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Chemistry

Preliminary phytochemical study and antiproliferative activity of extracts from the plant *Chloroleucon extortum* Barneby & J. W. Grimes (Jurema Branca)

Estudo fitoquímico preliminar e atividade antiproliferativa dos extratos da planta Chloroleucon extortum Barneby & J. W. Grimes (Jurema Branca)

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ABSTRACT

Due to the widespread use of medicinal plants and their derivatives in the daily lives of the world's population, a lack of knowledge about the bioactive compounds of many plant species still prevails because of the limited access to information and efforts to search for scientific evidence despite their widespread popular use. Therefore, the present work aims to conduct a phytochemical study of the species Chloroleucon Extortum Barneby & J. W. Grimes (Jurema Branca) and evaluate its cellular antiproliferative activity. As it is a plant native to the Brazilian Caatinga, phytochemical tests made it possible to observe the presence of secondary metabolites such as tannins, phenolic compounds, and coumarins extracted using different techniques. Cytotoxic activity was evaluated against cell lines such as HCT-116 (human colorectal cancer), HL-60 (acute promyelocytic leukemia), HeLa (human cervical cancer), and NCI-H292 (human mucoepidermoid carcinoma of the lung) and PBMC (human peripheral blood mononuclear cells). Thus, the extracts indicated moderate cytotoxic activity.

Keywords: Keywords: Medicinal plants; Secondary metabolites; Cytotoxicity

RESUMO

Devido ao amplo uso de plantas medicinais e seus derivados no cotidiano da população mundial, ainda prevalece o desconhecimento sobre os compostos bioativos de muitas espécies vegetais devido à falta de acesso às informações e aos esforços de busca por evidências científicas dadas. uso popular. Portanto, o presente trabalho tem como objetivo realizar um estudo fitoquímico da espécie Chloroleucon Extortum Barneby & J. W. Grimes (Jurema Branca) e avaliar sua atividade antiproliferativa celular. Por se tratar de

uma planta nativa da Caatinga brasileira, através de testes fitoquímicos foi possível observar a presença de metabólitos secundários como taninos, compostos fenólicos e cumarinas extraídos por diferentes técnicas. A atividade citotóxica foi avaliada contra linhas celulares como HCT-116 (câncer colorretal humano), HL-60 (leucemia promielocítica aguda), HeLa (câncer cervical humano) e NCI-H292 (carcinoma mucoepidermóide humano do pulmão) e PBMC (humano células periféricas células mononucleares do sangue). Assim, os extratos apresentaram atividades moderadas em termos de poder citotóxico.

Palavras-chave: Palavras chaves: Plantas Medicinais; Metabolitos secundários; Citotoxicidade

1 INTRODUCTION

In Brazil and other countries, the search and development of new drugs from natural products has grown, and various studies have been carried out to prove their activities, clarify mechanisms of action, or identify active components and investigate the possible toxic effects of different plant species (Aguiar & Veiga Junior, 2021). Without these studies, the use of some medicinal plants and their products can become a problem, as some of the substances they contain can trigger deleterious effects that result in severe and even fatal clinical conditions (Pinho et al., 2010).

The cytotoxicity of medicinal plants is the perfect example for this context, as the harmful effects they can have on the body are still unknown in some species. In this way, cytotoxicity can be assessed through changes in the process of cell division in the test organism and the incidence of chromosomal mutations (Silva et al., 2023). The detection of potentially cytotoxic substances and their probable effects on organisms is important in this study, as it contributes to understanding the biological behavior of the extracts evaluated. This way, we will be able to substantially reduce the number of animal tests, increasing the safety of the potentially clinical use of these plants (Zakerzadeh et al., 2017), as communities use several species from Brazil's different biomes without proper proof of their safety.

The Caatinga biome is a dry forest, green only during rainy periods, with thorny shrubs adapted to arid soils. Its vegetation covers the states of Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Sergipe, Bahia and Minas Gerais and has an

area of approximately 734,478 km^2 (Albuquerque & Silva, 2005). In this region, several ethnodirected studies have been conducted to verify the immense potential of the medicinal plants used in local folk medicine (Oliveira et al., 2021; Dos Reis et al., 2023).

