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## Antibacterial and antitumor activities of a lectin-rich preparation from *Microgramma vacciniifolia* rhizome



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### ABSTRACT

The rhizome of *Microgramma vacciniifolia* contains a lectin (carbohydrate-binding protein) called MvRL. Studies demonstrated that a MvRL-rich fraction did not show *in vivo* genotoxicity and acute toxicity in mice. This study aimed to evaluate the MvRL-rich fraction from *M. vacciniifolia* rhizome for antibacterial activity *in vitro* and *in vivo* as well as antitumor effect *in vivo* using the Ehrlich carcinoma model in mice. The fraction showed antibacterial activity against *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* with minimal inhibitory concentrations ranging from 31.2 to 125.0 µg/mL and minimal bactericidal concentrations from 62.5 to 200 µg/mL. The fraction was also effective *in vivo* against infection caused by these bacteria on *Tenebrio molitor* larvae considering the parameters evaluated. In regard to the antitumor activity, the treatments of Ehrlich carcinoma-bearing mice with the fraction at 100 and 200 mg/kg *per os* resulted in 62.58% and 75.43% of tumor inhibition, respectively. In conclusion, the MvRL-rich fraction showed *in vivo* antibacterial and antitumor activities and thus can be considered as an alternative of natural origin for the development of candidates for therapy.

### 1. Introduction

Morbidity and mortality are still closely correlated with the development of infections (Quiles et al., 2015) and the World Health Organization (2020) reports that, in 2017, there were 48.9 million cases and 11 million sepsis-related deaths worldwide. Pathogens of the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) are mainly correlated with nosocomial infections due to their ability to proliferate in the hospital environment (Tiwari et al., 2018). In addition, multidrug bacterial resistance has become a challenge in the therapy of diverse types of infections, increasing the costs of hospitalization as well as the associated mortality rates (Frieri et al., 2017). The emergence of multidrug-resistant strains arises researchers in the search for new therapeutic alternatives (Terreni et al., 2021) and the diversity of phytochemicals with antimicrobial properties put the plants in evidence

for antimicrobial drugs development (Turner et al., 2019).

The cancer remains the second leading cause of death worldwide (Martel et al., 2020). Despite the current availability of various chemotherapeutic substances, the therapies sometimes fail due to tumor cell resistance as well as complications due to side effects (Fontana et al., 2021). Tumor cell resistance causes relapses and metastases, which complicate the prognosis (Wu et al., 2019). Thus, it is essential the search for new molecules with antineoplastic properties and less toxicity; in this scenario, natural products from plants also have arisen interest of many research groups (Patriota et al., 2019).

*Microgramma vacciniifolia* is an epiphytic plant that belongs to Polypodiaceae family, cited in the literature for its astringent activities and recommendations to treat hemorrhages, expectorations, dysenteries, intestinal cramps, and hydrops (Santos and Sylvestre, 2006; Agra et al., 2008). The rhizome of *M. vacciniifolia* contains a lectin called MvRL. Lectins are carbohydrate-binding proteins that are broadly found in

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plants and possess several biological activities, including antimicrobial (Almeida et al., 2020) and anticancer (Patriota et al., 2021) properties. MvRL showed antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici* (Albuquerque et al., 2014a), insecticidal activity against *Nasutitermes corniger* (Albuquerque et al., 2012) and cytotoxic action against lung mucoepidermoid carcinoma (NCI-H292) cells (Albuquerque et al., 2014b).

Studies demonstrated that a MvRL-rich fraction (obtained by fractionation of saline extract from the rhizomes with ammonium sulfate) did not show *in vivo* genotoxicity and did not cause behavioral, hematological, and histopathological alterations in mice when evaluated for acute toxicity at a dose of 1,000 mg/kg b.w. (Silva et al., 2020). In addition, MvRL-rich fraction promoted central and peripheral analgesia as well as anti-inflammatory effects by interfering with bradykinin via and inhibiting inflammatory cell migration (Silva et al., 2021). In this work, we aimed to evaluate this MvRL-rich fraction for antibacterial activity *in vitro* and *in vivo* as well as antitumor effect *in vivo* using the Ehrlich carcinoma model in mice.

