ORIGINAL ARTICLE

EVALUATION OF THE ACUTE TOXICITY, ANTITUMOR AND ACUTE INFLAMMATION INHIBITORY EFFECTS OF *DRIMYS BRASILIENSIS* (WINTERACEAE)

Mariana Cardoso Oshiro[1]; Alícia de Souza Santos[2]
Alisson Macário de Oliveira[3]; Emanuelle Maria da Silva[4]; Helimarcos Nunes Pereira[5]
Jhonatta Alexandre Brito Dias[6]; Marcela Albuquerque de Oliveira[7]
Marília Gabriela Muniz Arruda[8]; Marcilene Souza da Silva[9]
Simone da Paz Leôncio Alves[10]; Ivone Antônia de Souza[11]

Highlights: (1) *D. brasiliensis* showed anti-inflammatory and antitumor activity in M. musculus mammals. (2) The leaf ethanolic extract of *D. brasiliensis* showed a high content of phenolic compounds. (3) *D. brasiliensis* presented low toxicity, reinforcing the consumption through traditional use.

PRE-PROOF
(as accepted)
This is a preliminary, unedited version of a manuscript that was accepted for publication in the Revista Contexto & Saúde. As a service to our readers, we are making this initial version of the manuscript available, as accepted. The article will still be reviewed, formatted and approved by the authors before being published in its final form.

http://dx.doi.org/10.21527/2176-7114.2024.48.14659

How to cite:
Oshiro MC, Santos A de S, de Oliveira AM, da Silva EM, Pereira HN, Dias JAB. et al. Evaluation of the acute toxicity, antitumor and acute inflammation inhibitory effects of *drimys brasiliensis* (winteraceae). Rev. Contexto & Saúde, 2024;24(48): e14659

[1] Federal University of Pernambuco. Recife/PE, Brazil. https://orcid.org/0000-0003-4972-7844
[2] Federal University of Pernambuco. Recife/PE, Brazil. https://orcid.org/0009-0007-3220-2861
[3] Federal University of Pernambuco. Recife/PE, Brazil. https://orcid.org/0000-0003-4152-150X
[4] Federal University of Pernambuco. Recife/PE, Brazil. https://orcid.org/0000-0003-0781-6558
[5] Federal University of Pernambuco. Recife/PE, Brazil. https://orcid.org/0000-0002-1376-7660
[6] Federal University of Pernambuco. Recife/PE, Brazil. https://orcid.org/0000-0003-4726-9990
[7] Federal University of Pernambuco. Recife/PE, Brazil. https://orcid.org/0009-0008-0148-2191
[8] Federal University of Pernambuco. Recife/PE, Brazil. https://orcid.org/0000-0003-3151-7953
[9] Federal University of Pernambuco. Recife/PE, Brazil. https://orcid.org/0000-0002-9332-3911
[10] Federal University of Pernambuco. Recife/PE, Brazil https://orcid.org/0000-0002-4487-3044
[11] Federal University of Pernambuco. Recife/PE, Brazil https://orcid.org/0000-0002-9330-4593

ABSTRACT

The genus *Drimys*, belonging to the Winteraceae botanical family, is widely recognized and valued. Its species have been used in folk medicine to treat various health problems, such as gastric pain, toothache and anemia. In certain localities, the dried leaves and berries of the fruit are used as condiments due to their spicy flavor. This study aims to evaluate the phytochemical profile, acute toxicity, anti-inflammatory and antitumor activities of the ethanolic leaf extract of *Drimys brasiliensis*. In this study, we conducted an analysis of the phytochemical profile and quantification of phenolic and flavonoids compounds. We evaluated the acute toxicity administering a dose of 2000 $mg.kg^{-1}$ in mice. Futhermore, we investigated the anti-inflammatory activity using experimental models of paw edema, peritonitis and air pouch, and carried out an antitumor test using the 180-sarcoma model. Chromatographic analysis revealed the presence of anthocyanidins, phenolic compounds, coumarins, anthracene derivatives, terpenes, naphthoquinones, saponins and triterpenes. The extract showed a large amount of phenolic and flavonoids compounds. We also observed low toxicity in rodents, with an LD50 greater than 2000 $mg.kg^{-1}$. Additionally, we verified that the extract showed inhibition of leukocytes and neutrophils at the tested doses of 50, 100 and 200 $mg.kg^{-1}$. In the paw edema model, the concentrations of 100 and 200 $mg.kg^{-1}$ were statistically different ($p<0.05$). In the 180-sarcoma model, the dose of 300 $mg.kg^{-1}$ resulted in tumor inhibition of 64.33%. In view of these promising results, further studies are needed to better understand the bioavailability and pharmacokinetics of the plant extract.

Keywords: Phytotherapy. Toxicity. Anti-inflammatory Agents; Antineoplastic.

INTRODUCTION

Inflammation plays a fundamental role in the tumor microenvironment and is considered one of the main contributing factors to carcinogenesis[1]. The imbalance in the oxide-reduction process caused by the excess of free radicals, such as reactive oxygen species (ROS), nitrogen (RNS) and chlorine (RCl), favors the transformation of normal cells into tumors[2]. The activation of the immune response and the production of cytokines, interleukins and growth factors modulate cell signaling promoting tumor growth[3]. In addition, tumor cells can appropriate pathways and mechanisms of the inflammatory process to drive their proliferation[4].

Recent studies have revealed the presence of bacteria and fungi colonizing tumor cells,

adding another element to the complex tumor microenvironment[5]. The combination of infection and inflammation plays a role in the development of certain types of cancer, such as gastric cancer associated with chronic *Helicobacter pylori* infection and hepatocellular carcinoma (HCC) related to chronic hepatovirus infection[6].

Innovative therapeutic approaches have emerged as alternatives to invasive and non-selective treatments. Therapeutic strategies that not only target cancer cells, but also influence the tumor microenvironment, could modulate the immune response, and interact with the tumor microbiota[7]. Among these approaches, non-steroidal anti-inflammatory drugs (NSAIDs) have been considered potential candidates in the prevention and treatment of cancer, since some have antitumor activity, as well as a potent anti-inflammatory effect[8]. Medicinal plants also show similar potential, as many species have antitumor and anti-inflammatory activities[9], including some species popularly used in the treatment of cancer[10,11].

