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Investigation of wound healing *in vitro* of specie *Brachymenium exile* (Dozy & Molk.) Bosch & Sande Lac.

Investigação da cicatrização *in vitro* da espécie *Brachymenium exile* (Dozy & Molk.) Bosch & Sande Lac.

Investigación de la cicatrización *in vitro* de la especie *Brachymenium exile* (Dozy & Molk.) Bosch & Sande Lac.

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Abstract
The *Brachymenium exile* is the species of Bryaceae family, the family of bryophytes best represented in cerrado biome. The potential for biotechnological and biopharmaceutical applications of bryophytes have been investigated sharply. The aimed to investigate the cicatrizing potential of ethanolic extract of *B. exile* by scratch assay. The *in vitro* study was using the spreading and migration capabilities of fibroblast cell line using the extract of *B. exile*. the extract of *B. exile* allowed the cellular migration in 76% more that the control and not citotoxic assay in fibroblast cell line (3T3-L1). The extract of *B. exile* showed activity potential wound healing *in vitro* assay.
Keyword: Bryophytes; Biological activities; Healing.

Resumo
Brachymenium exile é uma espécie da família Bryaceae, a família de briófitas mais bem representada no bioma cerrado. O potencial para aplicações biotecnológicas e biofarmacêuticas de briófitas tem sido investigado intensivamente. O objetivo foi investigar o potencial cicatrizante do extrato metanólico de *B. exile* por ensaio de migração celular. O estudo *in vitro* avaliou a capacidade de propagação e migração da linhagem celular de fibroblastos usando o extrato metanólico de *B. exile*. O resultado mostrou que o extrato de *B. exile* permitiu a migração celular de 76% das células e não apresentou citotoxicidade para as células da linhagem de fibroblastos (3T3-L1). O extrato de *B. exile* apresentou potencial de atividade cicatrizante significativo em ensaio *in vitro*.
Palavras-chave: Briófitas; Atividades biológicas; Cicatrização.

Resumen
Brachymenium exile es una especie de la familia Bryaceae, la familia de briófitas mejor representada en el bioma del cerrado. Se ha investigado intensamente el potencial de las aplicaciones biotecnológicas y biofarmacéuticas de las briófitas. El objetivo fue investigar el potencial cicatrizante del extracto metanólico de *B. exile* mediante ensayo de migración celular. El estudio *in vitro* evaluó la capacidad de propagación y migración de los fibroblastos utilizando el extracto metanólico de *B. exile*. El resultado mostró que el extracto de *B. exile* permitió la migración celular del 76% de las células y no presentó citotoxicidad para células del fibroblasto (3T3-L1). El extracto de *B. exile* mostró un potencial significativo para la actividad cicatrizante en un ensayo *in vitro*.
Palabras clave: Briófitos; Actividades biológicas; Curación.

1. Introduction

In Brazil, the cerrado is a large biome that extends over more than 2.000.000 km^2 and with a is a biodiversity rich in a variety of endemic botanical species (Silva et al., 2018). Among the plant species occurring in the cerrado approximately 478 species are bryophytes (Yano, 2014).

The Brachymenium exile is the species of Bryaceae family, the family of bryophytes best represented in cerrado biome, according to Carmo and Peralta (2016). Chemically the bryophytes have in a variety of biologically active compounds as terpenoids, phenols, glycosides, and fatty acids (Ludwiczuk & Asakawa, 2020). Even though bryophytes can be used in medicine, the use of bryophytes for applied research with implications for human health is not yet fully explored, so it is important to research the biological activities and check the potential for biotechnological and biopharmaceutical applications of bryophytes (Greeshma *et al*., 2017; Lu *et al*., 2019); Martínez-Abaigar & Nuñez-Oliveira, 2021).

According to Santos *et al.* (2021), wound healing is processes with occur the body human, in general synchronously in phases so hemostasis, inflammation, growth, re-epithelialization, and remodeling.

The present study aimed to investigate the cicatrizing potential of ethanolic extract of *B. exile* by using the spreading and migration capabilities of fibroblast cell line.