## 2. Materials and methods

### 2.1. Plant material

Rhizomes of *M. vacciniifolia* were collected in September 2016 at the Recife, Brazil (08°03'07" S, 34°56'59" W) and taxonomic recognition was certified at the herbarium Dárdano de Andrade Lima of the *Instituto Agrônomo de Pernambuco* (Recife), where an exsiccate (no. 63,291) is deposited. The access was recorded (A347889) in the *Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado* (SisGen).

### 2.2. Preparation of MvRL-rich fraction

MvRL-rich fraction was obtained as previously described (Albuquerque et al., 2012; Santana et al., 2012). The rhizomes were cleaned with distilled water and put to dry for 1 week at 28 °C and additional 3 days in an oven at 35 °C. Next, the material was triturated using a knife mill. The flour (100 g) was submitted to protein extraction in 150 mM NaCl (1 L) for 12 h at 28 °C under magnetic stirring. Then, the suspension was filtered and centrifuged (9,000 g, 15 min, 28 °C) and the saline extract corresponded to the supernatant. The MvRL-rich fraction was attained by treating the extract with ammonium sulfate at 60% saturation (Green and Hughes, 1955) for 4 h, followed by centrifugation (9,000 g, 15 min, 28 °C), collection of the precipitated fraction and resuspension in 150 mM NaCl. The fraction was dialyzed against distilled water for 6 h (two liquid changes) and then dried by lyophilization.

### 2.3. Protein concentration and hemagglutinating activity

Protein concentration was determined according to Lowry et al. (1951) using a standard curve of bovine serum albumin (31.25–500 µg/mL). Hemagglutinating activity (HA) was performed in tune with Albuquerque et al. (2012) using a suspension (2.5%, v/v) of human O-type erythrocytes in 150 mM NaCl. The HA was defined as the inverse of the highest dilution of sample that led to agglutination of erythrocytes. Specific hemagglutinating activity was defined as the ratio between the HA and protein concentration (mg/mL).

### 2.4. Evaluation of antibacterial activity

#### 2.4.1. Determination of minimal inhibitory (MIC) and bactericidal (MBC) concentrations

The minimal inhibitory concentration (MIC) of the fraction was determined according to İşcan et al. (2002) in 96-well microplates, with modifications. The microorganisms assessed were *Acinetobacter baumannii* (ATCC 19606), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC

700603), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 29213). The cultures were grown in Mueller Hinton Broth and final cell concentrations were adjusted to  $5 \times 10^5$  colony-forming units (CFU)/mL. The culture medium (170 µL) was added to each well and the MvRL-rich fraction (dissolved in 0.1%, v/v, dimethyl sulfoxide, DMSO) was serially two-fold diluted to achieve concentrations in the range 7.8–1,000 µg/mL. Next, 10 µL of the microorganism culture was added. The plates were incubated at 37 °C for 24 h and a resazurin solution (0.01%, w/v) was used to evaluate bacterial growth, which was indicated by color changing from blue to pink. The lowest concentration at which no color change occurred was recorded as the MIC. For MBC determination, aliquots (10 µL) of the wells (before resazurin addition) were transferred to Mueller-Hinton Agar plates and incubated for 24 h at 37 °C. The MBC was determined as the lowest concentration of protein fraction capable of preventing bacterial growth in 99.9% in comparison with initial inoculum. Two independent experiments were performed in triplicate.

#### 2.4.2. Time-kill analysis

To evaluate the kinetics of microbial death, bacterial suspensions ( $10^5$  CFU/mL) were incubated with the MvRL-rich fraction at  $\frac{1}{2}$  MIC and MIC for 37 °C for 24 h, and then seeded in a plate with Muller Hinton Agar medium. A growth control with no test sample was also performed. The growth was accompanied by visual counting of colonies after 1, 2, 4, 8, 12 and 24 h. Two independent experiments were performed in triplicate and time-kill curves were constructed by plotting log CFU/mL versus time (Bendali et al., 2008).

