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Chemistry

Phytochemical, cytotoxic, antileishmania and antimicrobial potentials of Rhodes grass (*Chloris gayana* Kunth)

Potenciais fitoquímico, citotóxico, antileishmania e antimicrobiano do capim-Ródes (*Chloris gayana* Kunth)

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ABSTRACT

The present study aimed to analyze the cytotoxic and antimicrobial activity of the plant *Chloris gayana* Kunth (Poaceae) proceeding from the ethanol extract, taking into consideration its major secondary metabolites, such as alkaloids, triterpenes, steroids, flavonoids, and tannins. The ethanol extract showed no antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida krusei*. However, the ethanol extract was able to potentiate the activity of amikacin against a strain of S. aureus MRSA (methicillin resistant *S. aureus*), suggesting a possible application of the extract itself or its isolated phytochemicals, as adjuvants of amikacin in the treatment of infections caused by MRSA strains resistant to this antibiotic. Potential antileishmanial activity was observed, but further research on the isolated action of these metabolites is needed.

Keywords: Antibacterial resistance; Antileishmania; Antimicrobial; Phytochemistry; Poaceae

RESUMO

O presente estudo buscou analisar a existência de atividade antimicrobriana e citotóxica da planta *Chloris gayana* Kunth (Poaceae) a partir do extrato etanólico, visto que essa apresenta importantes metabólitos secundários, como alcaloides, triterpenos, esteroides, flavonoides e taninos. O extrato etanólico não

apresentou atividade antimicrobiana contra *Staphylococcus aureus*, *Escherichia coli* e *Candida krusei*. No entanto, o extrato etanólico foi capaz de potencializar a atividade da Amikacina contra uma cepa de *S. aureus* MRSA, sugerindo uma possível aplicação deste extrato, ou de seus fitoquímicos isolados, como adjuvantes da amicacina no tratamento de infecções causadas por cepas MRSA resistentes a este antibiótico. Foi observado potencial atividade antileishmania, porém ainda há necessidade de aprofundar os estudos da ação isolada desses metabólitos.

Palavras-chave: Resistência antibacteriana; Antileishmaniose; Antimicrobiano; Fitoquímico; Poaceae

1 INTRODUCTION

The efficient treatment of bacterial infections by antibiotics have changed modern medicine. However, antibiotic-resistant bacteria have developed as a result of the abuse and overuse of these potent medications, posing a serious threat to public health (Zarei-Baygi and Smith, 2021). Pathogenic bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* showing resistance to third generation cephalosporins and fluoroquinolones have caused severe infections in hospitals and communities around the world (Rath *et al*., 2014; Edelsberg *et al.*, 2014; Mediavilla *et al*., 2012). Infections caused by yeasts of the genus *Candida*, resistant to conventional antifungals, have also been a great concern, mainly because of their greater severity in immunocompromised patients (Jensen *et al*., 2015).

Healthcare professionals and researchers have emphasized the need of using antibiotics correctly to preserve their efficacy (Ashiru-Oredope *et al*. 2022; Majumder *et al*., 2020). On the other hand, the search of new strategies for the treatment of infectious diseases has currently been treated as a priority, due to the high prevalence of resistant microorganisms (Ye and Chen, 2023; Breijyeh and Karaman, 2023; Hetta *et al*., 2023; Strommenger *et al*., 2014).

Leishmaniasis is a parasitic zoonosis belonging to the group of neglected tropical diseases (Mitropoulos; Konidas; Durkin-Konidas, 2010). Endemic in 98 countries, affecting more than 12 million people, the disease basically manifests itself in a cutaneous or visceral form (Alvar *et al*., 2012). The digenetic life cycle of

the parasite consists of interconnected sub-cycles that include: a flagellate form, the promastigotes, occurring in the phlebotomine vector and a flagellated oval form; the amastigotes, dominating the macrophages in the vertebrate host, which can affect rodents, canids, marsupials, ungulates and primates (Bray, 1974).