Drimys brasiliensis is a tree species belonging to the Winteraceae family and native to Brazil. This plant is found in high-altitude and riparian forests, in marshy and well-drained soils, and has been recorded from the Northeast (Bahia) to the South of the country (Rio Grande do Sul)[12,13]. Popularly known as "cataia", "casca de anta", "canela-amarga", "para-tudo" and "caá-tuya", there are reports that indigenous ethnic groups observed the tapir (*Tapirus americanus*) feeding on the bark of this species when sick, justifying the popular name given to the plant[14].

In folk medicine, reports indicate the use of D. brasiliensis leaves and bark to treat gastric problems such as intestinal pain, colic, constipation, among others. In addition, the plant is antiscorbutic, sudorific and a stimulant against physical and mental exhaustion[15].

In this context, this study aims to evaluate the phytochemical profile, acute toxicity, anti-inflammatory and antitumor activities of the ethanolic extract leaf of *D. brasiliensis* in *Swiss* albino mice (*Mus musculus*).

METHOD

Collection and Identification of Plant Material

The plant material was collected in the city of Curitiba-PR, under the geographical coordinates 25°26'56.7"S 49°14'19.1"W, in June 2018, during the morning day. The plant material was identified by the Forest Engineer Inti de Souza. Two exsiccates were made and deposited in the Herbarium Escola de Florestas Curitiba - EFC under the number 17757 and in the Herbarium of Biological Sciences of the Department of Biological Sciences of UFPE under

number 85343. It was registered with the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN) under registration number: ACE2610.

Extraction and yield

A sample of 100g of dried *D. brasiliensis* leaves was dynamically macerated at room temperature, approximately 27ºC, for a period of 12 hours, renewing the solvent. The solvent used for the extraction was ethyl alcohol, in a ratio of 1:9 (w/v). The resulting extract underwent a rotaevaporation process until the solvent was completely removed, resulting in a dry extract. To determine the yield of the process, the dry weight of the extract obtained was divided by the weight of the dry leaf sample initially used, and the result was expressed as a percentage.

Phytochemical Prospecting and Quantitative Analysis of Polyphenols and Flavonoids

Phytochemical prospecting of the *D. brasiliensis* extract was carried out to identify the main groups of secondary metabolites present. For this procedure was used the chromatographic method described by Wagner and Bladt (1996)[16].

In addition, the *D. brasiliensis* extract was subjected to the quantification of total phenolic and flavonoids compounds, following the methodologies established by Singleton (1999)[17]. The absorbance was measured in a spectrophotometer (SHIMADZU model), using a wavelength of 415 nm. The absorbance values obtained were compared with the calibration curves constructed from the quercetin and gallic acid standards.

Animals and ethical aspects

In this study, male and female *Swiss* albino mice were used aged between 8 and 12 weeks and weighing on average 25-35 g. All the experimental procedures are in accordance with Brazilian laws on animal experimentation and were submitted to the Ethics Committee on Animal Use of the Federal University of Pernambuco and received a favorable opinion in accordance with CEUA/UFPE letter 55/21.

Acute Toxicity Assessment

The acute toxicity test on mice was conducted in accordance with OECD TG 423 (2002)[18]. For this study, nine nulliparous female Swiss albino mice of the species M. *musculus* at 8 weeks of age were randomly distributed into three groups of three: a control group, a group

treated at a dose of 2000 mg.kg^{-1} and a repeat group at the same dose of 2000 mg.kg−1. The control group was given distilled water orally at a rate of 1 ml for every 100g of body weight. The treated group was given the leaf ethanolic extract of *D. brasiliensis* orally at a dose of 2000 mg.kg-1. The animals were fasted for 4 hours before and 2 hours after administration of the extract or vehicle, although they had free access to water. After administration of the extract, the animals were observed individually for any signs of toxicity immediately after 30 minutes until the first 4 hours of the study. In addition, periodic observation was carried out over a period of 14 days to check for the occurrence of mortality and signs of toxicity in the animals. Throughout the 14-day test period, the animals' body weight was recorded, as well as their water and food consumption. At the end of the test, the animals were euthanized by cervical dislocation and had their livers, spleens, kidneys and lungs removed for macroscopic evaluations and to determine their relative weights. In addition, blood samples were taken from the animals to assess hematological parameters (red blood cells, hemoglobin, hematocrit, VCM, HCM, CHCM, leukocytes, segmented leukocytes, eosinophils, typical lymphocytes, monocytes and platelets) and biochemical parameters (urea, creatinine, TGO and TGP).

Evaluation of anti-inflammatory activity

Carrageenan-induced paw edema

For this study, eight male mice were selected in each experimental group, including groups treated with different doses of leaf ethanolic extract of *D. brasiliensis* (50, 100 and 200 mg.kg^{-1}, orally), a negative control group (0.9% saline solution) and a positive control group (dexamethasone 0.5 mg.kg^{-1}). Before the start of treatment (time 0h), the basal volume of the right hind paw of all the animals was measured using a hydroplethysmometer (Ugo Basile model). After administration of the treatments, paw edema was induced by intraplantar injection of 100 µL of 1% (w/v) carrageenan, and the volume of the hind paw was measured at intervals of 30, 60, 120 and 180 minutes after edema induction. The results obtained were presented as the variation in paw volume (Δ mL), according to Ferreira (1979)[19]. The results obtained were compared and statistically analyzed using analysis of variance (ANOVA) followed by the Tukey test, where values of $p < 0.05$ were considered statistically significant.

Peritonitis

For this trial, six male mice were selected in each experimental group, including groups treated with different doses of the leaf ethanolic extract of *D. brasiliensis* (50, 100 and 200

mg.kg^{-1}, orally), a negative control group (0.9% saline solution, orally) and a positive control group (indomethacin 10 mg.kg^{-1}, orally). After administering the treatments, the animals received an intraperitoneal injection of 1% (w/v) carrageenan to induce the inflammatory process. After 4 hours, the animals were sacrificed and blood was collected from the peritoneal cavity using 3 mL of heparinized phosphate buffered saline (PBS). The leukocyte count was carried out using a hematology analyzer (model ABX Micros 60®). The results were expressed as the mean numbers of total leukocytes and neutrophils (105 mL-1) for each experimental group and the inhibition of leukocyte and neutrophil migration compared to the control experimental group expressed as a percentage, according to the methodology of Oliveira (2016)[20]. The results obtained were compared and statistically analyzed using analysis of variance (ANOVA) followed by Bonferroni, where p-values < 0.05 were considered statistically significant.

