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Phytochemical Prospecting and Biological Activity Evaluation of the Ethanolic Extract of the Mandacaru Cactus in the Lavrado of Roraima, Brazil

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Abstract - Lavrado refers to the savanna region in Roraima, crucial for biodiversity and water resource conservation. Comprising diverse vegetation around lakes and streams, often accompanied by buritizais and Mandacarus. This study focused on the phytochemical prospecting and biological evaluation of the ethanolic extract of *C. jamacaru*, collected in this region. The extract was assessed for antioxidant capacity using DPPH and ABTS methods, and for total phenolic compound content. The chemical profile was analyzed using the APCI-MS method. Toxicity was evaluated through CL50 analysis in an acute assay with Artemia salina. Phytochemical prospecting revealed the presence of secondary metabolites: phenols, tannins, alkaloids, flavonoids, sesquiterpenolactones, and other lactones, saponins, steroids, triterpenoids, flavones, flavonols, chalcones, aurones, and isoflavones. Antioxidant capacity was 63.8% for DPPH and 92.3% for ABTS. Total phenolic compound values were 102.4 mg GAE 100 g^{-1}. The ethanolic extract showed no lethality against A. salina at concentrations of 2250, 2000, 1500, 1250, 1000, 500, 250, and 125 µg mL^{-1}. Antimicrobial activity showed no inhibition against tested microorganisms at concentrations up to 1 mg mL^{-1}.

Keywords: Biodiversity. Antioxidant activity. Toxicity. Antibacterial activity. Mandacaru.

Prospecção Fitoquímica e Avaliação da Atividade Biológica do Extrato Etanólico de Cacto Mandacaru no Lavrado de Roraima, Brasil

Resumo - Lavrado refere-se à região de savanas em Roraima, crucial para a conservação da biodiversidade e dos recursos hídricos. Composto por vegetações diversas ao redor de lagos e igarapés, frequentemente acompanhados por buritizais e Mandacarus. Este estudo focou na prospecção fitoquímica e avaliação

biológica do extrato etanólico de *C. jamacaru*, coletado nessa região.O extrato foi avaliado quanto à capacidade antioxidante, determinada pelos métodos de DPPH e ABTS, e quanto ao teor de compostos fenólicos totais. O perfil químico foi analisado pelo método *APCI-MS*. A toxicidade foi avaliada a partir da análise de CL50 em ensaio agudo com *Artemia salina*. Na prospecção fitoquímica obteve-se a presença dos metabolites secundários: fenóis, taninos, alcaloides, flavonoides, sesquiterpenolactonas e outras lactonas, saponinas, esteroides, triterpenoides, flavonas, flavonóis, chalconas, auronas e isoflavonas. A avaliação da capacidade antioxidante foi de 63,8% para o DPPH e de 92,3% nas análises de ABTS. Os valores de compostos fenólicos totais foram de 102,4 mg EAG 100 g^{-1}.O extrato etanólico não apresentou letalidade frente *A. salina* nas concentrações de 2250, 2000, 1500, 1250, 1000, 500, 250 e 125 µg mL^{-1}. A atividade antimicrobiana não apresentou inibição nos microrganismos testados em concentrações de até 1 mg mL^{-1}.

Palavras-chave: Biodiversidade. Atividade antioxidante. Toxicidade. Atividade antibacteriana. Mandacaru.

Prospecto Fitoquímico y Evaluación de la Actividad Biológica del Extracto Etanólico del Cactus Mandacaru en el Lavrado de Roraima, Brasil

Resumen - Lavrado se refiere a la región de sabanas en Roraima, crucial para la conservación de la biodiversidad y los recursos hídricos. Compuesto por vegetación diversa alrededor de lagos e igarapés, a menudo acompañado por buritizais y Mandacarus. Este estudio se centró en la prospección fitoquímica y la evaluación biológica del extracto etanólico de C. jamacaru, recolectado en esta región. El extracto se evaluó en cuanto a la capacidad antioxidante, determinada por los métodos de DPPH y ABTS, y en cuanto al contenido de compuestos fenólicos totales. El perfil químico se analizó mediante el método *APCI-MS*. La toxicidad se evaluó a través del análisis de CL50 en un ensayo agudo con Artemia salina. En la prospección fitoquímica se encontró la presencia de metabolitos secundarios: fenoles, taninos, alcaloides, flavonoides, sesquiterpenolactonas y otras lactonas, saponinas, esteroides, triterpenoides, flavonas, flavonoides, chalconas, auronas e isoflavonas. La capacidad antioxidante fue del 63,8% para el DPPH y del 92,3% en los análisis de ABTS. Los valores de compuestos fenólicos totales fueron de 102,4 mg GAE 100 g^{-1}. El extracto etanólico no mostró letalidad frente a A. salina en concentraciones de 2250, 2000, 1500, 1250, 1000, 500, 250 y 125 µg mL^{-1}. La actividad antimicrobiana no mostró inhibición en los microorganismos probados en concentraciones de hasta 1 mg mL^{-1}.