Conventional treatments for leishmaniasis are expensive, limited, extremely toxic and there are already reports of parasite resistance to these conventional drugs, such as pentavalent antimonial and amphotericin B (Chappuis *et al*., 2007; Rijal *et al*., 2007; Alizadeh *et al*., 2008). For the development of new drugs for leishmaniasis, plants have been increasingly investigated and some secondary metabolites have already proven antileishmanial activity, such as tannins, alkaloids, flavonoids, terpenoids, among others (Sen; Chatterjee, 2011).

Chloris gayana Kunth (Poaceae), popularly known as Rhodes grass, is a native plant in Africa that was introduced in Brazil, characterized by its forage potential (Tamassia *et al*., 2001; Maciel *et al*., 2013). So far there is no scientific record of its medicinal use. However, several species of this family stand out for being used in therapeutics, for example: *Coix lacryma-jobi*, known as "*lady's tear*" and used against mycoses and as an antiseptic of the urinary tract; *Gynerium sagitum*, used against hair loss; and *Saccharum officinarum*, the popular "*sugarcane*", used against thrush (Fenner *et al*., 2006). *Cynodon dactylon* is another species of great importance for its numerous popular uses, such as the treatment of urinary tract infections, calculi, and prostatitis, along with its antimicrobial and antiviral activity (Singh *et al*., 2008).

Therewithal, *Cymbopogon citratus*, known as "*lemongrass*", is also noteworthy for its socio-economic and financial importance. It is widely used as a medicine in almost all continents and covers a wide range of indications, such as: fortifying, stomachic, antitussive, antigripal, analgesic, antiemetic, anticardiopathic, antipyretic, anti-inflammatory of the urinary tract, diuretic, antispasmodic and diaphoretic (Gomes and Negrelle, 2003; Duarte and Zaneti, 2004; Haida *et al*.,

2007). Some of these activities, such as stomatic, analgesic, antispasmodic and antimicrobial, have been confirmed through pharmacological tests. The spectrum of the use of substances extracted from *lemongrass*, especially the essential oil, is equally wide. A discovery that has been highlighted in the scientific literature refers to the action of the essential oil against leukemic cells (Gomes and Negrelle, 2003).

Despite the wide range of uses previously presented for some species in the family, veryfew have been studied, and much remains to be discovered. In this sense, studies regarding screening of biological activities of plants is markedly relevant to bioprospect and develop bioproducts with agricultural, food and health applications (Ferreira *et al*., 2023; Viana *et al*., 2022; Viana *et al*., 2023) Furthermore, the scope of this study was to analyze the cytotoxicity, antileishmania and antimicrobial activity of the plant *Chloris gayana* Kunth (Poaceae) proceeding from the ethanol extract, taking into consideration its major secondary metabolites, such as alkaloids, triterpenes, steroids, flavonoids, and tannins.

2 Materials and Methods

2.1 Chemicals

Dimethyl sulfoxide (DMSO: 99%; PubChem CID: 679), GIEMSA (PubChem CID: 13735) was purchased from Merck Chemical Company (Germany). Schneider's medium (PubChem CID: 2723893, RPMI medium (PubChem CID: 1640), fetal bovine serum (FBS; PubChem CID: 86289556), MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide; PubChem CID: 64965), Alamar blue (PubChem CID: 11077), and the antibiotics penicillin and streptomycin (PubChem CID: 71311919) were purchased from Sigma Chemical (St. Louis, MO, USA). The antibiotic amphotericin B (90%) was purchased from Cristália (São Paulo, SP, Brazil). Norfloxacin, Ciprofloxacin and Chlorpromazine were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2 Phytochemical Study

2.2.1 Collection and identification of the plant species

The aerial parts of *Chloris gayana* Kunth were collected at Campo Comprido Ranch, Cuité, Paraíba, Brazil. The botanical identification was performed by Prof. Dr. Carlos Alberto Garcia Santos, and an exsiccate was deposited in the Herbarium of the Education and Health Center, Federal University of Campina Grande, under the number 0150.

