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PHARMACOGNOSTIC PROFILE AND PHARMACOLOGAL ACTIVITIES OF *Eleutherine plicata*

PERFIL FARMACOGNÓSTICO E ATIVIDADES FARMACOLÓGICAS DE *Eleutherine plicata*

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ABSTRACT

Eleutrherine plicata is a medicinal plant which is characterized by presenting clinical evidence and therapeutic uses, biological activities, including antibacterial, antifungal, antioxidant, anti-inflammatory and analgesic effects. The purpose of this article is for pharmacognostic studies to identify chemical components, evaluate the cytotoxic, antimicrobial and anti-inflammatory potential of chloroform and methanolic extracts of *Eleutrherine plicata*. The extracts were produced using the *E.e plicata* bulb with hexane and methanol solvents according to the Brazilian Pharmacopoeia**.** In identification of secondary metabolites, colorimetric methods were used. The extracts were subjected to tests *in vitro* and *in vivo*. In cytotoxic assay, hemolysis was determined using concentrations of 150 to 2.34 µg / mL^{-1}. In anti-tumor test, to determine the cytotoxicity of extracts, Ehrlich's ascitic tumor cells were used (ETA). Cell viability was determined by the colorimetric method of MTT in 24 and 48 hours, using serial dilution of 1024 to 1 µg /mL^{-1} .Antibacterial activity was assessed using the disk diffusion methodology, in serial dilution of 1024 to 16 µg / $mL^{-1.}$ The anti-inflammatory activity was evaluated by neutrophil migration method and tested at concentrations of 1; 5 e 15 mg / kg^{-1}. Pharmacognostic tests showed the presence of tannins, anthraquinone glycosides and flavonid glycosides. In the cytotoxic assay, hemolysis was observed at concentrations of 150 to 75µg / mL^{-1}. ETA Test, in both extracts; showed a reduction in viability at the highest concentrations tested, at both times analyzed. Antimicrobial activity showed significant inhibition in methanolic extract on *E.coli* in concentrations of 1024 to 16 µg / $mL^{-1.}$ There was not anti-inflammatory activity for the tested concentrations of both extracts methanolic and hexanic. Both extracts showed a 70% reduction in cell viability in the cytotoxic assay. Since this trial was conducted with tumor cells, it arouses interest in antitumor investigation, which is a promising result; among the tests performed.
Key words: *E. plicata*, biological properties, pharmacognostic studies, hexanic and methanolic extracts

RESUMO

A *Eleutrherina plicata* é uma planta medicinal que se caracteriza por apresentar evidências clínicas e utilizações terapêuticas, atividades biológicas, incluindo efeitos antibacterianos, antifúngicos, antioxidantes, antiinflamatórios e analgésicos. O objetivo deste artigo foi realizar estudos farmacognósticos para identificar componentes químicos, avaliar o potencial citotóxico, antimicrobiana e antiinflamatório do clorofórmio e dos extratos metanólicos de *Eleutrherine plicata*. Os extratos foram produzidos utilizando bulbo de *E. plicata*, com solventes hexânico e metanólico de acordo com a Farmacopéia Brasileira. Na identificação de metabólitos secundários, métodos colorimétricos foram usados. Os extratos foram submetidos a testes *in vitro* e *in vivo*. No ensaio citotóxico, a hemólise foi determinada utilizando concentrações de 150 a 2,34 µg / mL^{-1}. No teste antitumoral, para determinar a citotoxicidade dos extratos, foram utilizadas células tumorais ascíticas de Ehrlich (ETA). A viabilidade celular foi determinada pelo Método Colorimétrico de MTT em 24 e 48 horas, utilizando diluição seriada de 1024 a 1 µg / mL^{-1}. A atividade antimicrobiana foi avaliada pela metodologia de disco difusão, em diluição seriada de 1024 a 16 µg / mL^{-1} A atividade antiinflamatória foi avaliada pelo método de migração de neutrófilos e testada nas concentrações de 1; 5 e 15 mg / kg^{-1}. Os testes farmacognósticos mostraram a presença de taninos, glicosídeos de antraquinona e glicosídeos de flavonídeos. No ensaio citotóxico, hemólise foi observada em concentrações de 150 a 75µg / mL^{-1}. Teste ETA, em ambos os extratos; apresentou redução da viabilidade nas maiores concentrações testadas, em ambos os tempos analisados. A atividade antimicrobiana apresentou inibição significativa do extrato metanólico em *E. coli* nas concentrações de 1024 a 16 µg / mL^{-1}. Não houve atividade antiinflamatória para as concentrações testadas de ambos os extratos metanólico e hexânico. Ambos os extratos mostraram uma redução

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de 70% na viabilidade celular no ensaio citotóxico. Por ser este ensaio realizado com células tumorais, desperta interesse na investigação antitumoral, o que é um resultado promissor; entre os testes realizados.
Palavras-chave: *E. plicata.* Propriedades biológicas. Estudos farmacognósticos. Extratos hexânico e metanólico.

1. INTRODUCTION

The inflammatory process is a dynamic and natural mechanism which the body's defense functions against invaders [1]. It is characterized as a immune system response stimuli harmful, such as pathogenic microorganisms, damaged cells, toxic compounds or irradiation [2], and acts by removing harmful stimuli and initiating the healing process [3]. Inflammation mechanism represents a chain of organized and dynamic reactions, including cellular and vascular events with specific humoral secretions. These pathways involve the infiltration of white blood cells (monocytes, basophils, eosinophils and neutrophils), plasma and fluids at inflamed site [4]. Exacerbated inflammation occurs when acute inflammatory mechanisms are unregulated, causing causing tissue damage [5].