Air pouch

In this trial, six male mice were selected per group, including the groups treated with the leaf ethanolic extract of *D. brasiliensis* (50, 100 and 200 mg.kg^{-1}, orally), the negative control group (0.9% saline solution, orally) and the positive control group (indomethacin 10 mg.kg^{-1}, orally). Each group of animals received a subcutaneous injection of 3 mL of sterile air in the back on the first and fifth day of the experiment. On the seventh day after the start of the experiment, 1% (w/v) carrageenan was injected into the air pouch of each animal. One hour after the administration of carrageenan, the animals received their respective treatments of the leaf ethanolic extract of *D. brasiliensis*, indomethacin or saline solution. After 6 hours of treatment, the animals were euthanized, then the exudates from the air pouch were collected using 3 mL of heparinized phosphate buffered saline (PBS). The leukocyte count was carried out using a hematology analyzer (model ABX Micros 60®). The results were expressed as the average number of total leukocytes (105 mL^{-1}) for each experimental group and the inhibition of leukocyte migration compared to the control experimental group expressed as a percentage, according to Cavalcante da Silva (2021)[21]. The results obtained were compared and statistically analyzed using analysis of variance (ANOVA) followed by Bonferroni, where p-values < 0.05 were considered statistically significant.

Evaluation of Antitumor Activity

In this study, six male mice were selected per group, including the groups treated with the leaf ethanolic extract of *D. brasiliensis* (200, 300 and 400 $mg.kg^{-1}$, orally), the negative control group (0.9% saline solution, orally) and the positive control group (methotrexate 10 $mg.kg^{-1}$, orally), according to the methodology of Stock (1955)[22]. The animals in the control and treated groups received 180-sarcoma cells by subcutaneous ascitic inoculation of the tumor in the axillary region. The treatments with the doses of *D. brasiliensis* extract and the positive and negative controls were started 48 hours after transplanting the tumors into the animals and lasted seven days. At the end of this period, the animals were sacrificed, the tumors removed, dried and weighed. Blood samples were taken by cardiac puncture to assess biochemical and hematological parameters. The animals were euthanized by cervical dislocation and had the tumors, liver, spleen, kidneys and lungs removed for macroscopic evaluations as well as to determine their respective relative weights. Tumor inhibition was calculated using the following equation (Eq. 1): TWI% = (C - T) / C x 100, where TWI% represents the percentage of tumor inhibition, C is the average weight of the tumors in the animals in the control group, and T is the average weight of the tumors in the animals in the test group. The results obtained were compared and statistically analyzed using analysis of variance (ANOVA) followed by the Tukey test, where values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Extraction and Yield

The yield of the leaf ethanolic extract obtained from *D. brasiliensis* was determined to be 10.04%. The obtention of high-quality and stable plant extract depends significantly on the extraction and drying methods used. In this context, cold maceration is an advantageous method, as it helps to preserve the stability of the secondary metabolites present in the extract. The ambient temperature used in this method prevent thermal degradation, as well as reducing the oxidation and loss of activity of the compounds, thus guaranteeing the preservation of their bioactive properties[23].

Phytochemical Prospecting and Quantitative Analysis of Polyphenols and Flavonoids

The phytochemical analysis carried out on the *D. brasiliensis* extract showed the presence of several classes of secondary metabolites, including anthocyanidins, phenolic compounds, coumarins, anthracene derivatives, mono/sesqui/diterpenes, naphthoquinones,

saponins and triterpenes. These results are consistent with previous studies investigating species of the Drimys genus, strengthening the characterization of the chemical composition of the leaf ethanolic extract of *D. brasiliensis*, as described by Mecchi and Lago (2013)*24*.

In the polyphenol and flavonoid quantification test, the *D. brasiliensis* species had a total phenol content of 52.30% and flavonoids of 3.55%. This result is in line with the findings of Bridi (2019)25, who also found high levels of phenolic acids and flavonoids in the Drimys winteri species. The presence of these substances suggests a significant oxidant-reducing potential, as demonstrated by Gastaldi (2018)26 in his study with the Drimys andina species. In addition, the presence of these compounds may be related to anti-inflammatory activity, as evidenced by Maleki, Crespo and Cabanillas (2019)27, which corroborates the findings found in this publication for *D. brasiliensis*.

Toxicity

The evaluation of the acute toxicity of the *D. brasiliensis* extract demonstrated its safety and non-lethality. The calculation of the lethal dose (LD_{50}) was determined using fixed cut-off values according to the OECD 423 methodology. The LD_{50} of the *D. brasiliensis* leaf ethanolic extract was established as higher than the dose of 2000 $mg.kg^{-1}$.

In the first 30 minutes of observation, the animals in the control group showed hyperactivity and in the 60 minutes of observation the animals in the control group no longer showed any effect indicating CNS stimulation. On the other hand, the animals in the group treated with the leaf ethanolic extract of *D. brasiliensis* showed signs of vibrissae movement, tail curling and piloerection in the first 30 minutes. After 60 minutes of observation, the animals showed expansion of the ear pinna, tail curling and piloerection, but these effects were not intensified. In relation to the CNS depressant signs (analgesia, catatonia, photophobia, gait reversal, loss of ear reflex, loss of corneal reflex, prostration, eyelid ptosis, decreased response to touch and sedation), no signs of decreased brain activity were observed in the animals in the control group or in the animals in the group treated with the leaf ethanolic extract of *D. brasiliensis* at a dose of 2000 $mg.kg^{-1}$. In the assessment of ANS related signs, the animals in the group treated with the leaf ethanolic extract of *D. brasiliensis* at a dose of 2000 $mg.kg^{-1}$ showed defecation within the first 30 minutes of observation. The animals in the control group, treated with distilled water, showed no signs of ANS alteration. In the first 30 minutes of observation, other behaviors such as self-cleaning, climbing, stereotyped movements and abdominal contortions were observed in the animals treated with the leaf ethanolic extract of

D. brasiliensis at a dose of 2000 mg.kg^{-1}. In the first hour of observation, the animals in the group treated with the leaf ethanolic extract of *D. brasiliensis* at a dose of 2000 mg.kg^{-1} showed behaviors such as self-cleaning and climbing. Other behaviors were not observed in the animals treated with distilled water. During the other observation periods over the 14 days of the test, no signs of toxicity were observed in the animals. In addition, no mortality was recorded during the test period. The animal's consumption of water and food was monitored and no statistically significant differences were observed between the mean consumption of the animals treated with distilled water and the animals treated with *D. brasiliensis*, according to Table 1.