2.2.2 Processing the plant and obtaining the crude ethanolic extract

The plant material was dehydrated at room temperature for 30 days and then grounded using a CienLab A-20-70 knife mill. The powder obtained (1524 g) was macerated in 96% ethanol. Then, the extractive solution was concentrated in a Quimis rotary evaporator model Q344B2 coupled to an Exipump vacuum pump, model AC (flow 37 L/min; max vacuum 600 mmHg; pressure: 20/25 psi), resulting in the crude ethanolic extract (Cg-EtOH, 90.68 g).

2.2.3 Partitioning of the crude ethanolic extract of *Chloris gayana* Kunth

Part of the crude ethanolic extract (60 g) was dissolved in a hydroalcoholic solution (MeOH:H_2O, 7:3), and subjected to partitioning with solvents in an increasing polarity gradient, using hexane, chloroform, and methanol, analytically pure obtained from Vetec (Brazil). Afterwards, the product was concentrated in a rotary evaporator, resulting in the respective phases: hexane (13.46 g), chloroform (5.15 g) and hydroalcoholic (22.65 g).

2.2.4 Preliminary phytochemical prospection

The crude ethanolic extract of the aerial parts of *Chloris gayana* Kunth and the respective phases obtained by partitioning (hexanic, chloroformic and hydroalcoholic)

were subjected to preliminary phytochemical screening. Table 1 briefly shows the list of characterization tests and reagents used for each class of metabolites.

The research of different types of secondary metabolites such as steroids, triterpenes, flavonoids, alkaloids, tannins and saponins were based on the methodology described by Matos (2009) and/or Biavati; Leite (2007).

Table 1 – Classes of metabolites and methods applied for phytochemical prospecting

METABOLITES	CHARACTERIZATION TEST	REAGENTS
Triterpenes and Steroids	*Liberman-Buchard*	Chloroform; acetic anhydride and concentrated sulfuric acid.
Saponins	*Foam index*	Distilled water
Flavonoids	*Shinoda / $FeCl_3$ / NaOH*	Ethanol 95%; acetic anhydride, concentrated hydrochloric acid and metallic magnesium; $FeCl_3$; Naoh; Distilled water.
Tannins	*Ferric chloride / gelatin*	2.0% ferric chloride; 2.0% gelatin.
Alkaloids	*Draggendorff /Bouchardat*	Bismuth carbonate; potassium iodide; concentrated hydrochloric acid and water

Source: Oliveira (2014)

2.3 Evaluation of the intrinsic antimicrobial activity

The intrinsic antimicrobial activity of Cg-EtOH was tested against Gram-positive (*Staphylococcus aureus* ATCC 25923 and SA10) and Gram-negative bacteria (*Escherichia coli* ATCC 25922), as well as against a yeast strain (*Candida krusei* ATCC 6258). Bacterial strains were maintained on Brain Heart Infusion Agar (BHIA, Himedia, India) slant at 4 ºC, and prior to assay the cells were grown overnight at 37 ºC in Brain Heart Infusion (BHI, Himedia, India). The yeast strain was maintained on a Sabouraud Dextrose Agar (SDA, Himedia, India) slant at 4 ºC and prior to assay the cells were grown for 24 h at 37 ºC in Sabouraud Dextrose Broth (SDB, Himedia, India).

Stock solutions were prepared by dissolving 10,000 μg of Cg-EtOH in 1 mL of dimethyl sulfoxide. This stock solution was diluted in sterile distilled water to obtain the test solution (1024 $\mu g \cdot mL^{-1}$). Minimal inhibitory concentrations (MICs) were determined by micro-

dilution assay in BHI broth with bacterial suspensions of approximately 105 CFU·mL^{-1} and compound solutions ranging from 8 to 512 μg·mL^{-1}.

Microtiter plates were incubated at 37 ºC for 24 h, then 20 μL of resazurin (0.01% w/v in sterile distilled water) was added to each well to detect bacterial growth by color change from blue to pink. MICs were defined as the lowest concentration at which no bacterial growth was observed. Antifungal assays were performed by micro-dilution method in SDB double concentrated with yeast suspension of approximately 105 CFU·mL^{-1} and compound ranging from 8 to 512 μg·mL^{-1}. Microtiter plates were incubated at 37 ºC for 24 h.