Neutrophils, effector cells of the innate immune system, known as the first line of defense against invading pathogens, are abundantly present in the circulation and comprise 50 to 70% of the total circulating leukocytes in humans. [6]. Rapid recruitment of neutrophils to inflammation sites and their phagocytize ability of invading microorganisms are well known, as well as cytotoxic content of granules in cells. In fact, these cells are crucial for orchestrated elimination of microorganisms and for the resolution of inflammation [7]. Inhibition of neutrophil influx is necessary to prevent the inflammatory reaction from persisting, because these cells harbor most destructive potency for tissue damage, but it requires regulation so that infectious agent is sufficiently combated [6].

Various anti-inflammatory mediators and recruitment of white cells to remove cellular or tissue debris influences the Resolution of inflammation. It is possible that resolution does not occur in acute phase, making it chronic [8]. Chronic inflammation performs critical role in the development of pathological conditions, including autoimmune, metabolic, neurodegenerative, cardiovascular diseases and cancer [9].

The majority of human population is affected by disorders related to inflammation. Although several agents are accessible to treat various inflammatory diseases, their prolonged use leads to serious adverse effects [10]. Management of inflammatory diseases with steroidal or non-steroidal drugs is the traditional clinical practice. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the initial stages of prostaglandin biosynthesis by inhibiting cyclooxygenase

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(COX). NSAIDs are important drugs used to reduce the undesirable consequences of inflammation [10]. Chronic use of NSAIDs is related to cardiovascular, gastrointestinal and renal toxicities [11]. Likewise, use of corticosteroids leads to hypertension, hyperglycemia, osteoporosis and growth retardation [12]. Toxicity and recurrence of symptoms at discontinuation is a major problem related to synthetic substances currently available [13].

In this sense, natural products extracted from plants with anti-inflammatory activity are considered an important source for development of new therapeutic agents. Many studies have reported the inhibitory effect of isolated plants and natural products on inflammatory processes [14].

Brazil has more than 45,000 species of plants, comprising from 20 to 22% of total number of plant species in the world, much of biodiversity is found in Amazon rainforest. Taking into account the pharmacological potential of Brazilian plant species not yet investigated, thus creating the opportunity to explore plants for discovery of new secondary metabolites capable of interfering in inflammatory response [15]. Flavonoid, polyphenolic, proanthocyanidins, alkaloids, terpenoids and steroids compounds are generally responsible for anti-inflammatory activities of plant extracts [16].

Eleutherine plicata (*E. plicata*) belongs to botanical familie is Iridaceae popularly known in the Amazon rainforest region, widely used in popular Brazilian phototherapy, it exists in the form of a heap with red bulbs like onion and its leaves are whole, pleated and simple, and its flowers are colored from white to pink and the red bulbs [17]. *E. plicata* is medicinal plant of popular use with great therapeutic potential.

The objective of the objective of this article/ paper was to identify the chemical components of *E. plicata* through pharmacognostic studies, cytotoxic, antimicrobial, anti-tumor and anti-inflammatory evaluation.

2. METHODS

2.1 Species registration *Eleutherine plicata*

The collection of the botanical sample of the species *E. plicata* in the Medicinal Garden of Faculdade Estácio Unijipa of Ji-Paraná, is located in the municipality of Ji-Paraná, state of Rondônia (10º 52'20.37"S 61º58'36.33"). The exsiccata was identified by Silva CAS and

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registered under the number 200081 in herbarium professor doctor Marlene Freitas da Silva da (of) Universidade do Estado do Pará (UEPA).

2.2 Extracts

The production of polar and nonpolar extracts, was based on the methodology described by the Brazilian Pharmacopeia V Edition [16]

2.2.1 Hexanic extract

For the preparation of the nonpolar extracts it was inserted in an amber glass; the dry and ground bulb of *Eleutherine plicata*. Then the hexane solvent (Synth) was added filling the volume, keeping all the extract soaked. Every 7 days, the liquid was filtered on filter paper, concentrating the filtrate in a rotary vacuum evaporator (Fisatom) at a temperature below 50ºC.

2.2.2 Methanol extract

For the preparation of the nonpolar extracts it was inserted in an amber glass; the dry and ground bulb of *E. plicata*. Then the methanolic solvent (Dynamic) was added, filling the volume, keeping the entire extract soaked. Every 7 days, the liquid was filtered on filter paper, concentrating the filtrate in a rotary evaporator (Fisatom) under vacuum at a temperature below 50ºC.

2.3 Pharmacognostic study of *Eleutherine plicata*

Pharmacognostic analyzes, to identify active metabolites present in the species, were based on methods described in the Brazilian Pharmacopoeia V Edition [16] These methods consist of colorimetric and physical reactions that express presence of secondary metabolites with therapeutic activities.