#### 2.4.3. In vivo evaluation of antibacterial activity

*In vivo* antibacterial activity of MvRL-rich fraction against the same strains described above was evaluated using the infection model in *Tenebrio molitor* larvae. The larvae were randomly assigned to groups ( $n = 10$  per group) and infected by injecting 10 µL of bacterial suspension ( $1 \times 10^5$  CFU/larva) into the last left proleg. After 2 h incubation at 37 °C, the larvae received into the last left proleg a single dose of 10 µL of the fraction at concentrations corresponding to MIC or  $2 \times$  MIC and the assays were incubated again at 37 °C. Infected larvae inoculated with the vehicle (0.1%, v/v, DMSO) were used as positive control while vehicle-treated uninfected larvae corresponded to negative control. The fraction was also inoculated in uninfected larvae to verify the occurrence of any mortality related to the sample. Mortality rates of each group were recorded daily for 5 days.

### 2.5. In vivo antitumor activity

#### 2.5.1. Animals

Female Swiss mice (*Mus musculus*), 50 day-old and weighing 30–35 g, were obtained from the bioterium of the *Departamento de Antibióticos* of UFPE. The mice were housed at the same laboratory at a temperature of 22 °C, with 12:12 photoperiod and *ad libitum* access to food (Purina, Nestlé Brasil Ltda., Brazil) and water. The Ethics Committee on Animal Experimentation of UFPE certified all the procedures employed (process no. 23076.042699/2016–72).

#### 2.5.2. Tumor transplantation and treatments

For the transplantation of Ehrlich carcinoma, the tumor cells were removed from a donor animal by aspiration of the ascitic fluid and introduction ( $25 \times 10^6$  cells/mL) into the recipient animals subcutaneously in the sub axillary region. After 48 h of implantation, the animals were divided into groups ( $n = 5$  per group) and treated orally for 7 days with distilled water (negative control) or with MvRL-rich fraction at 100 or 200 mg/kg. On the eighth day, the animals were weighed, anesthetized (80 mg/kg ketamine and 20 mg/kg xylazine, i.p.) and blood samples were collected by cardiac puncture for hematological analysis. Then, the mice were euthanized by cervical dislocation and tumors were collected and had their weight determined. The percentage of tumor reduction was calculated (Koniya and Funayama, 1992).

### 2.5.3. Hematological analyses

The blood samples from each animal were allocated into EDTA-containing tubes for hematological analysis [erythrocytes, leukocytes, hemoglobin, mean corpuscular volume (MCV) mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)] using an ABX-PENTRA-80 Automated Hematology Analyzer (Germany).

### 2.6. Statistical analysis

The data were expressed as mean of replicates  $\pm$  standard deviation (SD) or standard error (SE) values, which were calculated using GraphPad Prism version 8 for Windows (GraphPad Software, San Diego, CA, USA). Significant differences ( $p < 0.05$ ) between treatment groups were analyzed using one-way ANOVA followed by Tukey's test using the GraphPad Prism software. Kaplan-Meier survival curves of *T. molitor* larvae were submitted to log-rank test the same software.

## 3. Results and discussion

Natural products of plant origin have been the focus of studies aiming to find alternatives to fight the spread of microbial resistance (Hobson et al., 2021). Lectins are reported as antimicrobial agents by acting through different mechanisms and exerting effects such as growth inhibition, damage to cell integrity and inhibition of biofilm formation (Almeida et al., 2020).