2.5 Modulation of the resistance to Amikacin assay

For the evaluation of the Cg-EtOH as modulator of the resistance to Amikacin, MIC values for Amikacin against the SA10 strain were determined in the presence or absence of Cg-EtOH or Chlorpromazine (a known efflux pump inhibitor) at sub-inhibitory concentrations (1/8 MIC). Antibiotic concentrations ranged from 0.15 to 312 μg·mL^{-1}. Microtiter plates were incubated at 37 ºC for 24 h and readings were performed with resazurin as previously described.

2.6 Parasites and Mice

Leishmania (Leishmania) amazonensis (IFLA/BR/67/PH8) was used for the determination of the antileishmania activity. Parasites were grown in supplemented Schneider's medium (10% heat-inactivated fetal bovine serum (FBS), 100 U·mL^{-1} penicillin and 100 μg·mL^{-1} streptomycin at 26 ºC). Murine macrophages were collected from the peritoneal cavities of male and female BALB/c mice (4-5 weeks old), obtained from Medicinal Plants Research Center (UFPI, Teresina, PI, Brazil). Mices were maintained at a controlled temperature (24 ± 1 ºC) and light conditions (12 h light/dark cycle), with water and *food ad libitum*. All protocols were approved by the Animal Research Ethics Committee (CEEA-PI No. 008/2012).

2.7 Antileishmania Activity Assay

Promastigotes in the logarithmic growth phase were seeded in 96-well cell culture plates at 1×10^6 promastigotes per well. The Cg-EtOH was added to the wells in serial dilutions of 800, 400, 200, 100, 50, 25, 12.5 and 6.25 µg mL^{-1}. The plate was kept at 26 °C in a biological oxygen demand (BOD) incubator, and then the optical density was measured by colorimetric assay using resazurin after 24, 48 and 72h to determine the promastigotes killing (Valadares *et al*., 2011). The absorbances plate was read in a Biotek absorbance plate reader (model ELx800) at a wavelength of 550 nm. The results were expressed as promastigote killing percentage (%).

Amphotericin B (Amph B) was used as positive control in serial dilutions of 8, 4, 2, 1 and 0.5 µg·mL^{-1}. The negative control was Schneider's medium with promastigotes (1×106 cells/well). The cell viability was considered as 100% for the parasite. Assays were performed in triplicate and were repeated 3 times on different days.

2.8. Cytotoxicity Determination

Cytotoxicity of Cg-EtOH was assessed using the MTT test (Gonçalves *et al*., 2016). In a 96-well plate, 100 µL of supplemented RPMI 1640 medium and about 1×10^5 macrophages were added per well. They were then incubated at 37 ºC in 5% CO_2 for 4 h to allow cell adhesion. After this time, two washes with supplemented RPMI 1640 medium were performed to remove non-adhered cells. The Cg-EtOH was added in triplicate, after being previously diluted in supplemented RPMI 1640 medium to a final volume of 100 µL for each well at the tested concentrations (800, 400, 200, 100, 50, 25, 12.5 and 6.25 µg·mL^{-1}). Cells were then incubated for 48 h. At the end of the incubation, 10 µL of MTT [5 mg·mL^{-1}] diluted in RPMI 1640 medium was added at a final concentration of 5 mg·mL^{-1} (10% of volume, i.e., 10 µL for each 100 µL well), and was then incubated for 4 h at 37 °C in 5% CO_2. Afterwards, the supernatant was discarded, and 100 µL of DMSO was added to all wells. The plate was then stirred for about 30 min at room temperature to complete formazan dissolution. Finally, spectrophotometric reading was conducted at 550 nm in an ELISA plate reader.