2.4 Analysis of the antimicrobial activity of methanolic and hexane extracts of *Eleutherine plicata (*Herb)

The antibacterial activity was evaluated using the disk diffusion methodology, according to the methodology standardized by the *Clinical and Laboratory Standards Institute* [18]. The culture medium used was Mueller-Hinton agar and the microorganisms included standardized strains of *Escherichia coli* (Lot 17110R) e *Staphylococcus aureus* (Lot 1802R,

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New Prov). For test, disc diffusion was used, in concentrations of 1024 to 16 µg / mL^{-1} of methanolic and hexanic extract. After, bacterial suspensions were prepared in physiological solution (Eurofarma/Brasil), using the Mac Farland scale as a turbidity standard. Posteriorly were sown on the plates containing Mueller-Hinton agar (Kasvi), and discs were later deposited. The plates were incubated in a bacteriological incubator (SP Labor) at 37ºC for 24 hours. Zones of inhibition (halos) were measured with a ruler to an accuracy of 0.5 mm. Positive control, penicillin was used to *Escherichia coli* and ampicillin (Sensifar) for *Staphylococcus aureus* and how negative control saline. The formation of inhibition halos (HI) equal to or greater than 6 millimeters in diameter was considered, positive antibacterial activity.

2.5 Experimental Animals

For animal experiments, adult male albino mice (Swiss) were used with average weight of 22-24 grams. These animals belonged to the Central Animal Laboratory of the Catholic University Dom Bosco - UCDB in Campo Grande state of Mato Grosso do Sul - Brazil. All animals were accommodated in sanitary cages that are duly standardized from polypropylene, with the presence of sanitary shavings and stored at a temperature of (22 ± 2) ºC and in the condition of light-dark cycles (12h), the animals had free access to the standard pellet diet (Nuvilab® CR-1, Nuvital, PR, Brazil) and water *ad libitum.* The experimental procedure was approved by the UCDB's Ethics Committee on the Use of Animals (CEUA) through protocol 030/2017.

2.6 Hemolytic and cytotoxic properties of extracts *Eleutherine plicata (*Herb)

The assay for determining hemolytic activity was performed according to the methods of Park et al [19] with minor modifications. Murine erythrocytes were collected from Swiss mice, washed with 0.9% saline and centrifuged at 580 *g* at 4 ºC for 2 min. For the experiment, 8% blood was used, and distributed in 96-well plate wells, with the addition of different concentrations of methanolic and hexane extracts (150, 75, 37.5, 18.75, 9.37, 4.6, 2.34 µg / mL^{-1}). The positive control received Triton X-100 2% (Vetec) and negative control received saline 0.9%, and all groups were performed in triplicate. After 1 hour incubation at 37ºC, reading was performed at 540 nm in a microplate reader (Thermo Scientific Multiskan Britain).

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2.7 Ehrlich Ascite Tumor cells (EAT)

ETA cells were provided by the Department of Pathology at Universidade Estadual Paulista de Botucatu (UNESP) and stored in a freezer at a temperature of -80ºC in the concentration of 2 X 10^5/mL. Perform the anti-tumor tests, cells were thawed, centrifuged for 5 minutes at 580 RPM. Cells were resuspended in culture medium RPMI 1640. From that cell suspension, it was injected 200µL EAT cell intraperitoneal in two mice. After five days, the animals were euthanized e the cells were obtained from the peritoneal lavage, washed twice in culture medium RPMI 1640 supplemented with antibiotics (penicillin / streptomycin) (Gibco) and fetal bovine serum (SBF) (Gibco) and centrifuged for 5 minutes at 580*xg* at room temperature. Subsequently, the cells were counted in a Neubauer chamber. Cell viability was assessed using the Tripan blue exclusion method. The cells were considered suitable for the experiment, *in vitro* if presented at least 90% viability.

Cytotoxicity was determined using the MTT colorimetric assay [20]. For the assay, a suspension of ETA cells, containing 2 x 10^5 cells/mL were placed in a 96-well plate and incubated with RPMI 1640 containing serial dilution 512 to 1µg / mL^{-1} of methanolic extract and 512 to 1µg / mL^{-1} of hexane extract, incubated in a greenhouse CO_2 5%, at 37ºC. Cell viability was assessed after 24 and 48 hours of incubation. After the time, the supernatant was aspirated and 10 µL of the MTT solution was added (5 µg / mL^{-1}), and incubated for 4 hours, protected from light. After the incubation period, a solubilizing solution (0.01 mL) was added to solubilize the formazan crystals. The plates were shaken slightly at room temperature for 5−10 min and the reading was recorded at 540 nm (SpectraMax F3, Molecular Devices). Experiments in all the groups were performed in triplicate. The cell viability percentages were calculated in relation to the untreated cell controls.

2.8 Evaluation of neutrophil migration

The mice (n = 5) were pretreated with subcutaneous (s.c.) vehicle (0.1 mL s.c.), or methanolic or hexanic extract of *Eleutherine plicata (*Herb) (1, 5 e 15mg / kg^{-1} s.c.) 15 min prior to application of the inflammatory stimulus (carrageenan, 1.5% 0.1 mL i.p.). The negative control group received saline solution (0.9%, 0.1 mL i.p.). After 6 h, the animals were euthanized (deepening anesthesia with ketamine (Cetamin) 150 mg/kg and xylazine (Calmiun) 7.5 mg/kg) and the peritoneal cavity was washed with PBS/EDTA (0.3%, 3 mL). The total and

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differential cells present in the exudate were assessed. The total count was performed in a Neubauer chamber. The differential count was performed with slides of the exudate cell smear, which were stained and examined under an optical microscope (Nikon® Eclipse 80i, oil immersion lens, 100/1.25). The results are expressed as the number of cells × 10^5 [21].

