Anti-Leishmania activity of condensed tannins isolated from Mimosa tenuiflora and β-1,3-1,6 glucan isolated from commercial nutraceuticals

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Abstract

This study evaluated the anti-Leishmania activity of condensed tannins from *Mimosa tenuiflora* and *β*-glucan (1,3 − 1,6) from commercial nutraceuticals to identify less toxic alternatives against leishmaniasis, a neglected disease affecting millions in tropical and subtropical regions. Current treatments rely on toxic drugs, motivating research on natural compounds with leishmanicidal activity. *M. tenuiflora* is known for antimicrobial, antioxidant, and anti-inflammatory properties, while β-glucans are immunomodulatory polysaccharides capable of activating macrophages and enhancing pathogen control. Condensed tannins showed activity against promastigotes (IC_{50} = 24.56 µg/mL) but were ineffective against intracellular amastigotes, suggesting action on extracellular structures, possibly via membrane destabilization, nucleic acid alteration, or enzyme inhibition. β-glucan did not affect promastigotes but significantly reduced internalized amastigotes (IC_{50} = 17.94 µg/mL). Cytotoxicity was low for both compounds (CC_{50} >1,000 µg/mL), resulting in high selectivity indices: >40 for tannins (promastigotes) and > 55 for β-glucan (amastigotes).The anti-amastigote activity of β-glucan appears linked to immunomodulation via receptors such as Dectin-1 and TLR2 on macrophages, stimulating reactive oxygen species and proinflammatory cytokines production, which control intracellular parasites.In conclusion, condensed tannins demonstrated limited potential, acting only on promastigotes, while β-glucan was effective against amastigotes, the pathogenic form of leishmaniasis, with excellent cytotoxic safety. β-glucan (1,3–1,6) emerges as a promising candidate for novel therapeutic strategies, warranting further investigation of its immunological mechanisms and in vivo efficacy.

INTRODUCTION

Leishmaniasis is a complex of infectious and parasitic diseases caused by protozoa of the genus *Leishmania* and has a wide clinical spectrum depending on the species involved in the infection (WHO 2023). According to the WHO (2023), leishmaniasis can be divided into cutaneous and mucocutaneous leishmaniasis, characterized by the formation of dermal ulcers, and visceral leishmaniasis, the more severe clinical form of the disease that can lead to death if untreated.

Considered a serious public health problem because of its wide geographical distribution, this form of anthropozoonosis is endemic to 90 countries, with a high prevalence in tropical and subtropical regions, where it affects millions of people (PAHO).

Currently, chemotherapy is the most effective way to prevent diseases and mortality; however, even with the availability of different therapeutic options, there are still limitations (Ezatpour et al. 2015; Mendonça and Antônio 2020). The therapeutic arsenal available for the treatment of leishmaniasis involves five drugs, pentavalent antimonials, amphotericin B, pentamidine, paramomycin and miltefosine, which are associated with a series of side effects and restrictions due to their cardiotoxic, hepatotoxic, and nephrotoxic profiles, among others (Gervazoni et al. 2020; Silva et al. 2021; Garza-Tovar et al. 2020; Roatt et al. 2020; Ghorbani and Farhoudi 2018). Furthermore, prolonged treatment is painful because of its

invasive administration in most cases, which can culminate in treatment failure (Chappuis et al. 2007; Croft and Coombs 2003).

Given the existing limitations, natural compounds appear to be promising sources of new molecules with leishmanicidal potential (Shen and Hao 2020; Garza-Torvan et al. 2020; Gervazoni et al. 2020). Among the botanical species with therapeutic potential is *Mimosa tenuiflora* (Willd). Pois., popularly known as jurema-preta, is widely distributed in the semiarid region of Brazil and is traditionally used in folk medicine for the healing of wounds, skin infections and inflammatory processes (Bezerra et al. 2010; Hernandez et al. 2021). Previous phytochemical studies have shown that the bark of this species is rich in condensed tannins, which are known for their medicinal properties (Crepaldi et al. 2021; Santos et al. 2022; Borges et al. 2017; Azevêdo et al. 2013).