2.8. Statistical analysis

In all experiments, data were expressed as mean of IC50 or CC50 values and the standard error of the mean thereof. Significance determination was performed using One-way ANOVA following by Bonferroni's post-hoc test, and positively considered when $p<0.05$. All analyses and graphs plotting were performed using GraphPad Prism version 8.0 (GraphPad Inc., La Jolla, CA, USA).

3 Results and Discussion

3.1 Phytochemical Analysis

Phytochemical screening of crude ethanol extract (Cg-EtOH) and the hexagonal, chloroform and hydroalcoholic phases of *Chloris gayana* Kunth revealed the presence of tannins, alkaloids, steroids, flavonoids and triterpenes and absence of saponins, as observed in Chart 1.

Chart 1 - Phytochemical screening of the aerial parts of *Chloris gayana* Kunth. The sign (+) indicates presence and (-) absence of the chemical constituent

RESEARCH			**RESULT**		
SECONDARY METABOLITE	**TEST**	**EEB**	**PHASE HEX**	**PHASE $CHCl_3$**	**PHASE MeOH:H_2O**
Steroids	Lieberman-Burchard	+	+	-	-
TriterpenEs	Lieberman-Burchard	-	-	+	-
Flavonoids	Shinoda	-	-	-	-
	$FeCl_3$	-	-	-	+
	NaOH	+	+	+	+
Tannins	Soil chloride	+	-	-	+
Alkaloids	Dragendorff	+	+	+	-
	Bouchardat	+	+	+	-
Saponins	Foam Index	-	-	-	-

Source: Oliveira (2014)

The results were considered as positive by the formation of precipitates, appearance of staining or foaming, and negative by their absence.

3.2 Antibacterial Activity Assay and modulation of the Amikacin-resistance

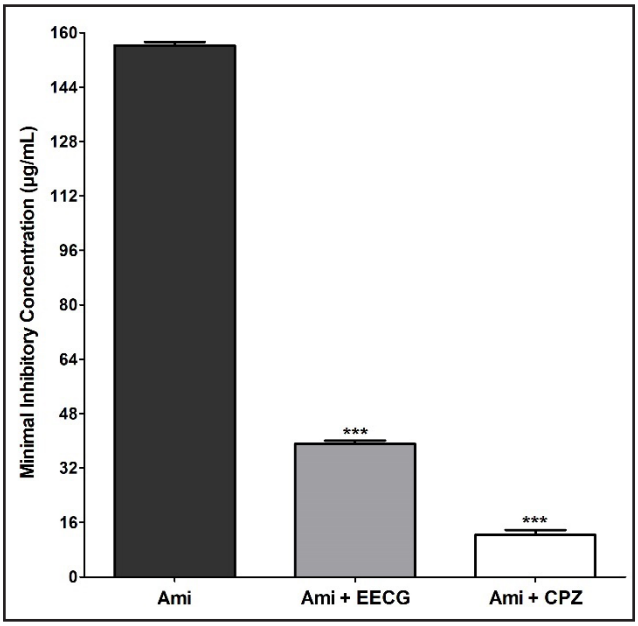
An earlier study determined that plant extract MIC values above 1000 $\mu g \cdot mL^{-1}$ are therapeutically irrelevant since it may be difficult to calculate the dose from that eliciting activity in vitro to that needed to be equivalent for the size of an adult person (Houghton *et al*., 2007). Taking this criterion into consideration, Cg-EtOH did not present clinically relevant antimicrobial activity in the tested concentrations, since the MIC values obtained against Gram-positive strains *S. aureus* SA10 and *S. aureus* ATCC 25923 were ≥1024 $\mu g \cdot mL^{-1}$. The extract was also inactive against Gram-negative strains *E. coli* EC13 and *E. coli* ATCC 25922 (both showing MIC values ≥1024 $\mu g \cdot mL^{-1}$). In addition, Cg-EtOH did not present antifungal activity against the *C. krusei* ATCC 6258 strain (MIC value ≥1024 $\mu g \cdot mL^{-1}$). Although these results are not considered as relevant, it is important to note that this is the first time that the antimicrobial activity of this species is evaluated.