The saline extract from *M. vacciniifolia* showed a protein concentration of 5.4 mg/mL and specific HA of 12 while the MvRL-rich fraction contained 14.2 mg/mL of protein and presented specific HA of 144. These data confirm the fraction as a preparation rich in the lectin MvRL. It was previously reported that the MvRL-rich fraction did not contain secondary metabolites of the classes of alkaloids, coumarins, cinnamic acid derivatives, flavonoids, tannins, and terpenes/steroids (Silva et al., 2020).

The MvRL-rich fraction was effective in inhibiting the growth of the bacteria evaluated with MIC values ranging from 31.2 to 125.0  $\mu\text{g/mL}$ ; bactericidal effect was also detected, with MBC ranging from 62.5 to 250.0  $\mu\text{g/mL}$  (Table 1). The *S. aureus* strain was the most sensitive to the fraction and the MBC/MIC ratio for all bacteria was 2 or 4, indicating that the fraction is a bactericidal drug (Levison, 2004). The time-kill curves of the bacteria treated with the fraction can be seen in Fig. 1. A reduction in the growth was observed already in the treatments at  $\frac{1}{2}$  MIC, mainly in the first 12 h; in some cases, a little growth recovery could be seen after 24 h. In the results obtained for the MIC treatments, bacterial growth was strongly inhibited in the first 12 h and the bacteriostatic effect remained even after 24-h incubation.

Lectin isolated from *Alpinia purpurata* inflorescence showed MIC of 200  $\mu\text{g/mL}$  against non-resistant *S. aureus* strains but did not affect the viability of *P. aeruginosa* strains up to the concentration of 400  $\mu\text{g/mL}$  (Ferreira et al., 2018). The lichen-derived lectin *Cladonia verticillaris* showed MIC of 229.9, 7.18 and 114.9  $\mu\text{g/mL}$  for *S. aureus*, *E. coli* and *K. pneumoniae* (Ramos et al., 2014). Lectin from *Moringa oleifera* seeds (WSMoL) presented a MIC of 250.0, 31.2, 7.8  $\mu\text{g/mL}$  for *E. coli*, *K. pneumoniae* and *S. aureus*, respectively (Ferreira et al., 2011; Coriolano et al., 2020). Finally, the lectin isolated from *Punica granatum* fruit

**Table 1**

Minimal inhibitory (MIC) and bactericidal (MBC) concentrations of the MvRL-rich fraction against bacterial species.

Bacteria	Concentration ( $\mu\text{g/mL}$ )		MBC/MIC ratio
	MIC	MBC	
<i>Acinetobacter baumannii</i> (ATCC - 19606)	62.5	250.0	4.0
<i>Escherichia coli</i> (ATCC - 25922)	125.0	250.0	2.0
<i>Klebsiella pneumoniae</i> (ATCC - 700603)	125.0	250.0	2.0
<i>Pseudomonas aeruginosa</i> (ATCC - 27853)	62.5	125.0	4.0
<i>Staphylococcus aureus</i> (ATCC - 29213)	31.2	62.5	2.0

presented MIC ranging from 6.25 to 12.5  $\mu\text{g/mL}$  against *S. aureus* strains (Silva et al., 2016, 2019). The results show that the MIC values found for MvRL-rich fraction are within the range found in evaluations with isolated lectins.

Invertebrate hosts, such as *Caenorhabditis elegans*, *Galleria mellonella*, and *Tenebrio molitor* have been used in studies on microbial infection due to advantages like easy commercialization and cultivation in the laboratory (Dinh et al., 2021; Giunti et al., 2021; Lozoya-Pérez et al., 2021). In addition, body temperature of *T. molitor* larvae (25-37°C) coincides with the human body temperature at which pathogens of medical importance develop (Lozoya-Pérez et al., 2021). Once the MvRL-rich fraction showed satisfactory results in the *in vitro* antimicrobial assay, we evaluated its *in vivo* antimicrobial potential using *T. molitor* larvae as bacterial host. The results (Fig. 2) showed that the infected larvae treated with the fraction had a longer life span than infected untreated larvae, revealing that the fraction also showed antibacterial activity *in vivo*.

