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# Antifungal activity of *Rhopalurus crassicauda* venom against *Candida* spp.

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ABSTRACT

Fungal infections are becoming a serious problem of human diseases, being one of the most important fungal pathogens the yeast of the genus *Candida*. So far, fungal infection treatment faces different challenges, including the limited number of therapeutic drugs. Scorpions are known to be a valuable source of biologically active molecules, especially of peptide-derived molecules with a variety of biological effects and useful, lead compounds for drugs development. Here, we pioneer described the antifungal effect of venom, mucus, and the major toxin (Rc1) from *Rhopalurus crassicauda* scorpion. These results support the potential for Rc1 to be further investigated as a novel antifungal therapeutic to treat *Candida* infections.

## Credit author statement

UZ performed the experiments and participated in the data interpretation. ISO interpreted the data and drafted the manuscript. IGF, FAC, KCFB, ECA, VMG and GBD participated in the data interpretation and wrote the manuscript. MBP coordinated and designed all the experiments, analyzed and interpreted the data. All authors read, corrected, and approved the final manuscript.

*Candida albicans* as well as other emerging non albicans species, including *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, and *Candida parapsilosis*, represent an important source of systemic infections throughout the globe. The body sites that *Candida* spp. preferably colonizes are the oropharynx, vagina, and skin, especially skin folds (axilla, groin, perineum). It can produce proteolytic enzymes (such as aspartyl proteinases and phospholipases) and toxins (such as mycotoxin) that affect host defenses and enhance their chances of colonizing and invading the host (De Bernardis et al., 1990; Schaller et al., 2003). Also, under favorable conditions, they can enter the bloodstream leading to deep-tissue infections (Conti et al., 2014; Turner and Butler, 2014; Whaley et al., 2016).

Fungal pathogens are a major cause of morbidity and mortality among several patient groups, including those hospitalized in the intensive care unit, solid organ and stem cell transplant recipients, and human immunodeficiency virus (HIV) infected patients. Also, there is a deepening appreciation of genetic risk factors, which may predispose to complications from fungal pathogens that occur among otherwise normal hosts. Most researches regarding novel antifungals have focused on patients who are known to be at greatest risk of invasive fungal infection, since the opportunistic mycoses are a cause of great concern to both clinicians and investigators (Pappas, 2010). The traditional pathogens in this group include *Aspergillus* species, *Cryptococcus* species, the zygomycetes, *Fusarium* species, and *Candida* species (Pfaller and Diekema, 2010).

Fungal pathogens cause life-threatening invasive diseases (*e.g.* fungaemia, meningitis, pneumonia), severe chronic conditions (*e.g.* chronic pulmonary aspergillosis, allergic bronchopulmonary aspergillosis), complex chronic respiratory conditions (*e.g.* asthma, chronic obstructive pulmonary disease) and recurrent infections, such as oral and vaginal candidiasis (Brown et al., 2012). In general, these infections are associated with high mortality, and successful clinical outcome requires