More than 596 shrub and tree species have been described in the Caatinga, as well as 1,788 herbaceous species, at least 180 of which are endemic, with the Fabaceae botanical family standing out (Joly, 1993), for which 603 species have been recorded to date. Despite the diversity of plant species, paradoxically, this is one of Brazil's most threatened and least studied and known biomes (Siqueira Filho, 2012). Chloroleucon extortum Barneby & J. W. Grimes, popularly known as 'Jurema Branca', is a Fabaceae species endemic to the Caatinga (Queiroz et al., 2009). However, there are currently no reported phytochemical or pharmacological studies on this species. Given this, this study aims to conduct a preliminary phytochemical study of the species and evaluate its antiproliferative activity.

2 METHODS

2.1 Materiais

Stem bark and leaves of Chloroleucon extortum Barneby & J.W. Grimes (Jurema Branca) were collected in the Carão community, Altinho, Pernambuco (08°35'13.5" S, 36°05'34.6" W), a semi-arid Caatinga region (Araújo et al., 2008). The exsiccate was deposited in the Herbarium of the Federal Rural University of Pernambuco (UFRPE) under number 49609.

2.2 Preparation and chemical characterization of the extracts

The samples were air-dried for two weeks for dehydration. After drying, the samples were pulverized in a Willye-type vertical knife mill (Adamo 340) and standardized in sieves, obtaining a particle size of 20 Mesh (1.2 mm). After this process, the samples were subjected to extraction by maceration every 48 hours in a ratio of 1:10 (m/v) with

80% ethanol, and the the solvent was replaced twice for a total of 3 macerations. The extracts were then filtered and subjected to evaporation under reduced pressure at 40 ± 5 °C until completely dry to obtain the crude ethanolic extract.

To obtain fractionated extracts of low (hexane), medium (ethyl acetate), and high polarity (methanol), one of the following extraction methods was used: Fractional extraction: The plant samples (100g) were macerated in a ratio of 1:10 (m/v) using the plant matrix and initially the extracting liquid hexane for 48 hours with 2 changes. The fraction was filtered and separated. The plant material was dried, and ethyl acetate was added, repeating the procedure carried out previously, the same using methanol; the respective fractions were collected individually and concentrated by evaporation under reduced pressure at a temperature of 40 ± 5 °C until completely dry.

Filter column chromatography: This was carried out in a Bucher funnel. A column was assembled by plugging the bottom with filter paper, filling it evenly with silica up to 2/3 of the funnel height, and overlapping it with filter paper. Afterward, an aliquot of the crude ethanolic extract (11.5 g for stem bark and 5 g for leaves) was incorporated into 1/3 of the total Silica gel 60 (Merck) that made up the column, forming the solid top. Elution was started, first with Hexane P. A (1 L), then with Ethyl Acetate P.A (1 L) and lastly, a final elution with Methanol P.A (1 L). All elutions respected the degree of polarity of the solvents, eluting from lowest to highest.

2.3 Determination of total phenolic content

The dry extract was diluted in methanol P.A. to a 1 mg/mL concentration in a 50 mL volumetric flask in triplicate. An aliquot of 0.2 mL (200 µL) of the diluted extract was added to a test tube. Subsequently, 500 µL of Folin-Ciocalteu reagent (10% aqueous solution), and 1 mL of sodium carbonate solution (7.5%) were added, and the volume was topped up to 10 mL with distilled water. After preparing this solution, it was stirred properly and left to stand at room temperature for 30 minutes, protected from light. After this period, the absorbance of the mixture was measured at 760 nm against a

blank prepared with distilled water. Tannic acid was used as a standard, and the total phenol content was expressed as milligram equivalents of tannic acid per gram of sample (mg TAE/g) (Amorim et al., 2008).

2.4 Determination of tannin content

The tannin content was determined according to the protocol developed by Amorim et al. (2008) and adapted for the species. The dry extract was diluted in methanol P. A at a concentration of 1 mg/mL in a 50 mL volumetric flask in triplicate. Subsequently, 1 g of casein was weighed and transferred to a 50 mL Erlenmeyer flask, adding 6 mL of the diluted sample and 12 mL of distilled water in triplicate. After 3 (three) hours of reaction under stirring, the solution was filtered into a volumetric flask, and the volume was made up to 25 mL with distilled water. An aliquot of 1 mL was removed, and the residual phenols were quantified using the Folin-Ciocalteu method. The difference between the total phenol content and the residual phenol content calculated the tannin content. Tannic acid was used as a standard.

