Rev Argent Microbiol. 2016;48(3):259–263

asociación argentina de microbiología aam

REVISTA ARGENTINA DE MICROBIOLOGÍA

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BRIEF REPORTS

Lethality of cytochalasin B and other compounds isolated from fungus *Aspergillus* sp. (Trichocomaceae) endophyte of *Bauhinia guianensis* (Fabaceae)

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Received 6 October 2015; accepted 8 April 2016
Available online 24 August 2016

KEYWORDS
Endophytic fungi;
Artemia salina;
Secondary metabolites

Abstract Endophytic fungi are fungi that colonize internal tissues of plants; several biologically active compounds have been isolated from these fungi. There are few studies of compounds isolated from endophytic fungi of Amazon plants. Thus, this study aimed the isolation and structural identification of ergosterol (**1**), ergosterol peroxide (**2**), mevalonolactone (**3**), cytochalasin B (**4**) and cytochalasin H (**5**) from *Aspergillus* sp. EJC 04, an endophytic fungus from *Bauhinia guianensis*. The cytochalasin B (**4**) and the diacetate derivative of cytochalasin B (**4a**) showed high lethality in the brine shrimp assay. This is the first occurrence of cytochalasins in Amazonian endophytic fungi from *B. guianensis*.
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PALABRAS CLAVE
Hongos endófitos;
Artemia salina;
Metabolitos secundarios

Letalidad de citocalasina B y otros compuestos aislados del hongo *Aspergillus* spp. (Trichocomaceae) endófito de *Bauhinia guianensis* (Fabaceae)

Resumen Los hongos endofíticos son hongos que colonizan los tejidos internos de las plantas; varios compuestos biológicamente activos se han aislado a partir de estos hongos. Existen pocos estudios de compuestos aislados de hongos endófitos de plantas amazónicas. Por lo tanto, este

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http://dx.doi.org/10.1016/j.ram.2016.04.002
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estudio tuvo como objetivo el aislamiento y la identificación estructural de ergosterol (**1**), peróxido de ergosterol (**2**), mevalonolactona (**3**), citocalasina B (**4**) y citocalasina H (**5**) a partir de *Aspergillus* spp. EJC 04, un hongo endofítico de *Bauhinia guianensis*. La citocalasina B (**4**) y el derivado diacetato de citocalasina B (**4a**) mostraron una alta letalidad en el ensayo de *Artemia salina*. Esta es la primera aparición de citocalasinas en hongos endófitos amazónica de *B. guianensis*.

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The *Aspergillus* genus has more than one hundred species, and belongs to the Ascomycota division, Deuteromycotina subdivision, Hyphomycetes class, Moniliales order, Moniliaceae family. The species are widely found in nature, they can be isolated from plants, soil, air and decaying matter[13]. Several species have been described as producers of toxic metabolites[3]. The *Aspergillus* fungi have been an important source of natural products useful for exploration in medicine, agriculture and industry. Several compounds with cytotoxic activity have been isolated from endophytic fungi[1,16,19]. Compounds of the xanthones class isolated from *Aspergillus sydowii* showed immunosuppressive activities[17], and the tensyuic A acid isolated from *Aspergillus niger* showed antimicrobial activity[6]. There are reports of the isolation of cytochalasin E from an endophytic fungus that showed cytotoxicity[18]. Petersen et al.[12] isolated from the fungus *Aspergillus sclerotioniger* cytochalasins sclerotionigrin A and B that showed cytotoxic activity in vitro against lymphocytic leukemia cells. The aims of this study were the isolation and structural identification of ergosterol (**1**), ergosterol peroxide (**2**), mevalonolactone (**3**), cytochalasin B (**4**), cytochalasin H (**5**), and the derivative 7,20-diacetyl-cytochalasin B (**4a**) from the endophytic fungus *Aspergillus* sp. EJC 04 cultures, and testing the lethality of isolated compounds against *Artemia salina*.