Palabras clave: Biodiversidad. Actividad antioxidante. Toxicidad. Actividad antibacteriana. Mandacaru.

Introduction

Cacti are succulent dicotyledons of a wide range of shapes and sizes, they can be trees, shrubs, vines, epiphytes or geophytes. Stems (stalks) can be columnar, plump, globular, tuberculate, rib-shaped,

winged or flattened, though they are usually segmented without leaves and have thorns (Barthlott and Hunt 1993).

In the Americas, cacti are found from the coastal plains to mountains with an altitude of about 3,000 m. Of the four areas identified as centers of cactus diversity, Brazil ranks third in this ranking. It is very common to associate the occurrence of cacti with places of extreme droughts; however, certain cacti inhabit other environments, for example, the shady and humid forests of the Amazon and the Atlantic Forest. Cacti are present in all major Brazilian biomes: Amazon, Caatinga, Cerrado, Atlantic Forest, Pampas and Pantanal (Zappi and Taylor 2020).

The state of Roraima is located in the northwest of the northern region of Brazil, with a predominance of the Amazon Rainforest; however, there is also a huge strip of "lavrado" in the central-eastern part (Barbosa and Campos 2011). The mandacaru (*Cereus jamacaru*) is a cactus that is native to Brazil and can reach up to 10 meters high. It has a woody trunk of about 60 cm in diameter, and many erect stems that form a compact top (Zara *et al*. 2012). It is abundant in the northeastern region of Brazil (Zara *et al*. 2012), but is also found in the "lavrado" of Roraima (Oliveira 2016; Passos 2019).

C. jamacaru has as its main phytochemical components two amines (tyramine and N-methyltyramine), in addition to the presence of hordenine and tyrosine. In its cladodes, there is the presence of flavonoids, tannins, anthraquinone saponins and often ß-sitosterol (Da Silva *et al*. 2017). This diversity of the metabolites, which have pharmacological activity, justifies their use as anti-inflammatory, antibacterial, sympathomimetic drugs and they also have a possible karyotonic activity (Davet *et al*. 2009; Necchi *et al*. 2012).

Cacti of the genera *Opuntia* and *Latifaria* are the most studied for the treatment of water, but these studies are considered recent when compared with other natural coagulants such as *Moringa oleifera* (Yin 2010). Natural coagulants, derived from cacti, have also become an alternative for the removal of organic contaminants in waters, since they are a natural, abundant, biodegradable and low-cost biomaterial (Rebah 2017).

Thus, this study consists of the phytochemical screening of the ethanolic extract of *C. jamacaru* collected in the "lavrado", in terms of its antioxidant capacity, its toxicity and antimicrobial activity, with the aim of using the powder of this cactus as a natural coagulant in the treatment of water for human consumption.