2.9 Statistical analysis

The results were treated statistically, the central tendency measure being used, average, along with the standard error. In order to verify significant differences between the averages, used the ANOVA test (p-value = 0.05) and the Bonferroni correlation, performed on Graphpad Prism 6.0.

3. RESULTS

3.1 Pharmacognostic study of *Eleutherine plicata (*Herb)

The pharmacognostic study was conducted according to the descriptions of the Brazilian Pharmacopeia V edition [16]. Through the experimental tests for the analysis of tannins present in the *E plicata*, it was possible to show the presence of this metabolite (Table 1). The results of analysis of reactions indicative of presence or absence of flavonoids in *Eleutherine plicata* showed that the species under study showed positive results for flavonoid glycosides.

Table 1- PHARMACOGNOSTIC STUDY OF *Eleutherine plicata(*Herb)

Presence / absence test	Reagent	Colour	Result
Tannins	Ferric chloride	Green colored precipitate	Positive
	Neutral lead acetate	Reddish brown with a small amount of viscous precipitate.	Positive
Flavonoid glycosides	Borntraeger	Yellow and red	Positive for the presence of anthraquinones in reduced form. Positive for the presence of anthraquinone in oxidized form

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Anthraquinone glycosides	Borntraeger	Yellow and red	Positive for the presence of anthraquinones in reduced form. Positive for the presence of anthraquinone in oxidized form.

In reaction with sodium hydroxide and in test with antimony chloride, obtained positive results for the presence of flavonoid glycosides, which, according to the methodology used, considers positive result when yellow color develops in reaction with sodium hydroxide and yellowish in color using antimony chloride.

Another class of glycosides analyzed were anthraquinones and the reactions showed a positive result for the presence of anthraquinone glycosides in reduced form (Table 1). Using Borntraeger's reagent to indicate the presence of anthraquinone glycosides, the color of reaction varies from yellow to red depending on the chemical form of anthraquinone. Develops yellow color with reduced anthraquinone and red color in the presence of oxidized anthraquinone. It was observed that, using the Borntraeger reagent, the color of medium became yellow and red which are indicative of the presence of anthraquinone in the reduced form and anthraquinone in the oxidized form.

3.2 Antimicrobial activity of *Eleutherine plicata (*Herb)

Antibacterial activity against species *E. coli* and *S.aureus* was evaluated using the disk diffusion methodology. The concentrations of 1024; 512; 256; 128; 64; 32 e 16 µg/mL^{-1} hexanoic or methanolic extracts and antibiotics ampicillin and penicillin as a positive control; and the negative control, the saline solution. The results were expressed in minimum inhibitory concentration (mm) (Table 2).

Table 2- Minimum Inhibitory Concentration (MIC) hexane and methanolic extracts of *Eleutherine plicata (*Herb) by the diffusion disk method.

	Concentration (µg/mL)							**Positive control**		**Control negative**
Strains	**1024**	**512**	**256**	**128**	**64**	**32**	**16**	**Amp**	**Pen**	**Physiological solution**

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E. coli (HE) 17110R	--	--	--	--	--	--	--	**	33*	--
S. aureus (HE) 1802R	--	--	--	--	--	--	--	37	**	--
E. coli (ME) 17110R	11*	10 *	--	--	--	--	--	**	40*	--
S. aureus (ME) 1802R	--	--	--	--	--	--	--	41	**	--

E. coli = Escherichia coli ; S. aureus = Staphylococcus aureus ;HE = Hexanic extract; ME= Methanolic extract ; Amp = Ampicillin ; Pen = Penicillin ; (--)There was no halo; (*)size of inhibition halo in millimeters; (**)Not tested on the strain.

The results demonstrate that the methanolic extract in the concentrations of 1024 and 512 µg/mL^{-1} presented antimicrobial activity against *E.coli*, with halos of 11 and 10 mm, respectively. However, the values found remained below the positive control (penicillin). The other concentrations and extracts did not show antibacterial activity in the tested concentrations (Table 2).

3.3 Hemolytic assay of hexane and methanolic extracts of *Eleutherine plicata (*Herb)

The hemolytic rate was assessed at concentrations of 150, 75, 37.5, 18.75, 9.37, 4.6 and 2.34 µg/mL^{-1} hexane and methanolic extracts of *E.plicata (*Herb) (Figure 1). The results showed that hexane extract of *E. plicata (*Herb) was hemolytic in the concentrations of 150 µg/mL^{-1} (81,57 ± 4,14%) and 75 µg/mL^{-1} (46,46 ± 4,47) greater than the negative control (PBS), but with values lower than the positive control (Triton 2%). While the methanolic extract did not have a hemolytic effect against erythrocytes; in any concentration assessed (Figure 1).