Table 1- Results of food and water consumption of the animals treated with distilled water and treated with the leaf ethanolic extract of *D. brasiliensis*.

Parameters evaluated	Distilled water (n=3)	D. brasiliensis (n=3)	D. brasiliensis* (n=3)
Water consumption (mL)	24.2±3.98	29.0±6.17	19.0±2.67
Food consumption (g)	12.3±1.61	13.6±1.2	13.1±1.2

Source: The author (2023). Legend: * repetition group that received a dose of 2000 mg.kg-^{1} leaf ethanolic extract of D. brasiliensis. Note: The values represent the mean ± standard deviation. No statistically significant differences ($p < 0.05$) were found between the groups.

The use of the leaf ethanolic extract of *D. brasiliensis* proved to be safe when administered orally. During the other observation periods over the 14 days of the test, no signs of toxicity were observed in the animals. In addition, no mortality was recorded during the test period. No changes were observed in blood parameters and showing no liver or kidney alterations, as shown in Table 2.

Table 2 - Hematological and biochemical parameters of mice in the group treated with distilled water and the group treated with leaf ethanolic extract of *D. brasiliensis*.

	Treatments	
Hematological parameters	Distilled water ± SD (n=3)	*D. brasiliensis* ± SD (n=6)
Red blood cells (million/µL)	5.24 ± 0.3470	5.01 ± 0.3089
Hemoglobin (g/dL)	15.86 ± 0.9843	15.06 ± 0.9498
Hematocrit (%)	47.66 ± 2.8674	45.33 ± 2.9249
VCM (fL)	94.43 ±0.7133	94.15 ± 1.3877
HCM (pg)	30.13 ± 0.0471	30.18 ± 0.0687
CHCM (%)	33.20 ± 0.0816	33.25 ± 0.1384
Leukocytes (cells/mm³)	8033.33 ± 758.6537	7350.00 ± 588.0759
Segmented (%)	45.33 ± 3.6817	44.16 ± 2.2669
Eosinophils (%)	1.66 ± 0.9428	1.66 ± 0.7453
Typical Lymphocytes (%)	51.66 ± 2.6246	52.5 ± 1.7078
Monocytes (%)	1.33 ± 0.4714	1.66 ± 0.7453
Platelets (cells/mm³)	167333 ± 5312.4592	176166 ± 9263.1288
Biochemical parameters		
Urea (mg/dL)	41.00 ± 1.2961	43.46 ± 2.3633
Creatinine (mg/dL)	0.64 ± 0.0748	0.62 ± 0.0689
TGO (U/L)	60.16 ± 2.3098	57.65 ± 6.0794
TGP (U/L)	124 ± 4.3909	121.86 ± 8.9878

Source: The author (2023). LEGEND: SD = standard deviation; "AST" - Aspartate Aminotransferase; "ALT" - Alanine Aminotransferase; "MCV" - Mean Corpuscular Volume; "MCH" - Mean Corpuscular Hemoglobin; and "MCHC" - Mean Corpuscular Hemoglobin Concentration. Note: No statistically significant differences were found ($p < 0.05$) compared to the control.

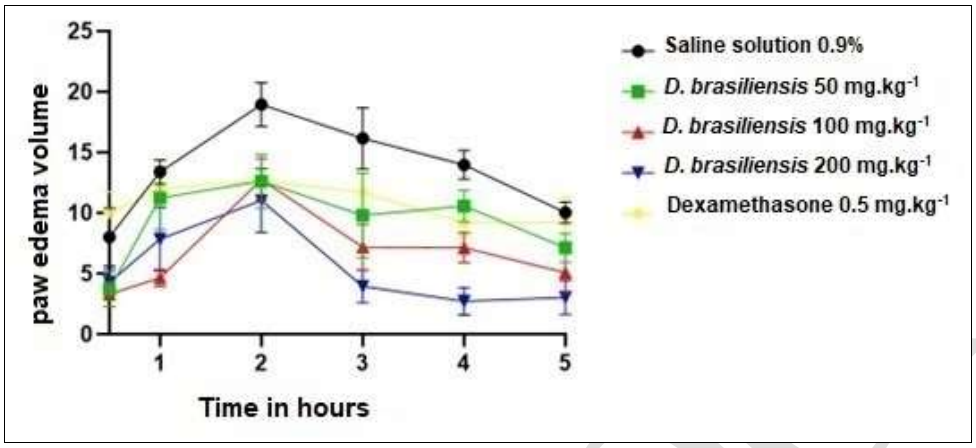
This result is in line with previous studies investigating the leaf ethanolic extract of the *D. angustifolia* species, as mentioned by Witaicenis (2007)[28]. In addition, another study investigated the effect of the *D. brasiliensis* bark extract and showed that it has no teratogenic effect, as well as no hemolytic activity, as evidenced by Fratoni (2018)[29]. However, it is important to note that the essential oil of *D. brasiliensis* has shown toxicity. As reported by Gomes (2013)[30], the dose of 1000 $mg.kg^{-1}$ administered to rodents resulted in signs of toxicity and CNS excitation, indicating that the internal use of the essential oil is not recommended. These findings provide information on the safety of consuming the leaves and bark of the plant, whether in the form of teas or tinctures, corroborating the traditional use of the species[31].

Evaluation of Anti-inflammatory Activity

In the paw edema model, the administration of carrageenan induced an acute and localized inflammatory response in the animal's paw, characterized by the sequential release of

inflammatory mediators in different phases. Graph 1 shows the variation in the volume of paw edema induced by carrageenan over a 5-hour period.

Graph 1 - Variation in paw volume of mice (M. musculus) in the paw edema model.



Source: The author (2023). Note: The concentrations of 100 and 200 mg.kg-1 of D. brasilienses were statistically different ($p<0.05$); ANOVA followed by Tukey test.