Previous *in vitro* studies have shown that condensed tannins are classified as phenolic compounds and demonstrate promising activity against pathogens (Monteiro et al. 2005; Pizzi 2021). These molecules can inhibit the growth of microorganisms through multiple mechanisms, including changes in nucleic acids, nutrient deprivation and destabilization of organelles important for cellular metabolism (Ucella Filho et al. 2022; Pizzi 2021). Furthermore, these molecules have antioxidant, anti-inflammatory and antimicrobial effects (Sanches et al. 2005). Given its ability to act as a pathogenic agent, future studies should investigate its anti-*Leishmania* activity for possible therapeutic improvement.

In addition to phenolic compounds, bioactive polysaccharides such as *β*-glucans (1,3 − 1,6) are also of interest in the therapeutic development of infectious diseases. *β*-Glucans can stimulate and activate macrophages and other cells of the innate immune system, resulting in an improved response against various pathogens (Castro; Calder; Roche 2021; Camilli; Tobouret; Quintin 2018). The adjuvant and modulatory effects of this molecule on the immune system suggest its potential in the development of new therapeutic strategies for leishmaniasis or the improvement of existing therapies.

In view of the above, the present study aimed to evaluate the anti-*Leishmania* activity of condensed tannins isolated from the bark of *M. tenuiflora* and *β*-glucan (1.3–1.6), aiming to contribute to the development of innovative and less toxic therapeutic alternatives for the treatment of leishmaniasis.

MATERIALS AND METHODS

Compounds studied

Condensed tannins isolated from the bark of *Mimosa tenuiflora*

The condensed tannins used in the present study were extracted by the Semi-Arid Research Center (NUPEÁRIDO), municipality of Patos, Paraíba-PB, at the domains of the Federal University of Campina Grande (UFCG). The material was subsequently purified at the Laboratory of Forestry Products Technology (LTPF) of the UFCG and transferred to the Federal University of Rondonópolis, Faculty of

Health Sciences, for studies of its biological activity. The entire procedure was performed according to the methods established by Paes et al. (2010) and Pereira et al. (2015).

β-glucan (1.3-1.6)

For the present study, a commercial nutraceutical rich in β-glucans (26 g) and amino acids, Bionutri AR-1®, was used. This product is obtained through a single fermentation process and is free of additives and preservatives, factors that classify it as a product of biotechnological innovation.

Evaluation of anti-*Leishmania activity in vitro*

Parasites

For *in vitro* analysis, a standard strain of *L. (L.) amazonensis* (IFLA/BR/1967/PH8) was used. The parasites were previously isolated from BALB/c mice previously infected and maintained in Schneider® medium (Sigma–Aldrich®) supplemented with 20% fetal bovine serum (FBS; Nova Biotecnologia) and 1% antibiotics (penicillin and streptomycin; Sigma–Aldrich®), with repetitions every three days until the 20th serial passage.

Preparation of compounds

Five micrograms (0.0050 g) of condensed tannins isolated from the bark of *M. tenuiflora* were diluted in 100 µL of dimethyl sulfoxide (DMSO). The nutraceutical used to evaluate the biological activity of *β*-glucan (1.3–1.60 on *L. (L.) amazonensis* was a dilution of 5 µg (0.0050 g) in 100 µL of phosphate-buffered saline solution (PBS). This dilution was selected because of the properties of the compound.

Analysis of anti-*Leishmania* activity

Anti-promastigote assay

In vitro susceptibility tests were performed on the promastigote forms of *L. (L.) amazonensis* in the log phase of growth in appropriate supplemented Schneider® medium (Sigma–Aldrich®). Growth inhibition was assessed by cell viability after the addition of MTT (3-(4-5-dimethylthiazol-2-yl)-2,5-diphenyltetrasodium bromide).

In 96-well plates, 100 µL of culture at a concentration of 10^{6} parasites/mL (19.75 parasites/mL) and 100 µL of the compounds to be tested were added at concentrations of 50–3.12 µL/mL. The plates were subsequently incubated at 26 °C for 72 h (Bosquiroli et al. 2015).

After 72 h of incubation, 5 mg/mL MTT was added to each well, after which the plates were stored again at 37 °C and 5% CO2 for 4 h, after which DMSO was added to dissolve the formazan crystals, after which the absorbance was measured with a spectrophotometer at a wavelength of 540 nm.

Anti-amastigote test

To evaluate the effects of the compounds against the amastigote forms, peritoneal macrophages from BALB/c mice were used, which were attached to circular coverslips at the bottom of 24-well plates supplemented with RPMI and infected with *L. amazonensis* promastigotes (IFLA)/BR/1967/PH8). Afterward, the plates were stored at 37 °C with 5% CO2 (Oliveira 2021).