2. Methodology

The ethanolic extract was obtained by cold static maceration. As an extraction liquid, 96ºGL ethyl alcohol was used. 100g of *B. exile* was washed in abundant in distilled water, subsequently, then it was put to dry at room temperature. After drying, the plant material was solubilized with the ethanolic solvent, placed in glass jars, and kept at room temperature for a period of seven days. After the extraction period, filtration was carried out on filter paper and later removal of the solvent with the aid of a rotary evaporator, in a thermostatic bath at a constant temperature of 50º C. Subsequently, the extract was placed in the freeze dryer for complete removal of water or solvent residues.

The fibroblast cell line (3T3-L1) was obtained from cell bank of Federal University of Rio de Janeiro (UFRJ). Cells were maintained at Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 4 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate and bovine calf serum to a final concentration of 10% in Atmosphere: air, 95%; carbon dioxide (CO2), 5% Temperature: 37°C Growth Conditions.

The cytotoxic assay was realized with described by Mosmann (1993). $2x10^5$ cells/well were seeded in 96 well plates and incubated for 48 hours at 37 ºC. Then ethanolic extract of *B. exile* were added per well and incubated for an additional 24 hours. The solutions of distilled water and ethanol (70%) where used who negative and positive controls tests, respectively. Elapsed 24 hours, the treatment solutions were removed and washed gain with PBS to remove any remaining traces. 10µl of MTT (5 mg/ml in PBS) solution and 90µl cell culture media DMEM was added and incubated for another 4 hours at 37 °C. Reaction was stopped by adding 100µl Dimethyl sulfoxide (DMSO) and finally absorbance was measured using ELISA reader at wave lengths: reference, 630nm and test, 570 nm. All experiments were carried out in triplicate.

The wound healing activity *in vitro* was investigated by spreading and migration capabilities of fibroblast cell line (3T3-L1) using a scratch wound assay which measures the expansion of a cell population on surfaces (Balocco & Meseguer, 2019). The 3T3-L1 cell line (concentration of 3x105 cells / mL) was seeded in 24-well plates containing coverslips coated with type 1 collagen (40 µg / mL) and incubated at 37 ºC for 2 hours. Elapsed the time, a linear wound was generated in the monolayer with sterile 100µl plastic pipette tip. PSB was used to washing the coverslips to remove cellular debris. DMEM medium with DMSO (0.25%) was used which control group, platelet derived growth factor (2 ng/mL), the MEBE (10 and 50 µg/mL), the commercial *Hypericum perforatum* oil (0.5 µg/mL) was added to a set of 3 coverslips per dose and incubated for

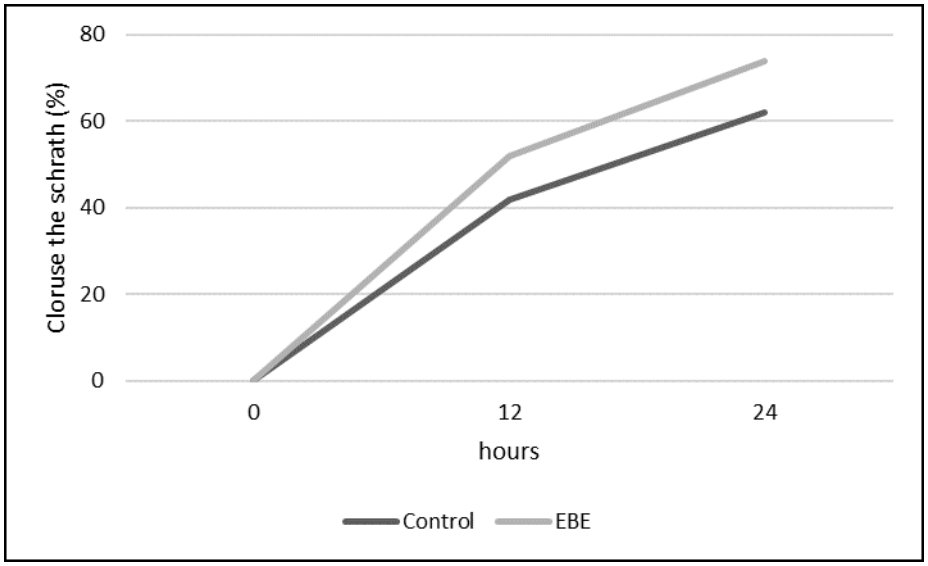
12h at 37 ºC with 5% CO_2. The cells were fixed with 4% paraformaldehyde for 15min and stained with 4′,6-diamino-2-phenylindole (DAPI) overnight. Three representative images from each coverslip of the scratched areas underreach condition were photographed to estimate the relative migration cells, according to Cavalcante *et al* (2020). The data were analyzed using NIS'S elements F 3.2 software. The experiments were performed at least in duplicate. The statistical analysis was done using to BioEstat 5.0 software. Data are expressed as values: mean ± SE of eight repetitions and analyzed by one way analysis of variance with $p < 0.05$ when compared to control indicated statistically significant difference in relation to the respective group using ANOVA followed by Tukey's comparison test ($p > 0.05$).