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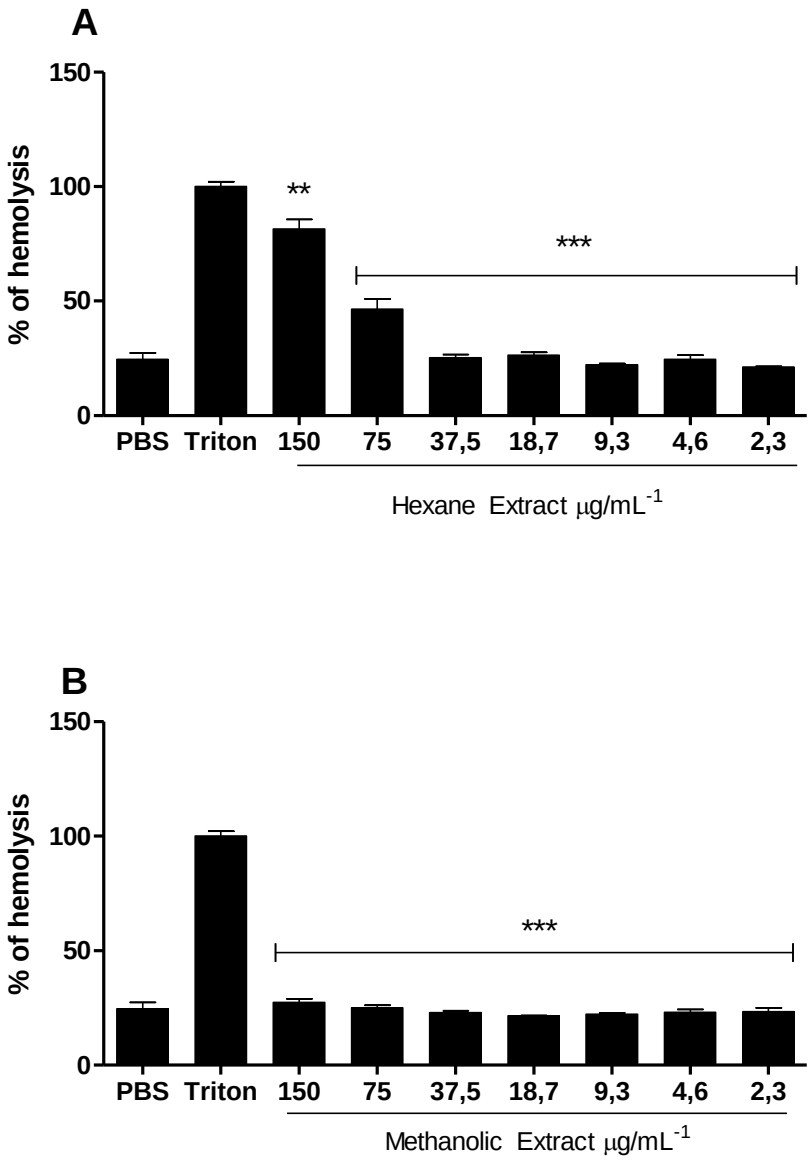

Figure 1: Determination of hemolysis treated with the hexane extract of *Eleutherine plicata (*Herb) (A) and methanolic extract (B). Results were expressed as percentage of hemolysis (mean±SEM). *P<0.05 when compared to the untreated control group (ANOVA followed by the Bonferroni test).

3.4 Evaluation of the cytotoxic activity of hexane and methanolic extracts of *Eleutherine plicata (*Herb)

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The cytotoxic effect of hexane and methanolic extracts was performed using EAT cells, in concentrations of 512, 256, 128,64, 32, 16, 8, 4, 2 and 1 µg/mL^{-1} ;incubated by 24 and 48 hours.

It was observed that the cell viability after 24 hours in the concentrations of 512 µg/mL^{-1} (35,73 ± 11,70%), 256 µg/mL^{-1} (39,91 ± 5,41%) and 4 µg/mL (36,81 ± 9,58%) were lower than the control. After 48 hours incubation with hexane extract, it is observed that in the concentrations of 512 µg/mL^{-1} (35,09 ± 0,74%), 256 µg/mL^{-1} (67,30 ± 9,42) and 128 µg/mL^{-1} (69,49 ± 2,05%) viability was lower compared to control (Figure 2, 3).