The ^{1}H and ^{13}C NMR experiments were recorded on a NMR spectrometer (Mercury 300, Varian, Oxford, Oxfordshire, UK) with $CDCl_3$ (Cambridge®) as solvent and standard. The MS spectra were carried out in the mass spectrometer using ESI (+) ion mode (Acquity TQD, Waters, Milford, MA, USA). The specific rotation was performed on a specific rotation equipment (Nova Instruments No. 1412, Piracicaba, Brazil).

The fungus *Aspergillus* sp. was obtained from a collection of ''Laboratório de Bioensaios e Química de Micro-organismos – LaBQuiM/UFPA''. This collection contains isolates from *Bauhinia guianensis*. The fungus was inoculated into a Petri dish containing PDA (Potato Dextrose Agar) culture medium (Himedia®) and incubated at 25 °C (BOD, Quimis®) for 8 days to reactivation. One strain is deposited with a code EJC 04. The fungus *Aspergillus* EJC 04 was identified by observing the morphology and microscopic aspects of the colony in an optical microscope and by DNA sequence through analyses of the ITS5 region.

Six Erlenmeyer flasks (1000 ml) containing 200 g of rice (Uncle Ben's®) and 75 ml of water per flasks were autoclaved for 45 min at 121 °C (autoclave Prismatec®). Small pieces of PDA containing mycelium of *Aspergillus* sp. were added to 4 Erlenmeyer flasks under sterile conditions, then, the Erlenmeyer flasks were incubated at 25 °C for 23 days, two Erlenmeyer flasks were used as control. Biomass was macerated with ethyl acetate (Tedia®) (3× 500 ml). The biomass was separated of the ethyl acetate solution by filtration. Then, the ethyl acetate extracts (10 g) were obtained after evaporation of the resulting solution in rotary evaporator at 45 °C (Quimis®). Part of the ethyl acetate extract (5.0 g) was fractionated on silica gel column using hexane/ethyl acetate (9:1, 4:1, 7:3, 1:1, 3:7), ethyl acetate/methanol (3:7, 1:1) and methanol, resulting in 9 fractions. The hexane/ethyl acetate 9:1 fraction (500 mg) was submitted on silica gel column chromatography eluted with hexane/ethyl acetate (9:1, 4:1, 7:3, 1:1, 3:7) and ethyl acetate, resulting in 92 fractions. The fractions were pooled (A1 to A8); the fraction A2 provided a white amorphous solid identified as ergosterol **1** (30 mg), and the A4 fraction provided a white amorphous solid identified as ergosterol peroxide **2** (35 mg). The hexane/ethyl acetate 8:2 fraction (600 mg) was submitted on silica gel column chromatography eluted with hexane/ethyl acetate (9:1, 4:1, 7:3, 1:1, 3:7) and ethyl acetate, resulting in 100 fractions pooled as B1 to B9, the fraction B2 afforded a yellow oil identified as mevalonolactone **3** (6 mg). The ethyl acetate fraction (2 g) was submitted on silica gel column chromatography, eluted with hexane/ethyl acetate (9:1, 4:1, 7:3, 1:1, 3:7), ethyl acetate, ethyl acetate/methanol (3:7, 1:1) and methanol, resulting in 120 fractions pooled as C1 to C12; fraction C5 afforded a white solid identified as cytochalasin B **4** (100 mg) and the fraction C7 gave a white solid identified as cytochalasin H **5** (5 mg). All compounds **1–5** were identified by NMR and MS spectrometric data.

The cytochalasin B (**4**) was acetylated; for this, 10 mg of compound **4** were removed and solubilized in 100 μl of pyridine (Tedia®), then it was added 250 μl of acetic anhydride (Tedia®) and kept at rest for 24 h at room temperature. After this period, the material was transferred to a separatory funnel, and it was added 25 ml of 5% HCl solution to remove excess pyridine, and extraction was performed with ethyl acetate (3× 15 ml). The ethyl acetate phase was further washed with distilled water (3× 25 ml) and after separation was added anhydrous sodium sulfate in the ethyl acetate phase, which after filtered was evaporated to obtain the acetylated product **4a**.

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