After 24 h, the compounds of interest were added at concentrations of 50–3.12 µL/mL for another 24 h. The coverslips were then fixed with Boiun's solution, stained with Giemsa and mounted on microscope slides with Entellan to evaluate the number of amastigotes within 200 macrophages under light microscopy. Each concentration of extract was tested in sextuplicate (Oliveira 2021).

The inhibitory concentration was calculated using linear regression, and the log10 of each inhibitor concentration was plotted in relation to the percentage activity with the software GraphPad Prism 5 (Oliveira 2021).

Cytotoxicity assay

To evaluate the cytotoxicity of each compound, NIH/3T3 cells (ATCC CRL-1658 mouse fibroblast lineage) were grown in 96-well plates ($1x10^4$ cells in 100 µL per well) in RPMI 1640 medium supplemented with penicillin, streptomycin and 10% fetal bovine serum for 12 h at 37 °C and 5% CO2 (Oliveira 2021).

After 12 h, the medium was replaced with fresh RPMI medium containing different concentrations of each compound (100 to 3.125 µL/mL) in quintuplicate, and the plates were incubated for 24 h. The viability/life control was determined by fibroblasts grown only in culture medium (Oliveira 2021).

Subsequently, 10 µL of resazurin was added to each well, followed by incubation for 4 h at 37 °C and 5% CO2. After incubation, 80 µL of dimethyl sulfoxide was added to dissolve the formazan crystals, and the absorbance was estimated with a spectrophotometer at a wavelength of 570 nm (Oliveira 2021).

The cytotoxic concentration (CC_{50}) was calculated by linear regression, and the log10 of each inhibitor concentration was plotted against the percentage activity with GraphPad Prism 5 (Oliveira 2021).

Statistical analysis

The results are expressed as the concentration of growth inhibition at 50% (IC_{50}) and were determined by nonparametric linear regression. One-way ANOVA was subsequently performed, followed by Tukey's post hoc test for comparisons between groups. The results were obtained using the statistical software GraphPad Prism 5.01®. The significance level was set at 5%.

RESULTS

Among the compounds evaluated, the condensed tannins isolated from the bark of *Mimosa tenuiflora* inhibited the growth of promastigote forms of *Leishmania (L.) amazonensis*, with a 50% inhibitory

concentration (CI_{50}) of 24.56 µg (Fig. 1-A). However, β-glucan (1.3–1.6) was not able to inhibit parasite growth in the same assay, with $_{an\ IC50}$ >50 µg (Fig. 1-B).

In the investigation of the activity on intracellular forms, a change in the profile of the biological activity of the compounds was observed, where *β*-glucan (1.3–1.6) was able to reduce the number of amastigotes internalized in murine macrophages, $_{with\ an\ IC50}$ of 17.94 µg (Fig. 2-B), whereas tannins did not reduce the number of amastigotes per macrophage (Fig. 2-A).

In terms of the cytotoxicity profile on murine fibroblasts, both compounds showed low cytotoxicity to NIH/3T3 cells (Fig. 3). In view of the results, it was possible to estimate the selectivity index (SI) of the compounds tested, where the condensed tannin presented an IS > 40.65 for the promastigote form, which was more selective for the parasite than for the cell, while *β*-glucan (1.3–1.6) was more selective for the amastigote form than for the cell, with an SI > 55.74 (Table 1).

Table 1
Anti-*Leishmania* activity on the promastigote and amastigote forms of *L. amazonensis* and selectivity index.

Compost	IC_{50} (µg/ml) *of L. amazonensis* promastigotes	CC_{50} (µg/ml) Murine NIH/3T3 fibroblasts	IS promastigote *L. amazonensis*	IC_{50} (µg/ml) *L. amazonensis* amastigotes	IS amastigotes *L. amazonensis*
Condensed tannin	24,59	> 1.000	> 40,65	> 50	-
B-glucano (1,3 – 1,6)	> 50	> 1.000	-	17,94	> 55,74

DISCUSSION

The present study is a preclinical evaluation of different compounds against the evolutionary forms of *L. amazonensis*, as well as their toxicity to 3T3 lineage cells. The *in vitro* evaluation of molecules on the different evolutionary forms of the parasite is essential, since amastigotes are the intracellular form involved in human pathogenesis, and the efficiency and toxicity profile of the molecule can be evaluated for later scaling up for *in vitro* studies.