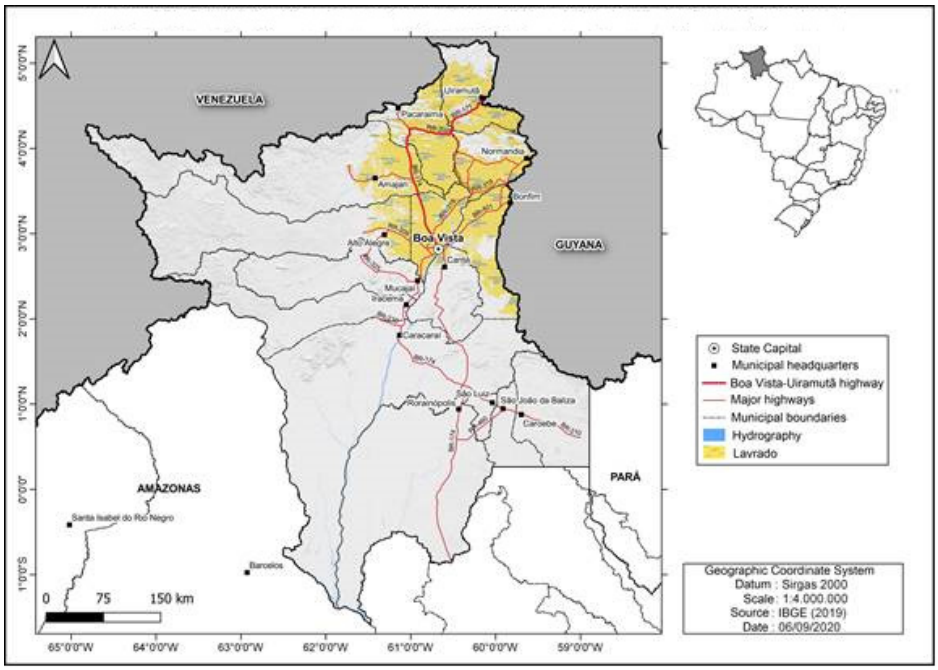
Materials and methods

This study was developed in the laboratories of the Graduate Program in Natural Resources (PRONAT) of the Federal University of Roraima.

Description of the area of cactus collection

Comprising an area of 230,104 km^2, the "lavrado" in Roraima borders Guyana and a part of Venezuela (Figure 1), with altitudes ranging between 400 and 800 meters in an extensive mountainous area of Precambrian origin (Miranda and Absy 2000).

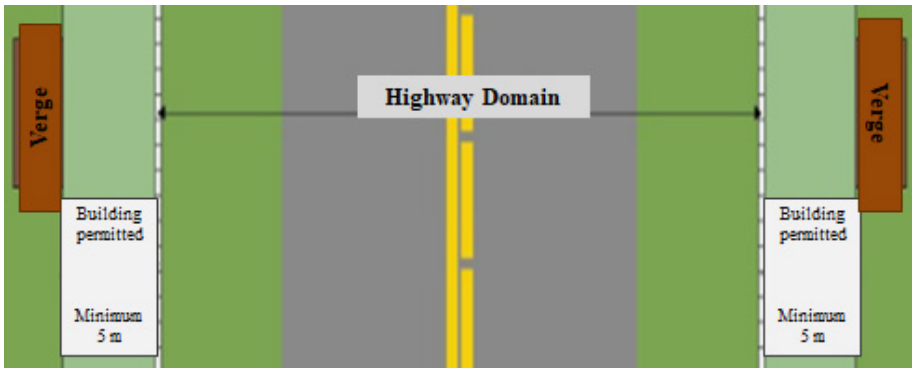
Figure 1. Map of the spatial distribution of the "lavrado" of Roraima and the Boa Vista-Uiramutã highway where the collections of cacti were carried out.



For the present study, cacti were collected in the "lavrado" on the verges of the BR 174, RR-202 and RR-171 highways between Boa Vista nd Uiramutã (Figure 1). Prior to the collection stage, a mapping of the occurrence of cacti in the studied area was carried out with the help of GPS equipment (Garmin, etrex 20). Such mapping was a primary factor in determining the species of cactus to be studied.

Considering that the path of the study runs through indigenous lands that require authorization for collection, it was decided to map the occurrence of cacti in the public domain, respecting the minimum limit of the verges of the highways. According to Resolution Nº. 9, of August 12, 2020, the domain of the highway is the physical basis on which a highway is based, which consists of the roadway, verges, hard shoulders, signage and the safety lanes. The limits are defined by an executive highway project, public utility decrees or expropriation projects. The verge, on the other hand, refers to the area along the public domain lanes of highways (Brasil 2020). The minimum limit of this strip is at least five meters according to Brazilian Law No. 13913/2019 (Brasil 2019) (Figure 2).

Figure 2. Illustrative scheme of the domain of highways and verges where the cacti were collected.



Source: Adapted from Empresa Gaúcha de Rodovias (2019).

