spray ionization–MS/MS. **Journal of Ginseng Research**, v. 40, n. 3, p. 220–228, 2016. Disponível em: <http://dx.doi.org/10.1016/j.jgr.2015.07.008>.

TALEB-CONTINI, S. H. et al. Antimicrobial activity of flavonoids and steroids isolated from two Chromolaena species. **Revista Brasileira de Ciencias Farmaceuticas/Brazilian Journal of Pharmaceutical Sciences**, v. 39, n. 4, p. 403–408, 2003.

TAYLOR, J. W. One Fungus = One Name: DNA and fungal nomenclature twenty years after PCR. **IMA Fungus**, v. 2, n. 2, p. 113–120, 2011.

VENKATACHALAM, M. et al. Partial characterization of the pigments produced by the marine-derived fungus Talaromyces albobiverticillius 30548. Towards a new fungal red colorant for the food industry. **Journal of Food Composition and Analysis**, v. 67, p. 38–47, 2018.

XIE, Y. et al. Antibacterial Activities of Flavonoids: Structure-Activity Relationship and Mechanism. **Current Medicinal Chemistry**, v. 22, n. 1, p. 132–149, 2014.