The results of the present study revealed that the condensed tannins isolated from the bark of *M. tenuiflora* significantly affected the promastigote form but were ineffective against the amastigote form. These results show that tannins directly affect structures present in or more exposed to the extracellular form of the parasite, such as surface components or specific metabolic processes of the promastigote. The mechanisms underlying the inhibition of promastigote growth have not been elucidated, but they may be the same as those in other microorganisms, such as bacteria and fungi, where this molecule inhibits growth through changes in nucleic acids, nutrient deprivation and destabilization of important organelles. in cellular metabolism (Molino et al. 2019; Huang et al. 2024).

Previous studies have shown that tannins can interact with membrane proteins, forming complexes and destabilizing their integrity, resulting in cell death by lysis (Ferreira and Evangelista 2021; Molino et al. 2019). In addition, these molecules can inhibit key enzymes involved in the metabolism of some microorganisms, preventing essential functions and slowing their growth. Since the inhibition mechanisms act on biological processes of organisms outside the intracellular environment, it is possible to explain the lack of inhibitory effects on the amastigote forms of *L. amazonensis*, showing that tannin has difficulty crossing the host cell membrane or maintaining intracellular activity, a fundamental characteristic for molecules with leishmanicidal activity.

In view of the above, the condensed tannins isolated from the bark of *M. tenuiflora* are more selective for promastigote forms than for amastigote forms, a factor that discards it as a molecule of interest in the prospect of anti-*Leishmania* compounds.

On the other hand, *β*-glucan (1.3–1.6) showed a distinct and potentially promising profile, as it showed significant inhibitory activity on the amastigote forms of *L. amazonensis* and zero toxicity on NIH/3T3 murine cells. Furthermore, *β*-glucan (1.3–1.6) was not able to inhibit the growth of the promastigote forms of the parasite. The profile observed in the present study suggests a selective action of the compound on the amastigote form, which is the pathogenic form in humans and other mammals

The selectivity observed for amastigotes may be related to the immunomodulatory effect of *β*-glucan (1,3 − 1,6). Previous studies report that this polysaccharide acts as a modulator of the innate immune response, interacting with recognition pattern receptors, such as Dectin-1 and Toll-like receptors, on phagocytic cells (Zimara et al. 2018; Brown et al. 2003; Yadav and Schorey 2006). This interaction results in increased production of reactive oxygen species and proinflammatory cytokines, factors that may contribute to the control of amastigotes internalized by macrophages (Castro; Calder; Roche 2021; Stothers et al. 2021).

Although no direct activity was observed for the promastigote forms, the ability of *β*-glucan (1.3–1.6) to reduce the number of parasites in infected macrophages is indirect. These results are corroborated by those of Patidar et al. (2020), who reported a reduction in the parasite load in murine peritoneal macrophages infected with *Leishmania donovani* and treated with *β*-glucan. In the same study, *in silico* evaluation revealed that the effect was due to the interaction between the *β*-glucan of *Saccharomyces cerevisiae* and Dectin-1 and TRL2 receptors, both of which are expressed by macrophages and dendritic cells, leading to the activation of different intracellular signaling pathways (IRAK-4, IKKα, IκB, LyN and SyK).

The absence of significant cytotoxicity to NIH/3T3 cells corroborates the findings of previous studies, which demonstrated that *β*-glucans are safe and biocompatible compounds and present a low risk to mammalian cells (Chan; Chan; Sze 2009). The results obtained reveal a relevant profile for the development of alternative therapies, considering the current limitations in the treatment of leishmaniasis, which culminate in therapeutic failure (Vetvicka and Fernandez-Botran 2018).

When the results concerning β-glucan (1.3–1.6) are considered, it is possible to discard its direct mechanism of action on the parasite, since the absence of activity on the promastigote forms of *L. amazonensis;* however, if necessary, further assays are needed to evaluate parameters such as phagocytosis, nitric oxide production and expression of macrophage activation markers to elucidate its mechanism of action on the amastigote forms.

In conclusion, the results highlight the importance of screening molecules with anti-*Leishmania* activity and investigating their action profiles on different evolutionary forms. Given its activity on amastigotes and low cytotoxicity, the investigated *β*-glucan (1.3–1.6) showed a more adequate profile for further studies, making it necessary to characterize the mechanisms involved and evaluate its efficacy in complex biological systems.