At 30 minutes after induction with carrageenan, the leaf ethanolic extract of *D. brasiliensis* at doses of 50, 100 and 200 mg.kg^{-1} were statistically significant in relation to the group treated with 0.9% saline solution. At 1 hour after induction with carrageenan, the leaf ethanolic extract of *D. brasiliensis* at doses of 100 and 200 mg.kg-1 were statistically significant in relation to the group treated with 0.9% saline solution. According to the graph, the maximum peak of inflammation was reached 2 hours after induction with carrageenan, and it was observed that the leaf ethanolic extract of *D. brasiliensis* at doses of 50, 100 and 200 mg.kg^{-1} and the group treated with dexamethasone 0.5 mg.kg^{-1} were statistically significant when compared to the group treated with 0.9% saline solution.

This same result was also observed in the third and fourth hours of observation after induction with carrageenan. In the last hour of observation, it was observed that the leaf ethanolic extract of *D. brasiliensis* at doses of 50, 100 and 200 mg.kg^{-1} continued to show statistically significant results when compared to the group treated with 0.9% saline solution. During this observation period, the animals in the group treated with dexamethasone 0.5 mg.kg $^{-1}$ no longer showed any variation in paw volume. The animals showed paw volume measurements close to the baseline measurements at the start of the test, demonstrating a recovery from the edema caused by the application of carrageenan. The leaf ethanolic extract of *D. brasiliensis* at doses of 100 and 200 mg.kg-1 was able to reduce the volume of paw edema

in mice induced by carrageenan in all the periods observed when compared to the vehicle 0.9% saline solution. The leaf ethanolic extract of *D. brasiliensis* at doses of 100 and 200 mg.kg-1 showed an anti-inflammatory effect. These results suggest that the *D. brasiliensis* extract has properties capable of modulating the inflammatory response, reducing the release of inflammatory mediators and, consequently, attenuating the development of carrageenan-induced paw edema.

The carrageenan-induced air pouch model was used as an approach to evaluate inflammatory processes like those found in rheumatoid arthritis, since the air pouch induced in the animals' backs resembles the synovial membranes of the connective tissue present in the joints[20].

Oral administration of *D. brasiliensis* extract considerably inhibited leukocyte migration in mice samples at all the doses evaluated, when compared to the negative control. Specifically, the doses of 100 and 200 mg.kg^{-1} of leaf ethanolic extract of *D. brasiliensis* showed a 64.05% and 76.91%, respectively, greater inhibition of leukocyte migration compared to the reference drug indomethacin at 52.50%, as shown in Table 3.

Table 3 - Effect of the leaf ethanolic extract of *D. brasiliensis* on leukocyte migration in acute inflammationin the air pouch model.

Groups	Dose mg.kg^{-1}	Cell migration (10^5 / ml)	Inhibition (%)
Control	-	1.77 ± 0.11	-
Indomethacin	10	6.54 ± 0.15*	52.50
Leaf extract *D. brasiliensis*	50	8.29 ± 0.23*	39.80
	100	4.95 ± 0.17*	64.05
	200	3.18 ± 0.24*	76.91

Source: The author (2023). Legend: Values represent the mean ± standard deviation (n=6).
* Statistically different from the negative control (vehicle); ANOVA followed by Bonferroni, $p<0.05$.

The pharmacological model of peritonitis is used to study the inflammatory response in the peritoneum, a serous membrane that lines the abdominal cavity. Peritonitis is characterized by inflammation of the peritoneum, usually caused by bacterial infection, traumatic injury or other inflammatory conditions. Oral administration of the *D. brasiliensis* demonstrated an effect in inhibiting the migration of leukocyte and neutrophil cells at all doses tested, when compared

to the negative control group. Specifically, the doses of 100 and 200 mg.kg^{-1} of the *D. brasiliensis* showed superior inhibition of the migration of leukocytes (56.38% and 77.05%), respectively, and neutrophils (60.00% and 64.21%), respectively, compared to the reference drug indomethacin for leukocytes (55.25%) and neutrophils (52.89%), as shown in Table 4.

Table 4 - Effect of the leaf ethanolic extract of *D. brasiliensis* on the migration of leukocytes and neutrophilsin acute inflammation in the peritonitis model.

Groups	Dose mg.kg^{-1}	Leukocytes (10^5 / ml)	Leukocyte inhibition (%)	Neutrophil (10^5 / mL)	Neutrophil inhibition (%)
Control	-	6.19 ± 0.20	-	3.80 ± 0.09	-
Indomethacin	10	2.77 ± 0.19*	55.25	1.79 ± 0.11*	52.89
Leaf extract *D. brasiliensis*	50	3.19 ± 0.10*	48.46	2.14 ± 0.10*	43.68
	100	2.70 ±0.14*	56.38	1.52 ± 0.16*	60.00
	200	1.42 ± 0.12*	77.05	1.36 ± 0.13*	64.21

Source: The author (2023). Legend: Values represent the mean ± standard deviation (n=6).
* Statistically different from the negative control (vehicle); ANOVA followed by Bonferroni, $p<0.05$.

The migration of leukocytes to the inflammatory site plays a crucial role in the cellular response during acute inflammation. This process is mediated by various inflammatory cytokines, including histamines, prostaglandins and bradykinins, among others. The inhibition of leukocyte and neutrophil migration may be an indication of the potential anti-inflammatory effect of the *D. brasiliensis*.

The results obtained suggest that the leaf ethanolic extract of *D. brasiliensis* has properties capable of modulating the inflammatory response, resulting in the inhibition of leukocyte and neutrophil migration. This inhibition can be attributed to the presence of bioactive compounds in the extract, which can interfere with the signaling of inflammatory cytokines and thus reduce the inflammatory response. Research by other authors has shown that drimanial sesquiterpenes present in *D. brasiliensis* extract exhibit anti-inflammatory activity[32]. The mechanism of action of this effect is related to a reduction in the activity of pro-inflammatory cytokines and a decrease in the activity of the transcription factor NF-κB, which is responsible for regulating the expression of pro- inflammatory cytokines[32,33]. In addition, it has been observed that D. winteri extract can increase gene expression of the anti-inflammatory cytokine IL-10[34].