On the other hand, when Cg-EtOH was added to the culture medium in a sub-inhibitory concentration, there was a 4x reduction in the MIC of Amikacin against the SA10 strain from 156 to 39 $\mu g \cdot mL^{-1}$ (Figure 1). A similar effect occurred when Cg-EtOH was replaced by Chlorpromazine, a known efflux pump inhibitor (Neyfakh *et al*., 1993), suggesting the occurrence of an efflux-mediated resistance mechanism. These results suggest that Cg-EtOH contains phytochemicals capable of inhibiting *S. aureus* efflux pumps.

Flow pumps are transmembrane proteins capable of pumping antibiotics into the external environment, reducing the intracellular concentrations of these drugs (Du *et al*., 2018), thus acting as an important mechanism of bacterial resistance to antibiotics. In *S. aureus*, several eflux pumps have been identified, including Nora, Norb, Norc, Tetk, Mepa, Msra, QacA, QacB, QacC, LmrS, Mdea and Sdrm proteins

(Hassanzadeh *et al*., 2017; Schindler and Kaatz, 2016). Among these, the LmrS flow pump is capable of extruding aminoglycosides (Floyd *et al*., 2010), such as Amikacin.

Figure 1 – Minimum Injunction Concentration (MIC) of Amikacin for MRSA strain SA10 in the absence, as well as in the presence, of Cg-EtOH or Chlorpromazine (CPZ). Each result represents the geometric means of three simultaneous experiments. $p<0.001$ versus Amikacin



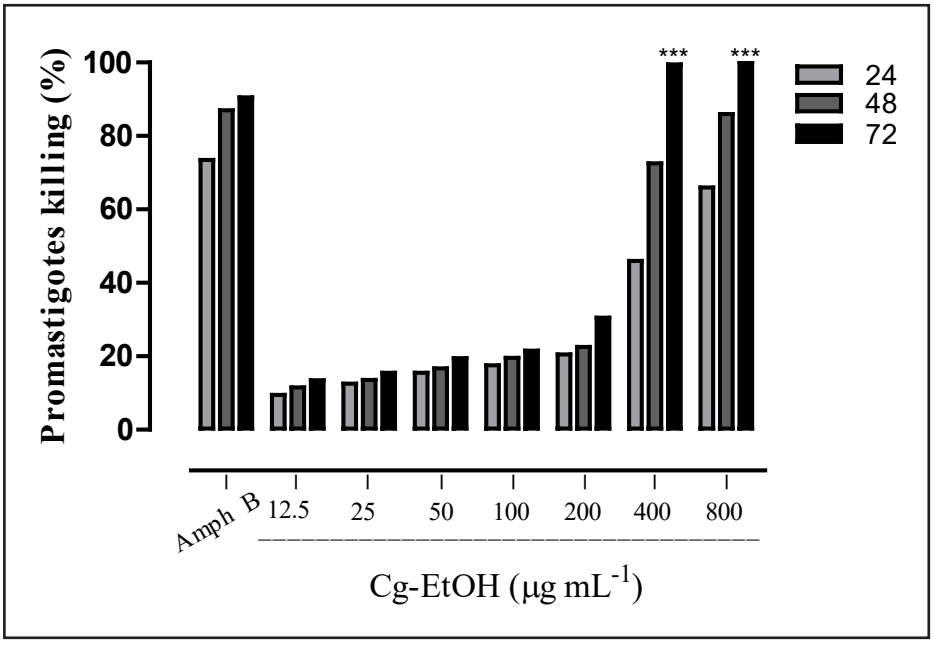
Source: Laboratório de Pesquisas em Microbiologia, Departamento de Parasitologia e Microbiologia, Universidade Federal do Piauí, Teresina, PI, Brasil

Efflux pump inhibitors have been proposed as a technological strategy to reverse the effectiveness of antibiotics, traditionally used against resistant strains due to the overexpression of resistance genes (Shriram *et al*., 2018). According to this strategy, when administered in combination with the antibiotic, the inhibitor could block the activity of the flow pump, allowing the permanence of the antibiotic in the intracellular environment and the binding in its cytoplasmic target (Silva *et al*., 2020; Ribeiro *et al*., 2019). The results obtained from the *in vitro* treatment of the MRSA SA10 strain with the combination Amikacin/ Cg-EtOH suggest that the combined use of Cg-EtOH phytochemicals with Amikacin could be effective in the treatment of infections caused by MRSA strains.