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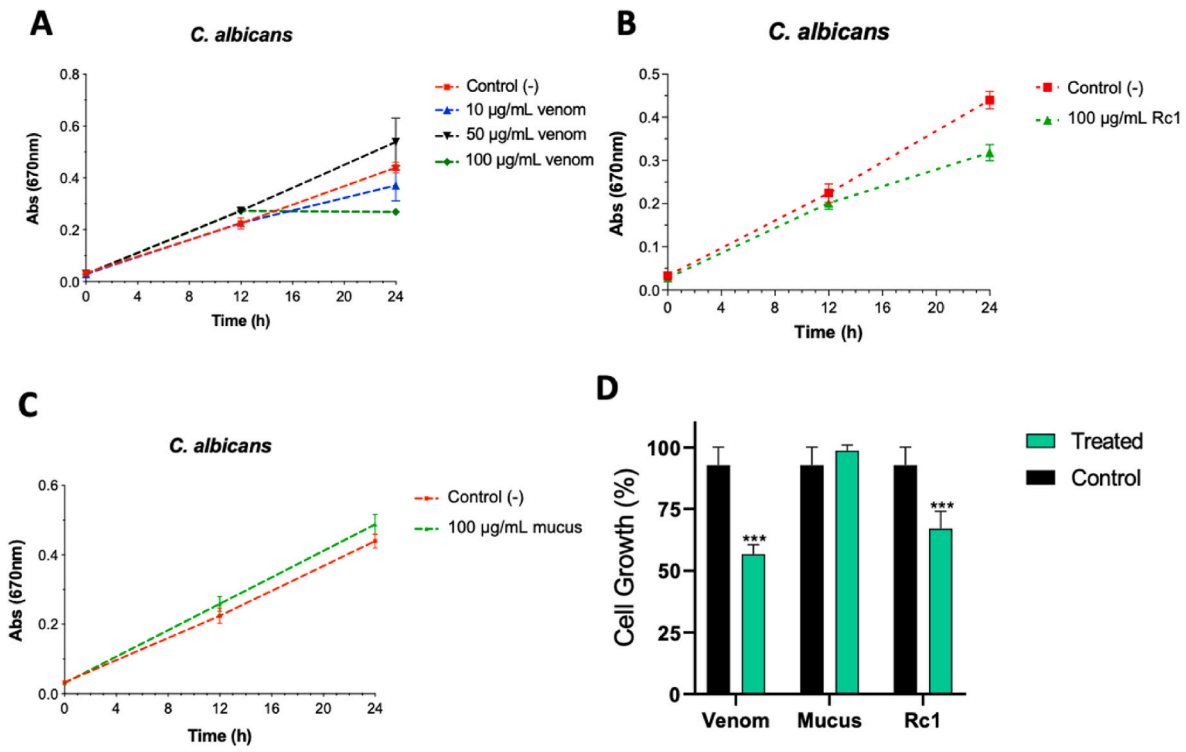


**Fig. 1. Inhibition of *C. albicans*.** Kinetic growth inhibition by **(A)** *R. crassicauda* venom (100 µg/mL), **(B)** Rc1 (100 µg/mL, corresponding to 14 µM), and **(C)** *R. crassicauda* mucus (100 µg/mL). **(D)** Growth inhibition after 24 h. Non-treated cells were used as controls. All experiments were performed in triplicate and data are expressed as the mean ± standard deviation. ***$p < 0.001$ when compared to control.

early diagnosis and effective antifungal therapy. However, antimycotic drugs are few, with chemical classes for invasive disease treatment limited to azoles, echinocandins, polyenes, and flucytosine (Odds et al., 2003).

Scorpion venoms are known source of components able to inhibit microorganism growing. In fact, many antibacterial and antifungal compounds have been isolated from scorpion venoms (Almaaytah et al., 2012; Machado et al., 2016). In a previous work, authors evaluated 11 scorpion-venom derived non-disulfide-bridged peptides which demonstrated activity against *Cryptococus neoformans*, *Candida* spp., and analogous activity against *C. albicans* biofilms (Guilhelmelli et al., 2016).

From *Tityus* species, *Tityus serrulatus* venom (Tsv) was capable of inhibiting filamentous fungi growth of *Aspergillus nidulans*, *Aspergillus terreus*, *Penicillium corylophilum*, and *Penicillium verrucosum* (Santussi et al., 2017). Ts1, the major toxin from Tsv, showed a dose-dependent inhibition against *A. nidulans* (Santussi et al., 2017). From *Tityus stigmurus*, two peptides present activity against fungi strains, stigmurin which has the ability to inhibit growth of *C. albicans*, *C. krusei* and *C. glabrata* (de Melo et al., 2015), and TistH was analyzed towards its antifungal properties against different strains of *C. albicans*, *C. tropicalis*, *A. flavus* and the filamentous fungus *Trichophyton rubrum* (Machado et al., 2016).

Inhabiting Amazonian savannah in the Brazilian northern and Guyana Southern (di Caporiacco, 1947; Martins et al., 2021), the scorpion *Rhopalurus crassicauda* Lourenço, 2002 (synonym *Rhopalurus amazonicus* Lourenço, 1986 and *Rhopalurus laticauda* Thorell, 1876) (Esposito et al., 2017; Martins et al., 2021) is a poorly studied species. There is only one study developed by Abreu et al. (2020) revealing the structure and function of the major toxin (Rc1 with ~7 kDa) and the isolation of a hyaluronidase. The same study also explored some activities of the whole venom (*e.g.* nociception and enzymatic activities) (Abreu et al., 2020). However, no antifungal activity has been explored so far. Based on these facts, this study aimed to describe a novel antifungal activity for *R. crassicauda* scorpion venom.