These findings reinforce the possibility that the *D. brasiliensis* has therapeutic potential in the context of inflammatory disorders. However, it is important to emphasize that further

studies are needed to deepen the understanding of the mechanisms of action involved and to evaluate the efficacy and safety of the plant extract.

Antitumor activity

The results of this test indicated that the intermediate dose of 300 mg.kg^{-1} of leaf ethanolic extract of *D. brasiliensis* showed tumor inhibition of 64.33%, higher than the other treatments. However, the dose of 400 mg.kg-1, the highest dose tested, showed a decrease in the inhibition rate, suggesting a drop in tumor inhibition, the effect observed was not dose-dependent, as shown in Table 5.

Table 5 - Tumor inhibition of treatments with *D. brasiliensis* and the reference drug methotrexate.

Treatments	*D. brasiliensis* 200 mg.kg^{-1}	*D. brasiliensis* 300 mg.kg^{-1}	*D. brasiliensis* 400 mg.kg^{-1}	Methotrexate 1.5 mg.kg^{-1}
Tumor Inhibition	36.42%	64.33%	16.62%	84.34%

Source: The author (2023).

Macroscopic analysis of the tumors removed from the animals revealed that they were solid, invasive, adherent to the epidermis and vascularized. The weights of the organs in the control groups and the groups treated with the leaf ethanolic extract of *D. brasiliensis* were recorded as absolute values (g) and relative values (%). No statistically significant differences were found in the analysis of variance (ANOVA) of the weights of the organs and tumors between the control groups and those treated with the leaf ethanolic extract of *D. brasiliensis*, as shown in Table 6.

Table 6 - Weight of organs and tumors of animals treated with saline solution, methotrexate and leaf ethanolic extract of *D. brasiliensis*.

		Treatments				
Organs	**Values**	**0.9% Saline solution**	***D. brasiliensis* 200 mg.kg^{-1}**	***D. brasiliensis* 300 mg.kg^{-1}**	***D. brasiliensis* 400 mg.kg^{-1}**	**Methotrexate 1.5 mg.kg^{-1}**
Liver	Absolute (g)	2.75±0.58	2.88±1.58	2.05±0.32	2.03±0.10	3.34±0.35
	Relative (%)	5.80±1.02	6.37±2.53	5.82±0.44	5.74±0.23	6.37±2.53
Spleen	Absolute (g)	0.38±0.13	0.40±0.19	0.25±0.04	0.24±0.05	0.23±0.03
	Relative (%)	0.80±0.25	0.89±0.03	0.71±0.06	0.66±0.13	0.45±0.04
Lungs	Absolute (g)	0.22±0.02	0.20±0.04	0.18±0.02	0.19±0.05	0.27±0.06
	Relative (%)	0.46±0.03	0.47±0.07	0.51±0.07	0.53±0.16	0.55±0.12
Kidney	Absolute (g)	0.51±0.04	0.50±0.10	0.40±0.06	0.36±0.02	0.56±0.06
	Relative (%)	1.09±0.09	1.16±0.13	1.14±0.13	1.02±0.05	1.12±0.15
Tumor	Absolute (g)	2.60±1.26	1.37±0.92	1.0±0.76	1.87±0.86	2.95±1.39
	Relative (%)	5.50±2.64	3.10±1.83	2.89±2.21	5.45±2.85	3.83±1.84

Source: The author (2023). Legend: Values represent the mean ± standard deviation (n=6). Note: Nostatistically significant differences ($p < 0.05$) were found between the groups.

The blood of the animals in the control groups and those treated with the leaf ethanolic extract of *D. brasiliensis* was analyzed and the statistical comparison (ANOVA) of the hematological and biochemical parameters revealed no statistically significant differences between the groups. It can therefore be assumed that ingesting the *D. brasiliensis* did not changed the weight or morphology of the organs of the animals tested, nor did it changed the blood parameters of the animals submitted to the experiment.

Although the results were not positive for oral administration, it is possible that alternating the route of administration could generate more promising results. The mechanism of action of the antitumor activity of *D. brasiliensis* is not yet fully understood. The genus *Drimys*, of which *D. brasiliensis* is a member, is known to contain sesquiterpenes responsible for antitumor activity[35]. There are studies with isolated compounds that show antitumor activity, such as the drimane sesquiterpenes from the species D. winteri, which showed that isolated compounds such as drimenol, isordrimenone and polygodial had the ability to reduce

cell viability by 7.37 μg.mL^{-1}, 4.71 μg.mL^{-1} and 4.00 μg.mL^{-1}, respectively[36]. Similarly, in another study with D. brasiliensis, 1-β-(p-coumaroyloxy)-polygodial, drimanial and 1-β-(pmethoxycinnamoyl)-polygodial demonstrated antiproliferative activity against different lines of sarcoma, carcinomas, leukemias and lymphomas[29].

However, the antitumor activity observed may be related to the anti-inflammatory properties of the plant extract. In the peritonitis studies, it was observed that *D. brasiliensis* was able to act on the terminal phase of acute inflammation, which is characterized by the recruitment of prostaglandins and cytokines, as well as inhibiting the migration of leukocytes and neutrophils. The correlation between pro-inflammatory cytokine signaling, the activation of transcription factors and tumor development is widely recognized[37,38]. Multitarget therapies aimed at interfering with the tumor microenvironment are promising alternatives for preventing the progression and metastasis of tumor cells[39]. Some plant species have potential as alternatives for antitumor treatment due to their anti-inflammatory properties[40]. In the specific case of *D. brasiliensis*, further studies should be carried out to establish the correlation between the anti- inflammatory and antitumor activity demonstrated by the species. In addition, it is important to elucidate the possible metabolic pathways, elimination routes and kinetics of the isolated compounds[41]. These studies are essential to integrate the data obtained in pharmacological trials and clinical studies, to guarantee the safety and efficacy of the potential phytopharmaceutical.