3.3 Antileishmanial Activity Assay

The inhibitory effects of GA and EA against *L. amazonensis* promastigotes showed a significant concentration-dependent decrease ($p<0.05$) in parasite viability, with approximately 100% of promastigote killing at concentrations of 800 $\mu g \cdot mL^{-1}$ after 72h of incubation for Cg-EtOH (Figure 2). The IC50 values for Cg-EtOH at 24, 48 and 72h of exposure were 2,464.877, 208,758 and 200,903 $\mu g \cdot mL^{-1}$, respectively. Amphotericin B (Amph B) was used as a positive control. The highest inhibitory effect of Amph B at concentration of 2 $\mu g \cdot mL^{-1}$ was observed after 48h of incubation.

Figure 2 – Effect of Cg-EtOH or amphotericin B (Amph) (2 $\mu g \cdot mL^{-1}$) on Leishmania amazonensis promastigotes. Cultures of log-phase promastigotes (1×10^6 per well) were incubated at 26 °C for 24, 48 and 72h in different Cg-EtOH concentrations. Data represent the mean percentage of promastigote killing ± standard error of 3 experiments carried out in triplicate. The same letter does not differ in Bonferroni's post-test ($p<0.05$)

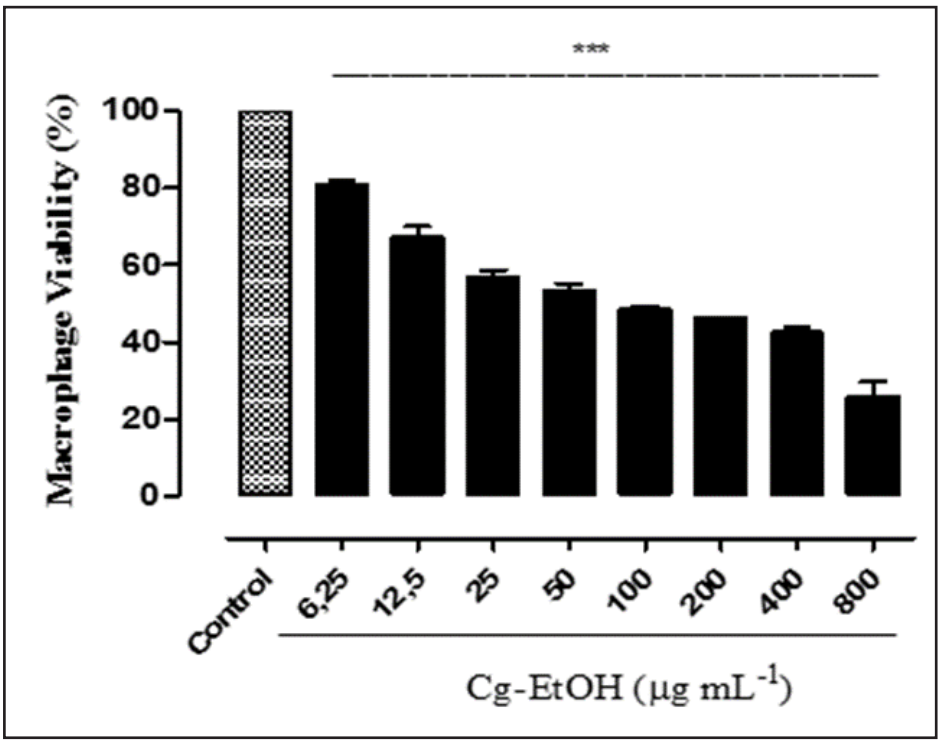


Source: Laboratório de Atividade Antileishmania, Núcleo de Pesquisas em Plantas Medicinais, Universidade Federal do Piauí, Teresina, PI, Brasil

3.4. Cytotoxicity on murine peritoneal macrophages

The cytotoxicity assessment of Cg-EtOH is shown in Figure 3. The Cg-EtOH significantly decreased the macrophage viability starting at concentrations of 6.25 μg·mL^{-1} ($p<0.05$), respectively. The mean cytotoxic concentration (CC_{50}) of Cg-EtOH is 95,914 μg·mL^{-1}.