Declarations

Acknowledgments

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Authors' contribution

All the authors of the present study contributed significantly to the design and preparation of the study.

Ethical aspects

To accomplish the present study, all the procedures presented are in accordance with the principles adopted by the Brazilian Society of Laboratory Animal Sciences (SBCAL).

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Conflict of interest

I declare that there are no conflicts of interest between the authors and the funding agency.

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Figures

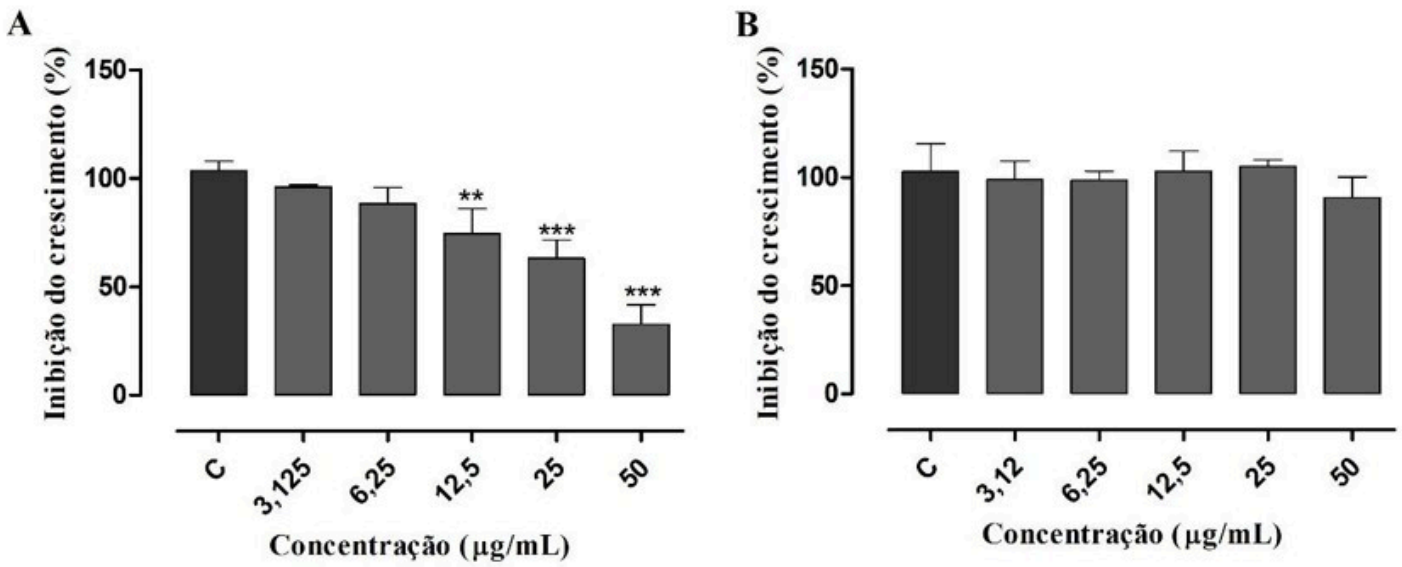

Figure 1

Evaluation of anti-*Leishmania*activity in promastigotes

A) Activity of condensed tannins isolated from *M. tenuiflora* on the promastigote form of *L. amazonensis*; B) *β*-glucan (1.3–1.6) activity on the promastigote form of *L. amazonensis.* ** and *** indicate p<0.01 and p<0.001, respectively (one-way ANOVA with Tukey's post hoc test). C = culture control with untreated promastigotes.