CONCLUSION

The ethanolic extract obtained from the leaves of *D. brasiliensis* showed anti-inflammatory activity mainly at doses of 100 and 200 mg.kg^{-1} and antitumour activity at a dose of 300 mg.kg^{-1} in *M. musculus* mammals, which can be attributed to the presence of sesquiterpene markers, recognized for their ability to demonstrate such therapeutic effects. In addition, the leaf ethanolic extract showed a significant content of phenolic compounds, which were the majority compounds. Low toxicity of the *D. brasiliensis* was observed, which reinforces the safety of consuming the leaves and bark, whether in the form of teas or tinctures, ratifying its traditional use. Future studies need to be carried out to deepen understanding of the bioavailability and pharmacokinetics of the plant extract. Furthermore, the promising results of this research point to the need for further investigation to elucidate the mechanisms underlying the interaction between the inflammatory process and the tumor microenvironment.

REFERENCES

1. Huang L, LaBonte MJ, Craig SG, Finn SP, Allott EH. Inflammation and Prostate Cancer: A Multidisciplinary Approach to Identifying Opportunities for Treatment and Prevention. Cancers. 2022;14(6):1367. doi:10.3390/cancers14061367

2. Neganova M, Liu J, Aleksandrova Y, Klochkov S, Fan R. Therapeutic Influence on Important Targets Associated with Chronic Inflammation and Oxidative Stress in Cancer Treatment. Cancers. 2021;13(23):6062. doi:10.3390/cancers13236062

3. Nigam M, Mishra A P, Deb V K, Dimri, D B, Tiwari V, Bungau S G, Bungau A F, Radu A-F. Evaluation of the association of chronic inflammation and cancer: Insights and implications. Biomedicine & Pharmacotherapy. 2023; 164: 115015. doi: 10.1016/j.biopha.2023.115015

4. Greten F R, Sergei I G. Inflammation and Cancer: triggers, mechanisms, and consequences. Immunity. 2019; 51 (1):27-41. doi: 10.1016/j.immuni.2019.06.025

5. Baek AE. Bacteria benefit tumor cells. Sci Signal. 2022;15(729):eabq4492. doi:10.1126/scisignal.abq4492

6. Shawki S, Ashburn J, Signs SA, Huang E. Colon Cancer: Inflammation Associated Cancer. Surg Oncol Clin N Am. 2018;27(2):269-287. doi:10.1016/j.soc.2017.11.003

7. Bujak JK, Kosmala D, Szopa IM, Majchrzak K, Bednarczyk P. Inflammation, Cancer and Immunity-Implication of TRPV1 Channel. Front Oncol. 2019;9. https://www.frontiersin.org/articles/10.3389/fonc.2019.01087

8. Ramos-Inza S, Ruberte AC, Sanmartín C, Sharma AK, Plano D. NSAIDs: Old Acquaintance in the Pipeline for Cancer Treatment and Prevention─Structural Modulation, Mechanisms of Action, and Bright Future. J Med Chem. 2021;64(22):16380-16421. doi: 10.1021/acs.jmedchem.1c01460

9. Fiorentino S, Urueña C, Fiorentino S, Urueña C. La fitoterapia como fuente de medicamentos reguladores del metabolismo tumoral y activadores de la respuesta inmunitaria. Rev Acad Colomb Ciencas Exactas Físicas Nat. 2018;42(163):132-144. doi:10.18257/raccefyn.542

10. Ochwang'i DO, Kimwele CN, Oduma JA, Gathumbi PK, Kiama SG, Efferth T. Cytotoxic activity of medicinal plants of the Kakamega County (Kenya) against drug-sensitive and multidrug-resistant cancer cells. J Ethnopharmacol. 2018;215:233-240. doi:10.1016/j.jep.2018.01.004

11. Teodor ED, Ungureanu O, Gatea F, Radu GL. The Potential of Flavonoids and Tannins from Medicinal Plants as Anticancer Agents. Anticancer Agents Med Chem. 2020;20(18):2216-2227. doi:10.2174/1871520620666200516150829

12. Flora and fungi of Brazil - Drimys J.R.Forst. & G.Forst. Accessed June 15, 2023. http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB15323

13. Tropicos | Name - !!Drimys J.R. Forst. & G. Forst. Missouri Botanical Garden.

Accessed June 15, 2023. http://legacy.tropicos.org/Name/40035760

14. Mariot A, Mantovani A, Bittencourt R, Ferreira DK, Reis MS. Population structure and annual current increment of casca-de-anta (Drimys brasiliensis Miers - Winteraceae) in Caçador, Santa Catarina, Brazil. Rev Bras Plantas Med. 2010;12:168-178. doi:10.1590/S1516-05722010000200008

15. Lorenzi H, Matos FJ de A. Plantas medicinais no Brasil: nativas e exóticas. Instituto Plantarum de Estudos da Flora; 2002

16. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. Springer Science & Business Media; 1996

17. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In: Methods in Enzymology. Vol 299. Oxidants and Antioxidants Part A. Academic Press; 1999:152-178. doi:10.1016/S0076-6879(99)99017-1

18. OECD. Test No. 423: Acute Oral Toxicity - Acute Toxic Class Method. Organization for Economic Co-operation and Development; 2002. Accessed June 15, 2023. https://www.oecd- ilibrary.org/environment/test-no-423-acute-oral-toxicity-acute-toxic-class-method_9789264071001-en

19. Ferreira SH. A new method for measuring variations of rat paw volume. J Pharm Pharmacol. 1979;31(9):648. doi:10.1111/j.2042-7158.1979.tb13616.x

20. Oliveira AM de, Nascimento MF do, Ferreira MRA, et al. Evaluation of acute toxicity, genotoxicity and inhibitory effect on acute inflammation of an ethanol extract of Morus alba L. (Moraceae) in mice. J Ethnopharmacol. 2016;194:162-168. doi:10.1016/j.jep.2016.09.004

21. Cavalcante da Silva G, Macário de Oliveira A, Soares de Freitas AF, Paiva PMG, Napoleão TH. Antinociceptive and Anti-Inflammatory Effects of Saline Extract and Lectin-Rich Fraction from Microgramma vacciniifolia Rhizome in Mice. Chem Biodivers. 2021;18(6):e2100125. doi:10.1002/cbdv.202100125

22. Stock CC, Clarke DA, Philips FS, Barclay RK. Sarcoma 180 inhibition screening data. Cancer Res. 1955;Suppl. 2:179-331

23. Negri LM, Spontón EA, Salgado O, Sancho A, Denoya GI. Effect of different drying conditions on volatile compounds of Canelo pepper (Drimys winteri). J Phytol. Published online September 16, 2020:56-61. doi:10.25081/jp.2020.v12.6297

24. Mecchi MC, Lago JHG. Chemical constituents derived from Drimys brasiliensis Miers (Winteraceae). Nat Prod Res. 2013;27(20):1927-1929. doi:10.1080/14786419.2013.782491

25. Bridi R, Giordano A, Peñailillo MF, Montenegro G. Antioxidant Effect of Extracts from Native Chilean Plants on the Lipoperoxidation and Protein Oxidation of Bovine Muscle. Mol Basel Switz. 2019;24(18):3264. doi:10.3390/molecules24183264

26. Gastaldi B, Marino G, Assef Y, Silva Sofrás FM, Catalán C a. N, González SB. Nutraceutical Properties of Herbal Infusions from Six Native Plants of Argentine Patagonia.