2.5 Determination of flavonoid content

The quantification of flavonoid content was based on the methodology described by Peixoto Sobrinho et al. (2008). The method is based on the reaction of the aluminum ion (Al3+) with flavonoid molecules in the sample, establishing the stable flavonoid-Al3+ complex, which is yellow and whose intensity is proportional to the flavonoid concentration. The dry extract was diluted in methanol P.A. at a concentration of 1 mg/mL in a 50 mL volumetric flask in triplicate. An aliquot of 0.2 mL (200 µL) of the diluted extract was transferred to test tubes to quantify the flavonoids. Subsequently, 0.120 mL (120 µL) of glacial acetic acid, 2 mL of pyridine solution (20%, v/v in methanol P.A), 0.5 mL (500 µL) of aluminum chloride reagent (5%, w/v in distilled water) were added. The volume was completed to 10 mL with distilled water in each tube. After preparing this solution, it was shaken well and left to stand for 30 minutes, protected from light,

at room temperature. After this period, the absorbance of the mixture was measured at 420 nm against a blank prepared with distilled water. The total flavonoid content was expressed as milligram rutin equivalents per gram of extract (mg ER/g).

2.6 Determination of coumarin content

The colorimetric test described by Osório and Martins (2004) with adaptations was used to quantify the coumarin content. 0.5 mL of the diluted extract (1.0 mg/mL) was transferred to test tubes. Subsequently, 2 mL of distilled water and 500 µL of lead acetate solution were added. The sample was shaken, and then 7 mL of distilled water was added. From this solution, 2 mL was transferred to new test tubes, and 8 mL of hydrochloric acid solution (37 % pure) was added. The samples were left for 30 minutes in a dark place at room temperature. The absorbance of the mixture was measured at 320 nm against a blank prepared with distilled water. The total coumarin content was expressed as milligrams of coumarin equivalent per gram of extract (mg EC/g).

2.7 Cytotoxicity

The cytotoxic activity was carried out at the Laboratory of Harmacotoxicological Prospection of Bioactive Products of the Department of Antibiotics at the Federal University of Pernambuco, where the experiment was carried out using the MTT method [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Mosmann, 1983; Alley et al., 1988). The non-neoplastic cell line was PBMC (human peripheral blood mononucleated cells), grown in RPMI 1640 culture medium. The human tumor cell lines used were HCT-116 (human colorectal cancer) and HL-60 (acute promyelocytic leukemia) maintained in RPMI 1640 culture medium; HeLa (human cervical cancer) and NCI-H292 (human mucoepidermoid lung carcinoma) maintained in DMEM culture medium.

The media was supplemented with 10% fetal bovine serum and 1% antibiotic solution (penicillin and streptomycin). The cells were maintained in an incubator at 37ºC in a humid atmosphere enriched with 5% CO2. HCT-116, NCI-H292, and HeLa

cells (2 x 105 cells/mL); HL-60 and PBMC (1 x 106 cells/mL) were plated in 96-well plates and incubated for 24 h to reach confluence, except for the plates containing HL-60. Samples dissolved in DMSO (final concentration of 0.1%) were then added to the wells at a final concentration of 50 µg/mL. The drug doxorubicin (5 µg/mL) was used as a standard. After 72 h of incubation, 25 µL of MTT (5 mg/mL) was added, and after 3 h of further incubation, the HL-60 plates were centrifuged, and the culture medium with MTT from all the strains was aspirated to add 100 µL of DMSO to each well. The absorbance was measured in a microplate reader at a wavelength of 560 nm. The experiments were carried out in quadruplicate, and the percentage of inhibition was calculated using the *GraphPad Prism* 8.1 *demo program*. Two independent experiments were carried out, and an intensity scale was used to evaluate the cytotoxic potential of the samples tested.

2.8 Statistical analysis

The levels of secondary metabolites were evaluated using Kruskal Wallis analysis of variance followed by multiple comparisons using Dunn's test. Differences were considered significant at the $p < 0.05$ level. The BioEstat 5.0 program was used for statistical analyses (Ayres et al., 2007).

3 RESULTS AND DISCUSSION

3.1 Extract yields

The extraction yields from the stem bark and leaves of *Chloroleucon extortum* (Jurema Branca) are demonstrated in Table 1.