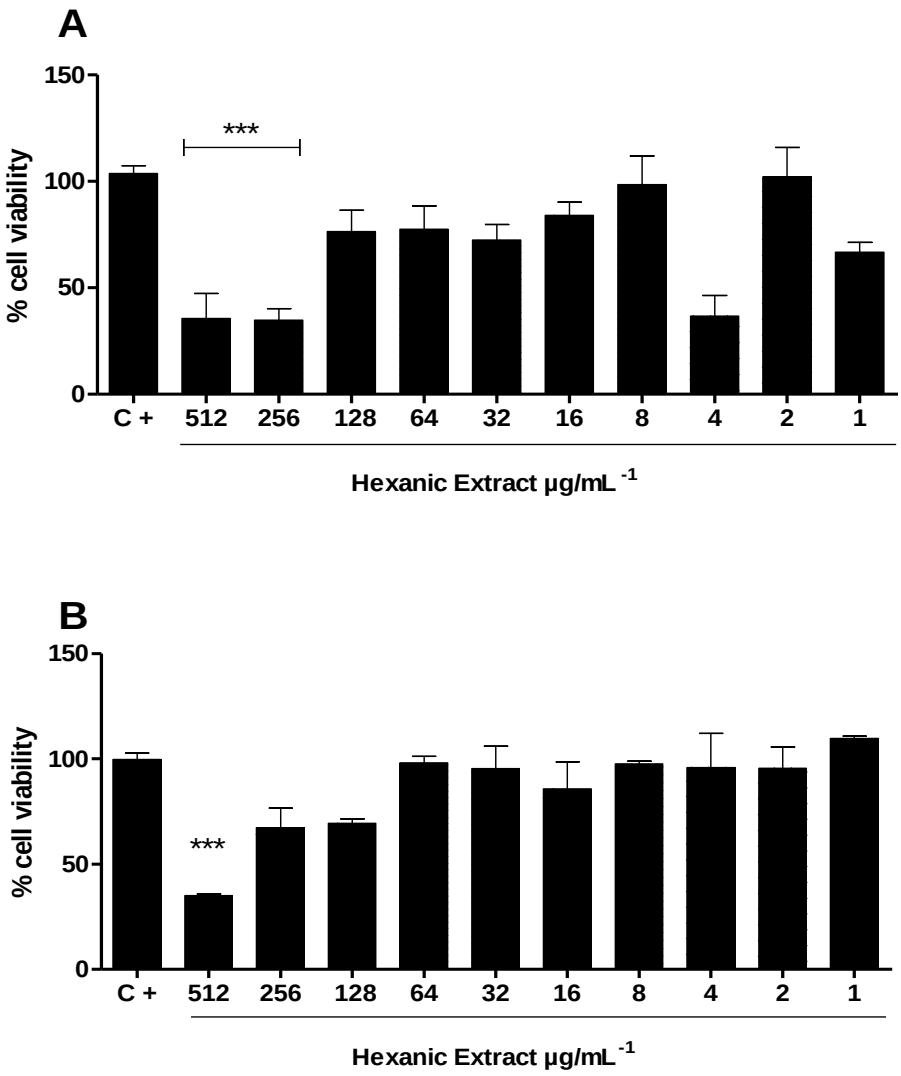

Figure 2 - Determination of cell viability treated with the hexane extract of *Eleutherine plicata (*Herb) for 24h (A) and 48h (B). Results were expressed as percentage of viable cells (mean±SEM). *P<0.05 when compared to the untreated control group (ANOVA

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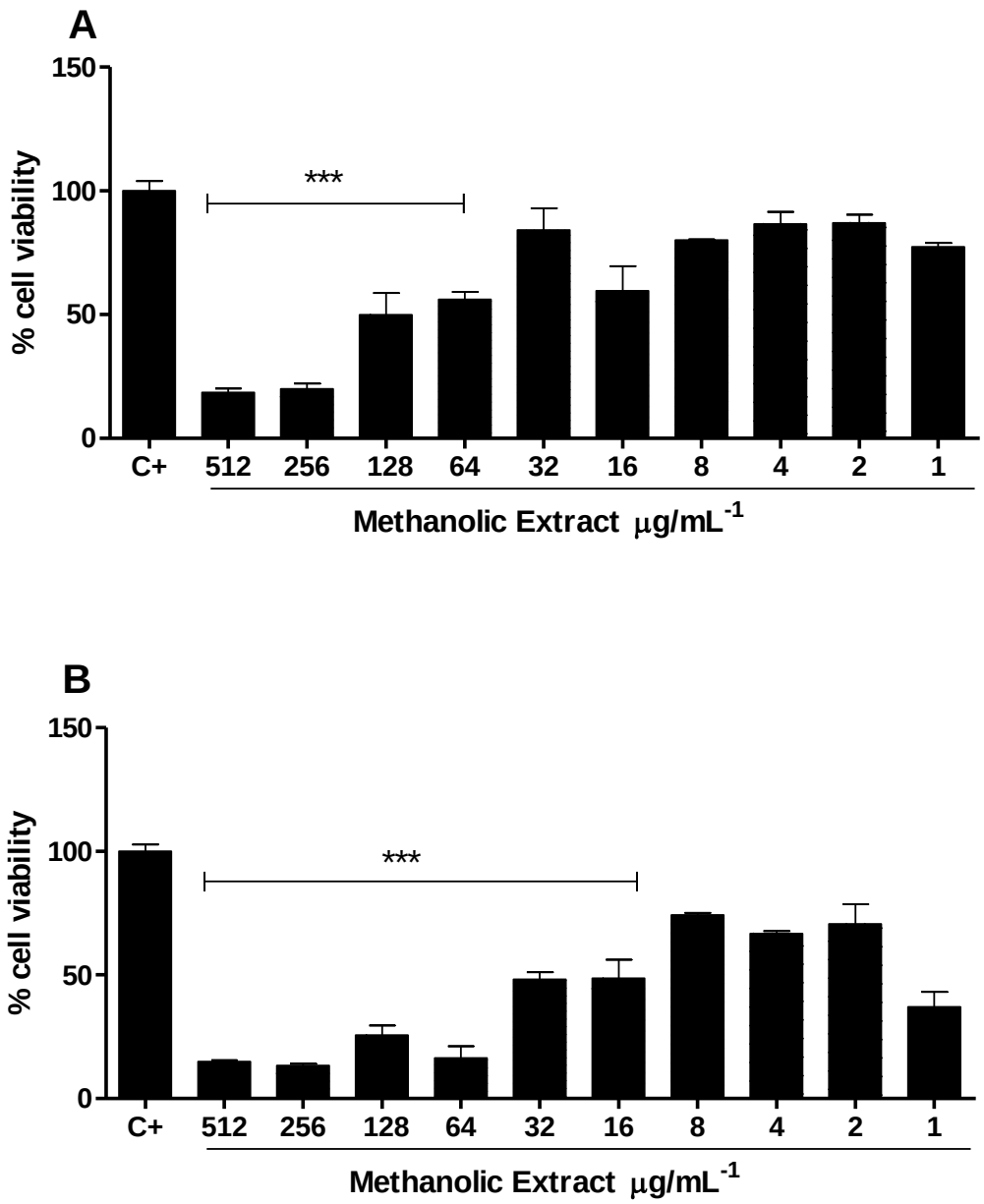

Figure 3 - Determination of cell viability treated with the methanolic extract of *Eleutherine plicata* (Herb) for 24h (A) and 48h (B). Results were expressed as percentage of viable cells (mean±SEM). *P<0.05 when compared to the untreated control group (ANOVA followed by the Bonferroni test).