Interaction of lectins with glycoconjugates may provoke internalization and induction of cellular responses across the surface of cells (Loh et al., 2017). The binding of lectins to cell wall components of Gram-positive and negative bacteria such as teichoic acids and lipopolysaccharides may trigger changes in permeability and nutrient uptake or induce oxidative stress induction (Almeida et al., 2020). Other cellular responses may include inhibition of protein or nucleic acid synthesis as well as inhibition of synthesis or plasma membrane functionality (Rezaei et al., 2012).

Plants have also been studied as source of new bioactive molecules against tumor cells and some drugs currently used in cancer chemotherapy are derived from plant, such as paclitaxel (Sharifi-Rad et al., 2021). The reduction of side effects underlies the search in recent years for phytochemicals with antitumor potential, which may have anticancer properties through several mechanisms (Kabeer et al., 2019). It was previous reported that MvRL exerted cytotoxic action on cancer cells without being toxic to human peripheral blood mononuclear cells (Albuquerque et al., 2014b). In the present study we evaluated the tumor inhibition properties of MvRL-rich fraction against Ehrlich mammary carcinoma in mice. Cells of this strain have high proliferation and may develop ascitic or solid tumor, which are characterized as undifferentiated, hyperdiploid and rapidly proliferating tumor with a predilection to metastasize to the lungs, liver, pancreas, kidneys, and bones (Chen and Watkins, 1970; Ozaslan et al., 2011; Mishra et al., 2018). The doses were chosen based on previous studies on the toxicity of the MvRL-rich fraction in mice (Silva et al., 2020, 2021).

Treatment with MvRL-rich fraction at the two doses assessed (100 and 200 mg/kg) significantly decreased the weight of Erlich carcinoma in comparison with untreated control (Fig. 3). The treatments with 100 and 200 mg/kg promoted 62.58% and 75.43% of tumor inhibition, respectively. Antiproliferative effects on tumor cells *in vivo* have been reported to other lectins; for example, a lectin from the seeds of *Moringa oleifera* (called MOSL) reduced the Ehrlich carcinoma in 55% when administered to mice at a daily dose of 4 mg/kg i.p. (Asaduzzaman et al., 2018). The *Kaempferia rotunda* lectin, at a dose of 2.5 mg/kg/day i.p., achieved a tumor growth inhibition percentage of 67% of Erlich carcinoma in mice (Kabir et al., 2011).

Anemia with decreased hemoglobin and hematocrit levels are often observed in chemotherapy patients as indicative of damage to hematopoietic cells. In this sense, we investigated whether MvRL-rich fraction would have interfered with hematological parameters in the tumor-bearing animals. Table 2 shows that the animals treated with the fraction presented an improvement in the red blood cells in comparison with the untreated group. Treatment with the lectin from *Trichosanthes cucumerina* seeds (1 mg/kg/day i.p.) also led to a 62% reduction in tumor growth and increase in red blood cells and hemoglobin content in animals with Ehrlich carcinoma (Kabir et al., 2012), likewise the lectin from *Momordica charantia* seeds (8 mg/kg/day i.p.), which promoted 75% inhibition of tumor proliferation and increased red blood cell and hemoglobin rates (Kabir et al., 2015).

Other common side effects of anticancer drugs are the damages to immune system cells, being leukopenia and lymphocytopenia common