Table 1 – Yield in percentage from extracts obtained from the stem bark and leaves of *Chloroleucon extortum*

Extractive method	Solvent	Stem bark Yield (%)	Leaves Yield (%)
Crude extract	Ethanol 80 %	14.84	17.47
Fractional Extraction	Hexane	0.24	2.78
	Ethyl acetate	0.83	1.4
	Methanol	7.87	9.63
Filter Column	Hexane	N/D	N/D
	Ethyl acetate	1.86	2.01
	Methanol	8.15	3.51

Caption: N/D - Not detected. Source: Authors

The extract yields varied according to the extraction method used and the use of different solvents. When comparing the extraction methods for both parts of the plant, the highest yields were obtained with the most polar solvent, specifically methanol, which indicated yields of 7.87 % and 8.15 % for the stem bark and 9.63 % and 3.51 % for the leaves. Simultaneously, the least polar solvent gave the lowest yields for both parts of the plant and for the different methods. According to Tiwari et al. (2011) and Oliveira et al. (2016), the extraction methods directly influence the extraction and, consequently, the total extract yield. This difference between the extraction yields using this solvent happens because the ethanol + water mixture behaves as an amphiphilic solvent and extracts both polar and medium-polar substances. Another characteristic is that using the maceration technique to extract natural products (in the case of fractional extraction) results in low yields (Karabegović et al., 2014; Bucar et al., 2013).

3.2 Phytochemical analysis

The phytochemical evaluation of the extracts was carried out using thin-layer chromatography with specific developer solutions for total phenols, flavonoids, tannins, and coumarins. After applying the developer solutions, it was possible to see the bands

of the compounds under 365 nm ultraviolet light. Therefore, if there is no previous chemical knowledge about the species under study, this analysis becomes important for recognizing the classes of secondary metabolites present (Table 2) (Simões et al., 1988).

Table 2 – Phytochemical screening of extracts of *Chloroleucon extortum stem barks and leaves obtained* by different extraction methods

Secondary Metabolite	**Stem bark**							**Leaves**						
Extractive method		**Fractional Extraction**			**Column**				**Fractional Extraction**			**Column**		
Statement type	**EB**	**H**	**Ac**	**M**	**H**	**Ac**	**M**	**EB**	**H**	**Ac**	**M**	**H**	**Ac**	**M**
Phenolic Compounds	+	-	+	+	+	+	-	+	-	+	+	+	+	+
Hydrolyzable Tannins	+	-	+	+	+	+	-	+	-	+	+	+	+	+
Condensed Tannins	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+
coumarins	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Key: EB = Crude extract; H = Hexane; Ac= Ethyl acetate; M= Methanol. (-) = Absence, (+) = Presence. Source: Author

The levels of total phenols, tannins, flavonoids, and coumarins in the stem bark and leaves of *C. extortum*, obtained by different extraction methods, were evaluated and the results expressed as the mean of the replicates accompanied by the standard deviation in mg/g tannic acid equivalent for total phenols and tannins, rutin equivalent for flavonoids and 1,2-benzopyrone equivalent for coumarins, as seen in Table 3.

Analyzing the results of the contents obtained through the different extractive techniques, it was possible to observe that the ethyl acetate fraction of the stem bark of the Jurema Branca species stood out in terms of total phenol content, with a content of 15.35 ± 3.19 mg EAT/g. Regarding flavonoid content, the ethyl acetate extract obtained using a filter column had 235.04 ± 26.66 mg ER/g, respectively. There was a statistically significant difference between the extracts obtained by different techniques. For tannin content, the ethyl acetate fraction exhibited a content of 13.60 ± 5.65 mg EAT/g; for coumarins, the hexane fraction exhibited a 27.23 ± 0.58 mg EC/g. However, the

observated content was not significantly different from the extracts obtained using different solvents.

Table 3 – Content of total phenols, tannins, flavonoids, and coumarins expressed as mean ± standard deviation/ concentration of extracts obtained from the stem bark and leaves of *Chloroleucon extortum* Barneby J. W. Grimes (Jurema Branca) by different extraction methods