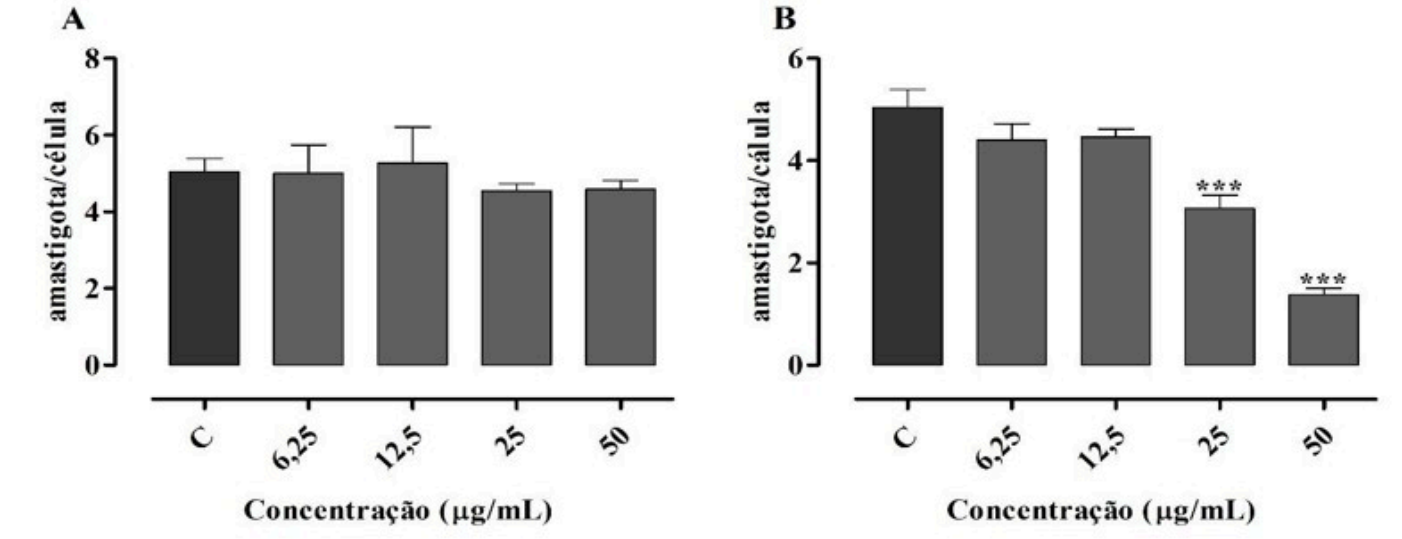

Figure 2

Evaluation of anti-*Leishmania*activity in intracellular amastigotes

A) Condensed tannins; B) *β*-glucan (1.3–1.6). *** indicates p<0.001 (one-way ANOVA followed by Tukey's post hoc test). C = control of infected macrophages without treatment.

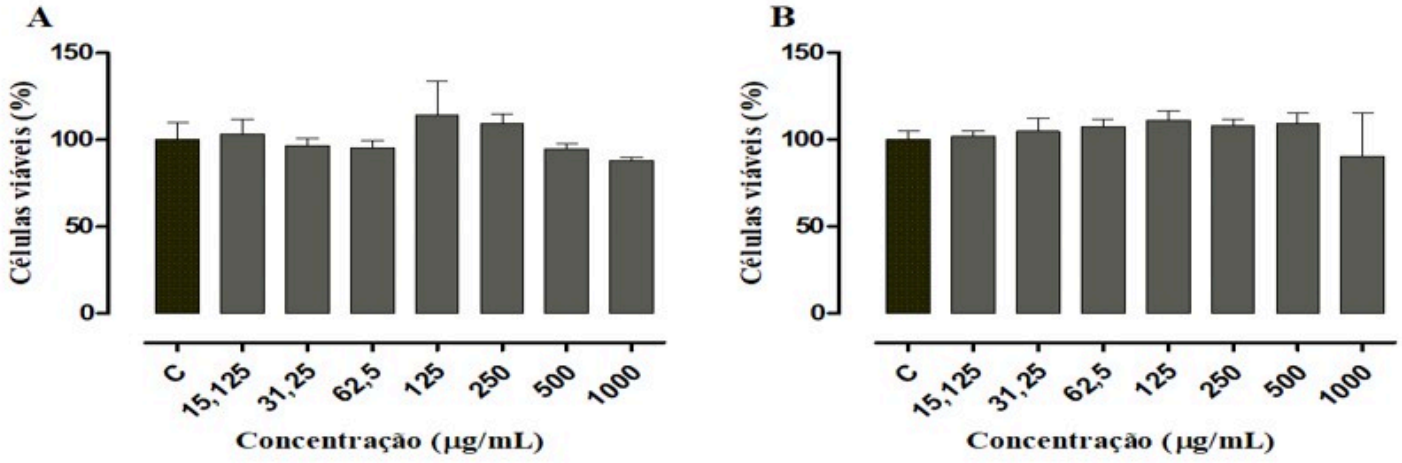

Figure 3

Cytotoxic activity on murine fibroblasts

A) Activity of condensed tannins isolated from *M. tenuiflora* on murine fibroblasts; B) *β*-glucan (1.3–1.6) activity on murine fibroblasts. C = culture control with cells without treatment.