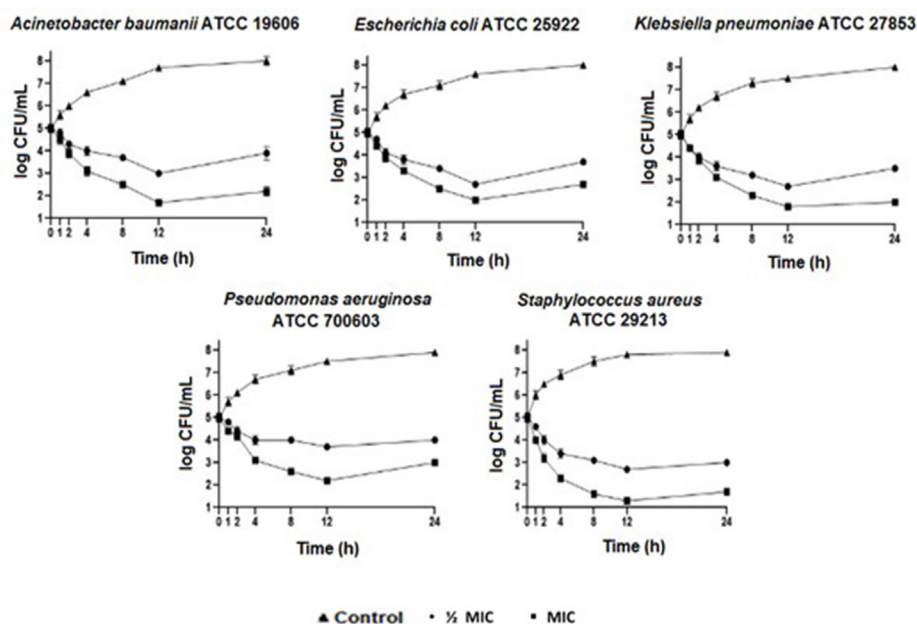


Fig. 1. Time-kill curves of bacteria treated or not with the MvRL-rich fraction from *Microgramma vacciniifolia* rhizome. Each point represents the mean  $\pm$  SD of two independent experiments performed in triplicate.

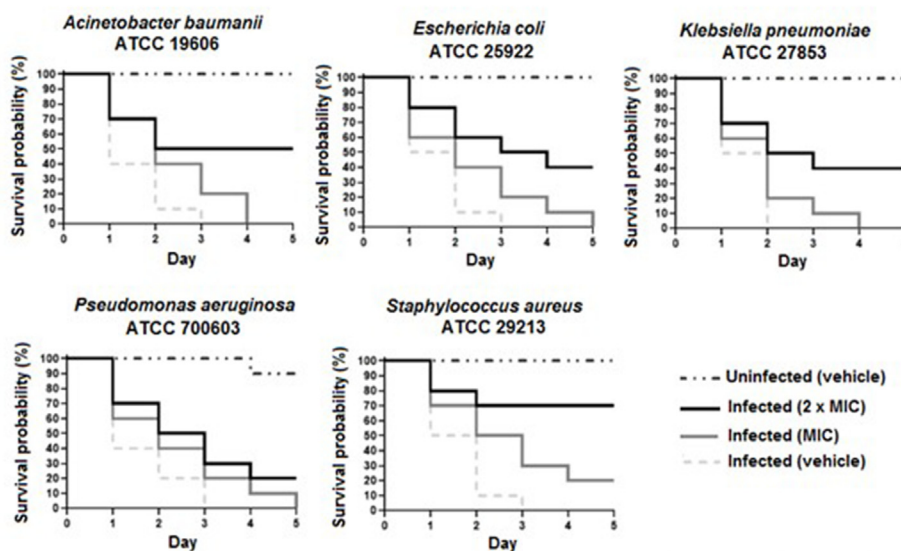


Fig. 2. Effects of the MvRL-rich fraction from *Microgramma vacciniifolia* rhizome on bacterial infection in *Tenebrio molitor* larvae. The Kaplan-Meier curves of uninfected insects treated with vehicle as well as infected insects treated with vehicle, or the fraction (MIC and  $2 \times$  MIC) can be seen. Survival log-rank test indicate a significant ( $p < 0.05$ ) increase in the survival time in the fraction-treated larvae in comparison with untreated infected larvae.