Extractive method	Stem bark				Leave			
	FT (mg EAT/g)	**TN (mg EAT/g)**	**Fla. (mg ER/g)**	**CUM (mg EC/g)**	**FT (mg EAT/g)**	**TN (mg EAT/g)**	**Fla. (mg ER/g)**	**CUM (mg EC/g)**
Crude ethanolic extract 80%	7.88 ± 1.86 24.92% a	0.29 ± 2.99 13.49% a	16.19 ± 1.22 7.54% a	22.18 ± 0.09 0.39% a	92.26 ± 6.62 7.18 a	71.40 ± 4.12 5.77% a	73.07 ± 6.74 9.23% a	22.58 ± 0.11 0.48% a
Hexane fraction	N/D	N/D	269.40±3.80 1.41% b	27.23 ± 0.58 2.19% b	N/D	N/D	272.85 ± 6.51 2.38% b	27.19 ±0.13 0.48 b
Ethyl acetate fraction	15.35 ± 3.19 20.80% b	13.60 ± 5.65 41.24% b	119.49 ± 6.40 5.36% a	23.27 ± 0.49 2.09% a	17.33 ± 4.07 23.49% b	25.75 ± 4.10 15.91% b	602.98 ± 15.44 2.56% a	26.55 ± 0.13 0.47% a
Methanol fraction	6.15 ± 2.62 42.65% a	4.93±2.21 44.82% c	31.72 ± 1.11 3.49% c	21.93 ± 0.11 0.51% c	102.08 ± 1.12 1.10% a	65.70 ± 3.97 6.07% c	113.63 ± 0.38 0.33 c	22.53 ± 0.13 0.59 c
Hexane column				S. R.				
Ethyl acetate column	15.29 ±5.38 35.21%d	13.26 + 5.32 40.12%d	235.04 ± 26.66 16.54% d	24.76 ± 0.38 1.51 D %d	45.67 ± 4.43 9.71%d	37.27 ± 6.62 17.15%d	331.15 ± 14.31 4.32 % d	23.46 ± 0.34 1.44 d
Methanol Column	N/D	N/D	6.34 ± 0.92 14.56 a	21.65 ± 0.18 0.82a	67.39 ± 1.66 2.47% d	52.22 ± 1.99 3.80 D	52.48 ± 3.21 6.12% a	23.24 ± 0.09 0.37% d

Key: FT = Total Phenols, TAN = Tannins, Fla = Flavonoids, CUM = Coumarins, N/A = Not detected, S.R= No yield. Equal letters in the same column indicate no statistical difference according to Kruskall Wallis (followed by Dunn), $p < 0.05$. Source: Provided by the author

About the extracts of Jurema Branca leaves, the methanolic extract expressed the highest content of total phenols (102.08 ± 1.12 mg EAT/g). Regarding flavonoid

content, the ethyl acetate fraction had 602.98 ± 15.44 mg EAT/g. However, there was no statistically significant difference between the extracts obtained using different solvents. For tannins, the methanol fraction had a content of 65.70 ± 3.97 mg EAT/g, and for coumarins, the hexane fraction had a content of 27.19 ± 0.13 mgEC/g. There was no statistically significant difference between the extracts obtained using different solvents.

Determining the content of secondary metabolites in plants for medicinal use is based on marking the quantities of a chemical class present in the plant's chemical constitution (Vigo et al., 2004; Freire et al., 2006). This is linked to biochemical, physiological, ecological, and evolutionary processes, as well as seasonality, circadian period, age, and development of the plant, in addition to the availability of nutrients and water in the soil (Miranda et al., 2016). These factors may, therefore, explain the differences observed in the results of the phytochemical evaluation of the samples studied. The study revealed several positive results about the presence of some secondary metabolites. Despite the differences between the extraction methods and their yields, it is also worth noting that there was favorable quantification of secondary metabolites in low-yield extracts, such as the extracts of the ethyl acetate fraction and the hexane fraction of both stem bark and leaves for flavonoid content. This is corroborated with the study by Silva (2009), which points that species from the Fabaceae family are rich in this type of metabolite.

Various studies have communicated that there is a wide variety of secondary metabolites in species from the Caatinga and point to the presence of phenolic compounds in various species from this biome. This indicates that these compounds may be related to many of the pharmacological activities popularly attributed to these species (Monteiro et al., 2006; Alencar et al., 2009). Araújo et al. (2008) also revealed the number of tannins and flavonoids in various species from the Caatinga, relating these to popular use. Phenolic compounds play an important role during plant development and in stressful situations. However, the presence of phenolic compounds in plants can lead to positive pharmacological responses, as these substances have proven

antioxidant, anti-inflammatory, and immunomodulatory properties. They are currently being studied as chemo-preventive agents (Alves et al., 2019).