Figure 3 shows the cell viability as a percentage (%) of the methanolic extract at 24 and 48 hours. The cell viability of the methanolic extract in the 24-hour period was lower in the concentrations of 16 (59,59 ± 9,98) , 64 (56,16 ± 2,97%) ,128 (57,72 ± 6,78%), 256 (19,99 ± 2,23%) and 512 µg/mL^{-1} (18,58 ± 1,64%). However, in the 48-hour period it is observed that in the concentrations of 1 (37,11 ± 6,08%), 16 (46,67 ± 7,54%), 64 (16,44 ± 4,79%), 128 (25,71 ± 3,87%) and 512 µg/mL^{-1} (14,91 ± 0,58%) cell viability was lower than control.

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3.5 Evaluation of the anti-inflammatory effect of hexane and methanolic extract *Eleutherine plicata (*Herb)

For the evaluation of the anti-inflammatory effect of *E. plicata (*Herb) hexanic and methanolic extracts of the plant were used in the following concentrations: 1, 5 and 15mg/kg^{-1} (Figure 4).

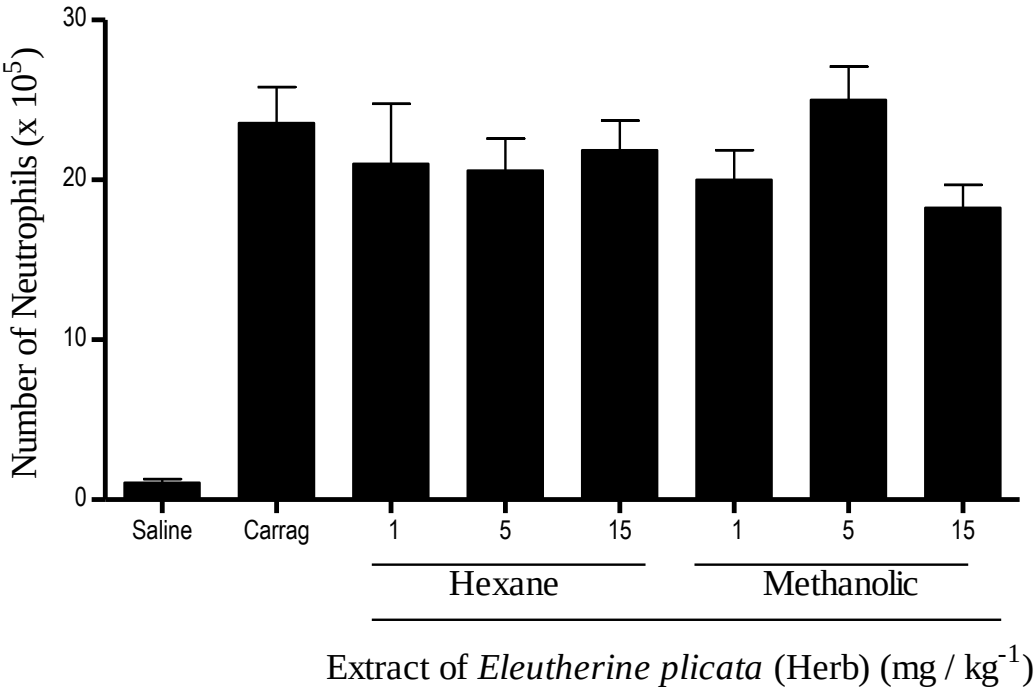

Figure 4 – Evaluation of the effect of hexanic and methanolic extract of *Eleutherine plicata (*Herb) on the migration of neutrophils into the peritoneal cavity of mice. Results were expressed as number of neutrophils (x10^5/mL) (mean ± SEM).

The anti-inflammatory effect of extracts of *E. plicata* in the concentrations evaluated did not show differences in neutrophil migration when compared to the positive control, therefore the Murapazinho plant (popular name) in both extracts did not demonstrate anti-inflammatory potential, in the tested concentrations.

4. DISCUSSION

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The use of medicinal plants in treatment of illnesses has followed the mankind through the ages because it is part of population culture. For several generations, the population of each region had the unique way of treating their pathologies using medicinal plants through popular wisdom. Thus, the use of plants has become a traditional practice of care commonly used by a significant part of the population for therapeutic purposes [22].

Plants have the ability to biosynthesize some substances, such as secondary metabolites, which function is: protection against predators, volatile attractors and supply of substance for plant pigmentation. Secondary metabolites present in plants are classified into: alkaloids, terpenes and flavonoids [23]. Biological activities with potential for the inflammatory process and in the anti-inflammatory activity of flavonoids are also described, this metabolite is found in plants and presents a high potential to act on both inflammation and immune system [24].

The species *E. plicata* presents indication of popular wisdom for illnesses related to stomach pain, colic, diarrhea and amoebiasis [25]. It is used in popular medicine in almost the whole country (Brazil), with great prominence in Amazon rainforest region, a habit initiated by indigenous populations in this region [9].