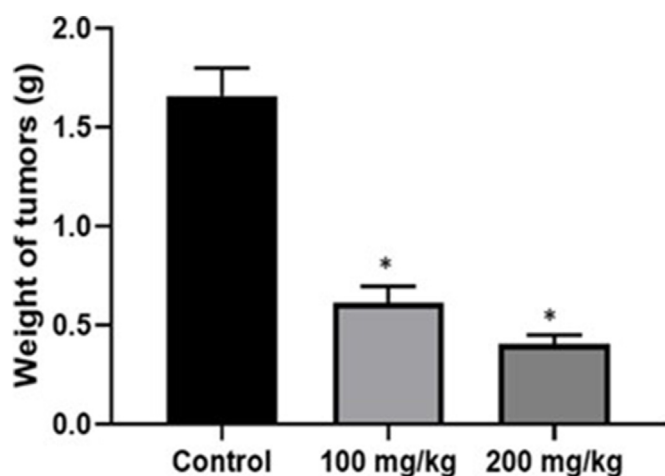
findings in patients during the chemotherapy. In the present study, an increase in leukocyte rates was observed in animals treated with the fraction in comparison with the untreated group (Table 2). Immunomodulation is one of the promising correlated effects in anticancer therapies, especially with phagocyte activation (Kou et al., 2016). These data stimulate future studies on the immunomodulatory potential of the MvRL-rich fraction.

#### 4. Conclusion

MvRL-rich fraction showed *in vivo* antibacterial and antitumor activities and thus can be considered as an alternative of natural origin for the development of candidates for therapy against invasive infections and cancer treatment.

#### CRediT authorship contribution statement

**Gabriela Cavalcante da Silva:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft. **Alisson Macário de Oliveira:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization. **Wêndeo Kennedy Costa:** Investigation, Methodology. **Antônio Felix da Silva Filho:** Investigation, Methodology. **Maira Galdino da Rocha Pitta:** Funding acquisition, Methodology, Resources, Validation, Visualization. **Moacyr Jesus Barreto de Melo Rêgo:** Funding acquisition, Methodology, Resources, Validation, Visualization. **Ivone Antônia de Souza:** Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Validation, Visualization. **Patrícia Maria Guedes**



**Fig. 3.** Weight of tumors after treatment of animals with Ehrlich carcinoma from control or treated for 7 days with MvRL-rich fraction at 100 or 200 mg/kg *per os*. Each bar represents the mean  $\pm$  SE of the weight of the tumor of each animal. (\*) indicates significant differences ( $p < 0.05$ ) between treatments and control.

**Table 2**

Hematological parameters of animals with Ehrlich carcinoma from control or treated for 7 days with MvRL-rich fraction at 100 or 200 mg/kg *per os*.

Parameters	Treatments		
	Control	MvRL-rich fraction	
		100 mg/kg	200 mg/kg
Erythrocytes ( $10^6/\text{mm}^3$ )	7.90 $\pm$ 0.20	8.98 $\pm$ 0.52*	9.07 $\pm$ 0.48*
Hematocrit (%)	38.30 $\pm$ 0.32	41.90 $\pm$ 0.21*	42.65 $\pm$ 0.42*
Hemoglobin (g/dL)	11.63 $\pm$ 0.08	12.83 $\pm$ 0.33*	13.09 $\pm$ 0.52*
MCV (fL)	48.43 $\pm$ 0.37	46.83 $\pm$ 0.59*	45.65 $\pm$ 0.74*
MCH (pg)	14.77 $\pm$ 0.41	15.00 $\pm$ 0.66	15.35 $\pm$ 0.51*
MCHC (%)	30.47 $\pm$ 0.25	32.10 $\pm$ 0.43*	32.06 $\pm$ 0.41*
Leukocytes ( $10^3/\text{mm}^3$ )	7.07 $\pm$ 0.19	10.03 $\pm$ 0.90*	11.97 $\pm$ 0.77*

MCV: mean corpuscular volume. MCH: mean corpuscular hemoglobin. MCHC: mean corpuscular hemoglobin concentration.

**Paiva:** Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Validation, Visualization. **Thiago Henrique Napoleão:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

#### 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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