3. Results and Discussion

The bryophytes are avascular plants is having many phytochemicals such as alkaloids, flavonoids, terpenoids, benzenoids, phenylpropanoids, acetogenins (Krishnan & Murugan, 2015). Some outstanding studies in bryophyte phytochemistry show the antimicrobial activity (Chen *et al*., 2018), antioxidant activity (Ganesh *et al*., 201) and antifungal activity (Labbé *et al*., 2005).

In this study, the healing activity of *B. exile* was investigated, and Scratch assay showed that the extract of *B. exile* allowed the cellular migration in 76% more that the control, according to Figure 1. The extract of *B. exile* not toxic in cytotoxic assay in fibroblast cell line (3T3-L1). According to Balocco and Meseguer (2019), the *in vitro* scratch assay is the method based on the observation that, upon creation of a new artificial gap, so called "scratch", on a confluent cell monolayer, the cells on the edge of the newly created gap will move toward the opening to close the "scratch" until neu cell-cell contacts are established again.

Figure 1 - Cell migration *in vitro* of fibroblast treated and non-treated with ethanolic extract of *B. exile* (EBE) by hours using *in vitro* scratch assay.



Source: Research data.

Bryophytes frequently grow in an unfavorable environment as the earliest land plants, and inevitably biosynthesize secondary metabolites against biotic or abiotic stress (Xié & Lou, 2009). According to Rowntree *et al*. (2011), the flavonoids are Major Classes of Bryophyte Secondary Metabolites with Approximate 360 example. The flavonoids a group of natural substances with variable phenolic structures, is attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function (Panche *et al.*, 2016).

According to Fernandez e Serrano (2019), bryophytes have anti-inflammatory activity and activity of anti-carcinogenic, are also producing antibiotics and antivirals. The rich flora of bryophyte of Brazil and the wide geographical distribution with about 1524 species, allows you to explore the potential of other biological activities.

In this study the methanolic extract of specie of bryophyte *B. exile* showed potential wound healing activity *in vitro*, allowed the cellular migration in 76% in fibroblast treated with extract. The showed results not toxic in cytotoxic assay with fibroblast no treated with ethanolic extract. Chemical study to analyzed specie to extraction, isolation and characterization of compounds is recommended to investigate the wound healing activity *in vitro* and *in vivo assay.*

The bryophytes elaborate several biologically active secondary metabolites, furthermore, these metabolites have interesting biological properties, therefore, it is necessary to expand the bioprospecting of bryoflora due to its biotechnological and phytochemical can boost the production of new molecules with a potential therapeutic (Asakawa & Ludwiczuk, 2018; Alves *et al.*, 2020).

4. Conclusion

The extract of *B. exile* showed potential wound healing activity *in vitro* assay. Its necessary explorer the wound healing activity of compounds isolated of specie and other biological activities in *in vitro* and *in vivo* studies.

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