Plant Foods Hum Nutr Dordr Neth. 2018;73(3):180-188. doi:10.1007/s11130-018-0680-3

27. Maleki SJ, Crespo JF, Cabanillas B. Anti-inflammatory effects of flavonoids. Food Chem. 2019;299:125124. doi:10.1016/j.foodchem.2019.125124.

28. Witaicenis A, Roldão EF, Seito LN, Rocha NP, Di Stasi LC. Pharmacological and toxicological studies of Drimys angustifolia Miers. (Winteraceae). J Ethnopharmacol. 2007;111(3):541-546. doi:10.1016/j.jep.2006.12.025

29. Fratoni E, de Athayde AE, da Silva Machado M, et al. Antiproliferative and toxicological properties of drimanes obtained from Drimys brasiliensis stem barks. Biomed Pharmacother Biomedecine Pharmacother. 2018;103:1498-1506. doi:10.1016/j.biopha.2018.04.103

30. Gomes MRF, Schuh RS, Jacques ALB, et al. Biological assessment (antiviral and antioxidant) and acute toxicity of essential oils from Drimys angustifolia and D. brasiliensis. Rev Bras Farmacogn. 2013;23:284-290. doi:10.1590/S0102-695X2012005000142

31. Matos FJ de A. Plantas medicinais: guia de seleção e emprego de plantas medicinais do nordeste do Brasil; volume II. In: Plantas medicinais: guia de seleção e emprego de plantas medicinais do nordeste do Brasil; volume II; 1989:144-144. Accessed June 15, 2023. https://pesquisa.bvsalud.org/portal/resource/pt/lil-85607

32. Burgos V, Paz C, Saavedra K, Saavedra N, Foglio MA, Salazar LA. Drimenol, isodrimeninol and polygodial isolated from Drimys winteri reduce monocyte adhesion to stimulated human endothelial cells. Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc. 2020;146:111775. doi:10.1016/j.fct.2020.111775

33. Yang W, Chen X, Li Y, Guo S, Wang Z, Yu X. Advances in Pharmacological Activities of Terpenoids. Nat Prod Commun. 2020;15(3):1934578X20903555. doi:10.1177/1934578X20903555

34. Burgos V, Paz C, Saavedra K, et al. Drimys winteri and isodrimeninol decreased foam cell formation in THP-1 derived macrophages. Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc. 2020;146:111842. doi:10.1016/j.fct.2020.111842

35. Abu-Izneid T, Rauf A, Shariati MA, et al. Sesquiterpenes and their derivatives-natural anticancer compounds: An update. Pharmacol Res. 2020;161:105165. doi:10.1016/j.phrs.2020.105165

36. Russo A, Cardile V, Graziano ACE, et al. Antigrowth activity and induction of apoptosis in human melanoma cells by Drymis winteri forst extract and its active components. Chem Biol Interact. 2019;305:79-85. doi:10.1016/j.cbi.2019.03.029

37. Liu J, Zhang X, Cheng Y. Dendritic cell migration in inflammation and immunity. Cell Mol Immunol. 2021;18: 2461-2471. doi: doi.org/10.1038/s41423-021-00726-4

38. Zhao H, Wu L, Yan G, Chen Y, Zhou M, Wu Y, Li Y. Inflammation and tumor progression: signaling pathways and targeted intervention. Signal Transduct. Target. Ther. 2021;6(1):263. doi: 10.1038/s41392-021-00658-5

39. Mantovani A. The Inflammation - Cancer Connection. FEBS J. 2018;285(4):638-640. doi:10.1111/febs.14395

40. Gandhi Y, Kumar R, Grewal J, Rawat H, Mishra SK, Kumar V, Shakya SK, Jain V, Babu G, Sharma P, Singh A, Singh R, Acharya R. Advances in anti-inflammatory medicinal plants and phytochemicals in the management of arthritis: A comprehensive review. Food Chemistry Advances. 2022; 1: 100085. doi: 10.1016/j.focha.2022.100085

41. Chandra S, Gahlot M, Choudhary AN, Palai S, Almeida RS, Vasconcelos JEL, Santos FA V, Farias PAM, Coutinho HDM. Scientific evidences of anticancer potential of medicinal plants. Food Chemistry Advances. 2023;2: 100239. doi: 10.1016/j.focha.2023.100239

Submitted: June 16, 2023

Accepted: November 7, 2023

Published: May 15, 2024

Author contributions:

Mariana Cardoso Oshiro: Investigation, Writing – original draft, Writing – review & editing

Alícia de Souza Santos: Investigation

Alisson Macário de Oliveira: Investigation

Emanuelle Maria da Silva: Investigation

Helimarcos Nunes Pereira: Investigation

Jhonatta Alexandre Brito Dias: Investigation

Marcela Albuquerque de Oliveira: Investigation

Marília Gabriela Muniz Arruda: Investigation

Marcilene Souza da Silva: Investigation

Simone da Paz Leôncio Alves: Investigation

Ivone Antônia de Souza: Supervisão: Investigation

All authors approved the final version of the text.

Conflict of interest: There is no conflict of interest.

Financing: Does not have financing

Corresponding author:
Mariana Cardoso Oshiro
Federal University of Pernambuco
Av. Artur de Sá, S/N. Cidade Universitária – Recife/PE, Brasil
E-mail: marianac.oshiro@gmail.com

Editora: Dra. Christiane de Fátima Colet
Editor-in-chief: Dra. Adriane Cristina Bernat Kolankiewicz