Studies carried out with the species *E. plicata (*Herb) the main groups of organic compounds were identified with the presence of: alkaloids, anthraquinones, flavonoids, foamed saponin, tannins, organic acids, reducing sugars, azulenes, carotenoids, catechins, depsides and depsidones, benzoquin derivatives, coumarin derivatives, steroids and triterpenoids , cardiac glycosides, lactones, polysaccharides, proteins and amino acids and purines [26]. When carrying out phytochemical studies with *E. plicata*, hexane and chloroform fractions showed as main chemical components naphthoquinones, anthraquinones, steroids and triperpenes, these data proving the presence of such metabolites through pharmacognostic studies [27]. Tannins and saponins are substances known for some of their outstanding characteristics, such as the astringency and anti-nutritional factor of tannins and the emulsifying function of saponins. However, the characteristics of common knowledge are few in face of the diversity of mechanisms of action and possible uses that are still unknown [28]. In phytochemical studies, the presence of tannins was observed in the ethanolic extract of *E. plicata (*Herb) [29].

The presence of flavonoid glycosides through sodium hydroxide reactions and reaction with antimony chloride may be related to antitumor activity, since flavonoids are responsible for inhibiting anti-inflammatory cells [30]. The results of analysis of reactions indicative of presence or absence of flavonoids in *E. plicata (*Herb) showed that species showed positive results for alkaloids.

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Anthraquinones are often orange compounds and can be observed *in situ*. They can be present as drugs, either in free forms (aglycone or genins) or linked to glycids (in the form of anthraquinone glycosides) [31]. Corroborating with this data; the bulb *E. plicata* showed a positive result for the presence of anthraquinone glycosides in reduced form.

The literature is scarce regarding anti-inflammatory studies of the species *E. plicata (*Herb) however, there are reports of antioxidant and antimicrobial action, as well as several studies for the genus *Eleutherine.*

There are records of antibiotic sensitivity tests with extract extracted from five solvents (ethyl acetate, chloroform, butanol, ethanol and water) from the *E. bulbosa* (Merr) evaluating antimicrobial activity against 16 types of human pathogenic bacteria. It was identified that butanol presented the best result with a minimum inhibitory concentration (MIC) from 46 mg/mL^{-1} to 187 mg/mL^{-1}, butanol and aqueous extracts showed higher rates in their zone of inhibition compared to the antibiotics gentamicin and ciprofloxacin used in the study. The authors also carried out toxicological screening with the species *Pseudomonas fluorescens* in effective concentrations, having its greatest inhibition for the butanol extract of 22.0 mm and approximately one standard deviation of 2.6 mm [10].

Study carried out with ethanolic extract of *E. plicata (*Herb), tested in human liver cancer cell line (HepG2), there was a direct correlation between concentration and cytotoxicity. While the relationship between the exposure time has been described that as longer as the time, as lower as the cytotoxicity was. This fact suggested that the event involved in cytotoxicity could be reversed over time [31].

It was observed that fractions show antimicrobial activity only using methanolic extracts in concentrations of 1024 and 512 $\mu g/mL^{-1}$ with halo values of 11 and 10 mm, respectively, but only in *E.coli* but less effective than the positive control (penicillin) that had a halo of 40 mm.

Studies carried out by Upadhyay et al. through the methanolic extract produced through the plant drug of *E. indica*, used in the evaluation of skin healing in experiments with an animal model using Swiss mice and Wister rats. In the evaluation of the study it was identified that the methanolic extract of *E. indica* showed an accelerated wound healing activity, as evidenced by the rapid rate of wound contraction and higher hydroxyproline content in the granulation tissue [32].

However, in the neutrophil migration test in mice; both with hexane and methanolic extract; no anti-inflammatory activity was observed at the tested concentrations. The peritonitis

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test was performed, due to the presence of flavonoids which indicate the possible presence of anti-inflammatory action. However, no effective results have been presented that evidence such an effect

According to studies carried out the species *E. plicata* and also registered with National List of Medicinal Plants of Interest to SUS (RENISUS); through popular use, it is indicated for the treatment of diarrhea [33,34]. However, in the present study, antidiarrheal activity was not evaluated. The species *E. plicata* presents viability for new studies, according to data published through the empirical knowledge of the population, and mainly in the Amazon rainforest region, which still suffers from some precarious health services.

CONCLUSION

The pharmacognostic tests of the *E. plicata (*Herb) identified the presence of secondary metabolites, such as: tannins, flavonoids and anthraquinones, confirmed, through colorimetric reactions the presence of tannins, anthraquinone glycosides and flavonoid glycosides, which demonstrate their biological potential.

Antimicrobial activity, despite presenting inhibition halos in the methanolic extract, was not significant compared to the control. However, in the hemolytic tests it was identified that the hexanic extract of the bulbs of *Eleutherine plicata*, did not present hemolysis in low concentrations.

The cytotoxic assays for both extracts showed a reduction in viability quite evident in some concentrations. Since the trial was conducted with tumor cells, it arouses interest in an investigation in this line of research. It is worth mentioning that the species is used empirically by the population of the Amazon rainforest as an anti-tumor in the treatment of cancer, emphasizing even more studies in this line of research, seeking to identify the mechanisms of action involved in the process. On the other hand, the anti-inflammatory test of both extracts did not present satisfactory results in any concentration tested.

In general, based on the results of this article/paper, the interest and continuity of the research will be relevant in line with the mechanisms involved in the antitumor activity presented by both extracts.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

Ethical statement Animals assays were approved by the Animal Ethics Committees of the Universidade Católica Dom Bosco, Brazil (AECs/UCDB), number 030/2017.

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