Tannins have a range of activities due to their astringent properties. They exert an antidiarrheal and antiseptic effect internally, as well as externally waterproofing the most exposed layers of the skin and mucous membranes, acting as bioprotective molecules of the underlying layers. In addition, they can also perform pharmacological activities with antimicrobial, antifungal action, helping to heal inflammatory processes of burns and wounds as well as helping as an effective antidote for poisoning (Souza et al., 2018).

Flavonoids, another class mentioned above, also have pharmacological actions that have already been shown in several studies, such as analgesic, anti-allergic, hepatoprotective, antimicrobial, and anti-inflammatory. These actions can inhibit the cyclooxygenase (COX) and lipoxygenase pathways, which play an important role as inflammatory mediators (Balestrin et al., 2021; Uddin et al., 2020). They can also play an antitumor role, possibly by controlling cell proliferation and blocking oncogenesis through mechanisms that modulate enzymes in the carcinogenic metabolic pathway (Mohana et al., 2018; Amado et al., 2011). The mechanism of antiviral action of this class is through the ability to interact with the virus. This ability ranges from binding upon entry into the host cell through bonds with glycoproteins of the viral envelope and cell receptors. As a result, its chemical structure is modified and the virus binding site is blocked (Dos Santos & Rodrigues, 2017).

Another representative of secondary metabolites contained in plants in general are coumarins, for which various studies point to a diversity of biological studies. For example, a series of coumarin derivatives (4-hydroxy, 7-hydroxy, and 3-carboxy coumarins) expressed better activity against Gram-positive rather than Gram-negative bacteria, while some were more effective specifically against Bacillus subtilis and Staphylococcus aureus (Lin et al., 2012). In another study, two new coumarin compounds presented significant antifungal activity when compared

to fluconazole (Al-Amiery et al., 2012) and tested against Candida albicans and Aspergillus niger species.

Coumarins have also demonstrated to have anticancer activity. In a study carried out by Ahmad et al. (2014) to evaluate the anticancer activity on the A2780 cell line of human epithelial ovarian carcinoma, the extracted coumarin inhibited the proliferation of 50% of the cells in vitro at a concentration of 0.64 mg/mL. Kim and colleagues (2008) observed that esculetin (6,7-dihydroxy coumarin) has a potent cytotoxic effect on the HT-20 cell line (human rectal colon adenocarcinoma) with a dose and time-dependent effect so that treatment with 55 µg/mL reduced cell viability by 50%.

3.3 Cytotoxicity

Regarding the cytotoxicity analyses of the species under study, the percentage of growth inhibition of PBMC cells treated with extractives from "Jurema Branca" varied between 17.04 ± 0.88% and 38.66 ± 1.81%. None of the extracts can be considered cytotoxic at a concentration of 50 µg/mL, as they exhibited below 50% inhibition of human peripheral blood mononucleated cells, as seen in Table 4.

Although there are, to date, no specific studies on the cytotoxicity of *Chloroleucon extortum*, similar results have been observed in related species of the *Fabaceae* family. The ethanolic extract of the stem bark of *Piptadenia stipulacea* ("jurema-branca" in another popular sense) expressed IC_{50} values of 37.96 µg/mL against HCT-116, 37.6 µg/mL against PC-3, and 27.82 µg/mL against HL-60, evidencing antitumor activity in human cell lines comparable to those observed in the present study (Santos et al., 2022).

The use of alternative in vitro methodologies is often used to replace in vivo test protocols. In this sense, the cytotoxicity tests described in ISO 10993-5 (2009) are essential to analyze the behavior of numerous substances in cells of interest through the cell growth inhibition method (Adan, Kiraz, & Baran, 2016).

According to Aritizabal et al. (2013), extracts from plant species can be considered advantageous for biological research when they present absent cytotoxicity against

healthy cells. In this aspect, the low inhibition of normal cells evaluated after treatment with extracts and fractions of "Jurema Branca", are of paramount importance for the biological study of species from the Caatinga biome. This is due to the scarcity of biological tests related to this species. Thus, this manuscript can be considered a pioneering study in ensuring the use of stem bark and leaves extracts, as both lacked cytotoxic effects on the hematopoietic system.