*R. crassicauda* scorpions were collected in Boa Vista - RR (Brazil, 2°49′14.88″ N and 60°40′19.20″ W). The species were kept at Medical School of Federal University of Roraima, received water daily and were fed with cockroaches or crickets twice a month, normally, 5 days prior to venom milking. Venom milking was performed by electrical stimulation of venom gland using electrical pulses (18 V) (Abreu et al., 2020). Pooled venom was stored at −20 °C until its use.

All the procedures involving the scorpions were in accordance with the ethical principles in animal research adopted by *Sistema de Autorização e Informação em Biodiversidade* (SISBIO), under registration number 57491/9.

The soluble crude venom (supernatant without mucus - 2 mg of proteins) was obtained, applied on a reversed-phase C18 column (10 mm × 250 mm) and the major peak was rechromatographed on another C18 column (2.1 mm × 250 mm) to obtain Rc1, as described by Abreu et al. (2020). The soluble venom and the toxin Rc1 (which represents 24% of the total protein of the soluble venom) were submitted to antifungal assays.

Yeast cells *C. albicans* (CE022) and *C. parapsilosis* (CE002) were preserved in the *Laboratório de Fisiologia e Bioquímica de Microrganismos* (LFBM), *Universidade Estadual do Norte Fluminense Darcy Ribeiro* (UENF), Campos dos Goytacazes, RJ, Brazil, were cultured in Sabouraud broth and quantified in a Neubauer chamber. To monitor the effect of proteins on the growth of yeasts, cells ($10^4$ in 1 mL Sabouraud broth) were incubated in the presence of the venom (10, 50 and 100 µg/mL), toxin Rc1 or mucus (100 µg/mL), at 28 °C in 200 µL microplates (Broekaert et al., 1990). The positive control was performed with fluconazole 100 µg/mL, getting 100% inhibition (data not shown).

Through the growth kinetics, it was observed that 100 µg/mL of *R. crassicauda* venom or Rc1 venom (representing 14 µM) were statistically able to inhibit the growth of *C. albicans* after 24 h, differently from mucus, which did not show the ability to inhibit the growth of the microorganism (Fig. 1A–D).