Figure 3 – Cytotoxicity of Cg-EtOH on the viability of murine peritoneal macrophages. Peritoneal macrophages were seeded at 1×10^5 per well in 96-well microplates and incubated for 48h in the presence of Cg-EtOH at concentrations 800 to 6.25 μg·mL^{-1}. Viability was determined using 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) assay. The optical density ± standard error of 3 experiments carried out in triplicate. *p<0.05; **p<0.01; and ***p<0.001



Source: Laboratório de Atividade Antileishmania, Núcleo de Pesquisas em Plantas Medicinais, Universidade Federal do Piauí, Teresina, PI, Brasil

Considering the relevant scenario of leishmaniasis, the introduction and development of new compounds are fundamental, especially those with low toxicity

and high availability, considering mainly the neglected and forgotten populations of public authorities, which are the most affected by the disease. In the investigation of new compounds with antileishmania activity, plants and their derivatives have advanced considerably in investigations (Sen; Chatterjee, 2011), due to being easily available and of low cost.

There are more than 250,000 plant species that have potent chemotherapy properties (Kayser; Kiderlen; Croft, 2003; Salem; Werbovetz, 2006). Plant derivatives with antileishmanial activity consist of alkaloids, terpenes, flavonoids, benzopyrans, phenolics and sesquiterpene lactones (Jesus *et al*., 2012; Ribeiro *et al*., 2015; Rodrigues *et al*., 2013; Rodrigues *et al*., 2015).

The ethanolic extract of *Chloris gayana* Kenth presented antileishmanial activity on promastigote forms of *L. amazonensis*. This is possibly due to the action of the alkaloides, tannins and triterpenes present in this extract. Alkaloids and terpenoids are characterized by acting on the mitochondria of the parasite causing inflammation and inhibiting the process of cellular respiration (Gantt *et al*., 2001; Guimaraes *et al*., 2010), and terpenoids are also able to act on leishmanial DNA by inhibiting important enzymes such as topoisomerases (Misra *et al*., 2010). Tannins are classically known for presenting immunomodulatory activity (Kolodziej; Kiderlen, 2005).

When evaluating cytotoxicity on murine macrophages, Cg-EtOH demonstrated potential to minimize the viability of these cells by the MTT method, and therefore its CC_{50} value is lower than the value of IC_{50} on the promastigote forms of *L. amazonensis*. Those that present more selectivity for the parasite than for host cells are considered in the search for new drugs for antileishmanial activity (Carneiro *et al*., 2012; Medeiros *et al*., 2011). In our study, Cg-EtOH was proven to be more selective for macrophages than for the parasite. However, by demonstrating significant antileishmanial activity, perspectives are opened for new approaches to study other components of this plant or its isolated compounds.

4 CONCLUSIONS

Chloris gayana Kunth is a plant with potential for therapeutic application, considering the presence of important classes of secondary metabolites such as alkaloids, triterpenes, steroids, flavonoids, and tannins. Although Cg-EtOH did not present antimicrobial activity in the tested strains, it attenuated the resistance of a strain of *S. aureus* MRSA to the antibiotic Amikacin. This result indicates a possible application of this extract, or its isolated phytochemicals, as adjuvants of Amikacin in the treatment of infections caused by MRSA strains resistant to this antibiotic. In addition, Cg-EtOH demonstrated potential antileishmanial activity, however, being more toxic to murine macrophages than to the parasite. These results encourage further studies with this plant, to determine which substances present in the extracts can contribute to biological activities and modulatory activities aiming at a future pharmaceutical application.

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