In parallel, studies with species of the genus *Mimosa* (such as Mimosa tenuiflora) also confirm the relative safety of plant extracts against normal cells. Recent assays reported that nanoparticles synthesized with *M. tenuiflora* extract maintained the viability of HUVEC cells above 80% at concentrations similar to those used in the present study, reinforcing the absence of toxicity in non-tumor cells (Rodrigues et al., 2023).

The cytotoxic profile of extracts and fractions of "Jurema Branca" for cancerous lines (HL-60, HCT-116, HeLa, NCI-H292) was also evaluated, and the results are expressed in Table 4. Of the twelve extracts evaluated, only the crude stem bark extract (JB1) presented an inhibition percentage of 65% for human colorectal cancer (HCT-116) and acute promyelocytic leukemia (HL-60) lines. This activity may be related to coumarins, a class of metabolites secondary substances present mainly in phytochemical quantification and which show, in addition to antimicrobial activity, cytotoxic and anticancer activity described in the literature (Ahmad et al., 2014; Kim et al., 2008).

In research with Piptadenia stipulacea, tannins were identified as relevant components for bioactivity. On the other hand, flavonoids such as rhamnetin and luteolin, identified in the ethanolic extract, were also correlated with cytotoxic effects, aligning with the results obtained here (Santos et al., 2022; Carvalho et al., 2023).

The extract from the AcOEt stem bark filter column (JB2) and the fractionated hexane leaves extract (JB10) also indicated inhibitory action for the HCT-116 lineage (58.5 ± 3.1 % and 51.8 ± 2.8 %, respectively). The JB10 extract is rich in flavonoids, as quantified in the phytochemical screening; these secondary metabolites are phenolic

compounds well known in plants for presenting antioxidant and anticancer activity (Ayad & Akkal, 2019; Muniyandi et al., 2019; Lima et al., 2020).

Table 4 – Percentage of cell growth inhibition (IC%) of Jurema Branca extracts in tumor cell lines at a concentration of 50 µg/mL using the MTT colorimetric method after 72 hours of treatment

Extracts	HCT-116	HL-60	HeLa	NCI-H292	PBMCs (%IC)
JB1	65,5 ± 1,8	65,6 ± 3,1	19,3 ± 0,8	20,4 ± 1,7	21,7 ± 0,6
JB 2	58,7 ± 3,1	21,8 ± 0,7	20,9 ± 1,8	26,3 ± 1,2	26,2 ± 1,8
JB 3	36,5± 2,9	25,5 ± 0,2	9,5 ± 0,4	18,0 ±1,0	22,2 ± 0,9
JB 4	38,4 ± 2,8	21,6± 1,0	35,0 ± 0,7	15,8 ± 1,5	17,0 ± 0,9
JB 5	36,7 ± 0,7	30,7 ± 0,2	31,8 ± 3,5	25,4 ± 2,9	20,1 ± 1,1
JB 6	13,4 ± 1,0	50,5 ± 2,7	32,8 ± 1,7	19,4 ± 1,4	18,9 ± 0,9
JB 7	28,6 ± 1,3	40,7 ± 0,5	21,8 ± 2,5	14,2 ± 1,7	25,0 ± 2,6
JB 8	30,2 ± 1,2	43,6 ± 0,4	48,3 ± 3,9	8,8 ± 0,6	38,7 ±1,8
JB 9	37,3 ± 0,1	47,7 ± 2,0	21,7 ± 1,2	23,4 ± 1,6	24,1 ± 1,6
JB 10	51,8 ± 2,7	51,6 ± 1,1	20,5 ± 1,4	17,3 ± 0,4	26,0 ± 2,5
JB 11	16,1 ± 1,1	31,5 ± 3,0	22,4 ± 3,6	16,6 ± 0,8	26,3 ± 2,1
JB 12	48,1 ± 0,5	29,7 ± 0,1	3,0 ± 3,2	24,6 ± 1,3	20,5 ± 1,5
DOX	81,8 ± 0,5	92,0 ± 0,4	85,0 ± 1,3	94,3 ± 0,3	.....