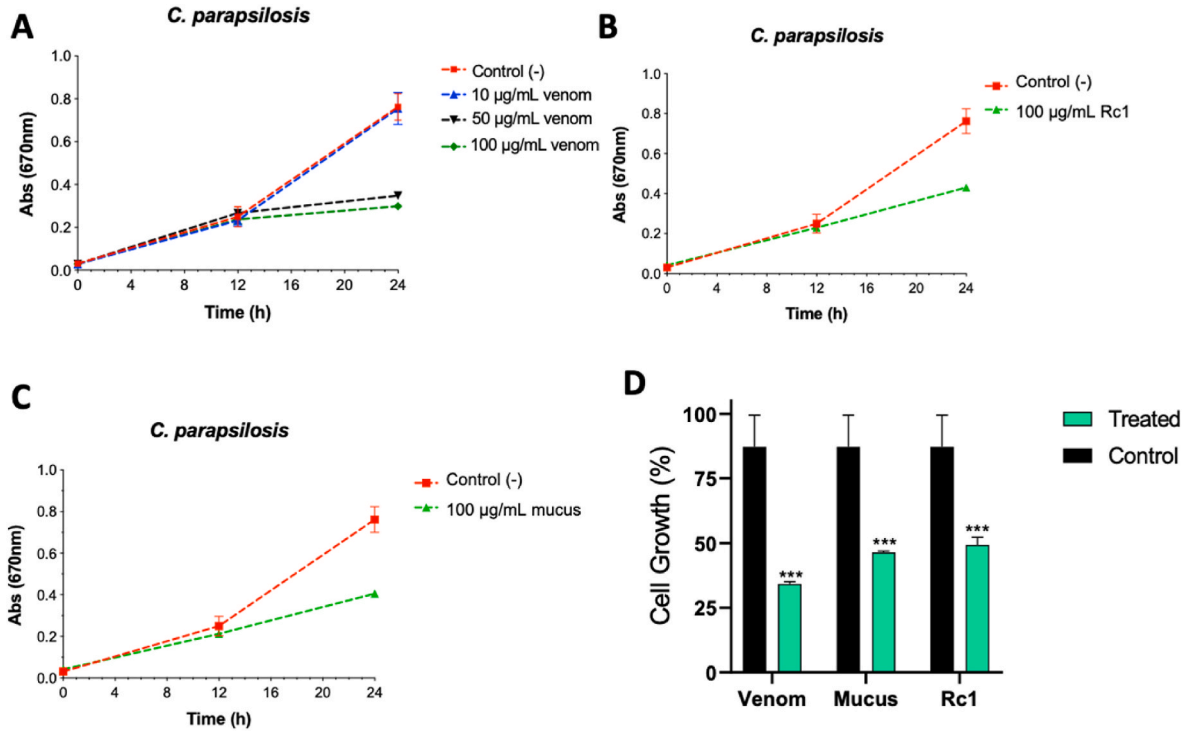


**Fig. 2. Inhibition of *C. parapsilosis*.** Kinetic growth inhibition by **(A)** *R. crassicauda* venom (100 µg/mL), **(B)** Rc1 (100 µg/mL, corresponding to 14 µM), and **(C)** *R. crassicauda* mucus (100 µg/mL). **(D)** Growth inhibition after 24 h. Non-treated cells were used as controls. All experiments were performed in triplicate and data are expressed as the mean ± standard deviation. ***$p < 0.001$ when compared to control.

When the same treatment was performed on *C. parapsilosis*, it was observed that *R. crassicauda* venom and mucus, as well as Rc1, were statistically able to inhibit its growth in 24 h, except for the 10 µg/mL of venom (Fig. 2A–D). When at a concentration of 50 µg/mL, *C. albicans* has a higher growth than the control (Fig. 1A). Dimorphic transitions with the development of pseudo hyphae could explain our failure to detect growth inhibition in this yeast species: pseudo hyphae formation might disrupt absorption measurements (Zottich et al., 2011).

Rc1 showed inhibitory activity in the order of magnitude of micromolar concentration, such as those reported for scorpion venom toxins. Serrulin, a glycine-rich native peptide of the *T. serrulatus* scorpion hemolymph, showed the minimal inhibitory concentration (MIC) of 1.5–3 µM and 3–6 µM against *C. albicans* and *Aspergillus niger*, respectively (Oliveira et al., 2019). Ts1, the major toxin from *T. serrulatus* venom, showed 100% inhibition against *A. nidulans* from 4.36 µM (Santussi et al., 2017). Stigmurin from *T. stigmurus* venom showed MIC of 37.5 µM against *C. albicans*, and its analogues of 4.69–9.38 µM (Parente et al., 2018). Other manuscripts report that the antifungal activity depends on fungal strain (de Melo et al., 2015; Guilhelmelli et al., 2016; Machado et al., 2016) and antifungal agent (Ahmadi et al., 2020). The scorpion venom D-amino acid analogue dKn2–7 from *Mesobuthus martensii* showed an amphotericin B-like killing kinetics for *C. albicans* with rapid onset of antifungal activity (Snyder et al., 2021). It is interesting to note that *R. crassicauda* venom showed inhibitory activity against *C. albicans* and *C. parapsilosis* at a concentration 10–50 times lower than that reported for *T. serrulatus* venom (1 and 5 mg/mL) against the growth of *A. nidulans, A. terreus, P. corylophilum*, and *P. verrucosum* (Santussi et al., 2017).

In conclusion, this study pioneer investigated the venom, mucus, and the major toxin of *R. crassicauda* scorpion for antifungal potential against *C. albicans* and *C. parapisilosis* strains. The isolated toxin, Rc1, exhibited the most potent antifungal efficacy against both strains tested. Future studies involving a wider range of *R. crassicauda* venom-derived toxins and analysis of peptide effects on host tissues are required to better determine if the scorpion venom could be a source of potential and novel antifungal drugs.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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