Data are presented as mean ± standard deviation of the percentage of cell growth prevention of cells: HCT-116 (human colorectal cancer), HL-60 (acute promyelocytic leukemia); HeLa (human cervical cancer); NCI-H292 (human mucoepidermoid carcinoma) treated with organic extracts about the negative control. JB1= Crude ethanolic stem bark extract, JB2= Hexane stem bark fractionated extract, JB3= Ethyl acetate stem bark fractionated extract, JB4= Methanol stem bark fractionated extract, JB5= Ethyl acetate stem bark filter column extract, JB6= = Methanol stem bark filter column extract, JB 7= Crude ethanolic extract leaves, JB8= Fractionated extract hexane leaves, JB 9= Fractionated extract ethyl acetate leaves; JB 10= Fractionated methanol leaves extract, JB 11= Ethyl acetate leaves filter column extract, JB 12= Methanol filter column extract, Dox = doxorubicin. Source: The author

Colon and rectal cancer rank third among the most common cancers in Brazil. In the Northeast, the incidence of cases is 10.99 per 100 thousand inhabitants, prevalent in males (Inca, 2022). Therefore, knowing the cytotoxic profile of this extract in the HCT-116 lineage represents a gain for identifying new plants that present components with biological action in their phytochemical constitution. During a comparative analysis between the ethanolic extracts of the stem bark (JB1) and the leaves (JB7) to inhibit

the growth of HL-60 cells, the JB7 extract inhibited 20% less of these cells, and the JB1 extract could be considered more promising for continuity of studies in this lineage.

Similarly, a recent study evaluated species of the genus *Mimosa* (*M. tenuiflora, M. pteridifolia, and M. caesalpiniifolia*) and demonstrated the selectivity of ethanolic extracts against tumor lines such as HCT-116 and MDA-MB-231, with lower toxicity on L929 fibroblasts. This provides relevant comparative context for species of the same subfamily (Alves et al., 2025).

Furthermore, it is possible to observe that the fragments of the fractionated MeOH stem bark extract (JB6) and the fractionated hexane leaves extract (JB10) expressed the ability to inhibit HL-60 cells by 50.5 ± 2.7 and 51.64 ± 1, 12%, respectively. These data corroborate the percentages of inhibition found in fractionated extracts from the leaves of the species *Bauhinia rufa*, which also belongs to the Fabaceae family, whose inhibitory activity was above 50% against the HL-60 cell line (Silva Júnior et al., 2018).

The twelve "Jurema Branca" extractives revealed a similar inhibition profile for the NCI-H292 lineage, with inhibition values below 30%. These findings corroborate the study by Silva (2013), who, when evaluating the cytotoxic activity of methanolic extracts from the leaves of *Senna alata* and *Senna siamea*, did not observe a satisfactory effect of EC50 against the human lung carcinoma line. The samples tested in the HeLa lineage also exhibited a low percentage of inhibition of the proliferation of these cells. Only the sample from the AcOEt leaves filter column (JB8) stood out, with a 48% cell growth inhibition profile. According to Rodrigues et al. (2014), samples with a cellular inhibition profile below 50% cannot be considered cytotoxic; therefore, for NCI-H292 and HeLa cells, the samples under study did not present activity.

The AcOEt stem bark filter column products (JB2), MeOH stem bark fractionated extract (JB6), and hex leaves fractionated extract (JB10) tested at a concentration of 50 µg/mL inhibited around 50% of cell proliferation for at least one tested carcinogenic lineage. In this work, the fractions that displayed the best results compared to the strains submitted to the studies were those that presented higher levels of flavonoids

and coumarins (JB2, JB6, JB8, and JB10). Therefore, it is possible to infer that these metabolites are responsible for the cytotoxic activity in the evaluated cancer cells.

4 CONCLUSION

The present study provided relevant information about the secondary metabolites present in the stem bark and leaves of the species, which is the first step towards isolating important active ingredients. It was possible to qualify and quantify them through specific techniques. By phytochemical characterization, the Jurema Branca leaves proved to be the richest part in terms of the presence of the evaluated markers.

Regarding cytotoxicity tests, it was clarified that *Chloroleucon extortum* extracts did not show cytotoxicity on normal cells, being more selective for tumor cells. The crude stem bark extract (JB1) showed a moderate percentage of inhibition, both for cells from the colorectal cancer line (HCT-116) and the acute promyelocytic leukemia line (HL-60). The ethyl acetate extract of the leaves obtained by the filter column indicated 48% inhibition of cell growth against the human cervical cancer line (HeLa). With these results, the importance of preliminary screening of plant extracts used in folk medicine was shown, revealing the potential of the species lacking this approach, thus requiring more in-depth scientific investigations. It is essential to stimulate these studies in search of new therapeutic and safe alternatives.

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