Preparation of the extract

For the study, about 1,200 g of the aerial part of *Cereus jamacaru* was used. Thorns from plant material were removed with a knife, then the cladodes were washed under running water, cut, and placed on trays. The pieces were dried in an air circulation oven at 37 ºC for 3 days, and then ground to a powder and stored in a dark and hermetically sealed container. The ethanolic extract of the cladodes of the cactus was prepared using the maceration method. A total of 320 g of powdered cactus was added to 3.0 liters of absolute ethyl alcohol, and left for 7 days. After this period, the extract was filtered through analytical filter paper and concentrated under reduced pressure in a rotary evaporator. Then, the obtained extract was stored and conserved until the completion of the analyses (Martins *et al*. 2022).

Analysis of the chemical profile using high-resolution mass spectrometry with atmospheric pressure chemical ionization (APCI-MS)

The ethanolic extract of *C. jamacaru* was solubilized in HPLC-grade methanol to generate a stock solution of 1,000 ppm. Aliquots (10 µL) of this solution were transferred to vials containing 1 mL of methanol. Then, 5 µL of the diluted solutions were analyzed by direct insertion in the trap ion mass spectrometer (LCQ Fleet), which was equipped with an APCI source operating in positive and negative modes. The analytical parameters used were: discharge current: 5 µA; vaporizer temperature: 320 ºC; capillary temperature: 220 ºC; sheath gas: 30 psi; aux gas: 10 Arb, mass range, m/z 100-1000. MS/MS spectra were acquired using helium as the collision gas and energy ranging between 20-30%.

Qualitative classification of secondary metabolites

Phytochemical prospection was performed by adapting the methodology proposed by Matos *et al*. (2001). The ethanolic extract was tested for phenols, tannins, phenolic substances, flavones, flavonols, chalcones, isoflavones, saponins, free steroids, free pentacyclic triterpenoids and alkaloids (Table 1).

Table 1. Chemical tests in ethanol extract.

Component	Procedure
Phenols and Tannins	3 mg of ethanol extract was dissolved in 5 mL of distilled water. It was filtered and transferred to a test tube, adding 1 to 2 drops of 1% $FeCl_3$. Any change in coloration or formation of precipitate was indicative of a positive reaction.
Alkaloids	0.2g of the extract was used and dissolved in 1 mL of methanol. It was then homogenized and alkalized with 10 mL of 10% calcium carbonate solution. Next, 25 mL of chloroform was added, and after homogenization, the mixture was filtered through a separation funnel using paper previously soaked in chloroform. The filtrate was shaken with 7 mL of 2% HCl. The upper layer was separated for characterization reactions (precipitation) with Bouchardat, Mayer, and Dragendorff reagents. A drop of the reagent was placed next to another acidic solution on a microscope slide, and the result was observed by visualizing the reaction under ultraviolet light.

Flavones, Flavonols, Aurones, Chalcones, and Isoflavones	Approximately 0.03 g of the ethanol extract was weighed and diluted in 5 mL of methanol, adding a drop of 10% ferric chloride. After waiting for a few minutes, the formation of precipitate or change in coloration between green, brownish-green, greenish-yellow, red-purple, and red indicated a positive test for flavones, flavonols, aurones, chalcones, aurones, and isoflavones.
Flavonoids	Dissolve a few milligrams of the extract in 10 mL of methanol, where the extract was filtered. Then, add 5 drops of concentrated hydrochloric acid and magnesium shavings. The appearance of a pink color in the solution indicated a positive reaction.
Sesquiterpenolactones and Other Lactones	Dissolve a few milligrams of the extract in 3 mL of methanol. Add 12 drops of a 10% alcoholic solution of hydroxylamine hydrochloride and two drops of a 10% methanolic solution of KOH. Gently heat in a water bath for 2 minutes. Then cool and acidify with 1N HCl solution. Add 1 drop of 1% $FeCl_3$. The appearance of a violet color indicated a positive reaction.
Steroids and Triterpenoids	Dissolve a few milligrams of the extract in 10 mL of chloroform. Filtration was performed over activated charcoal. Then transfer the filtrate to a completely dry test tube. Add 3 drops of acetic anhydride and shake gently. Add drops of concentrated H_2SO_4 and shake gently again. If there is a rapid development of colors, ranging from fleeting blue to persistent green, it indicates a positive result.
Saponins	Dissolve approximately 0.08 g of the ethanol extract in 5 mL of hot water and vigorously shake for 2 minutes in a closed tube. If the foam layer remained stable for more than half an hour, the result was considered positive for foamy saponins.

Sequestering activity of 2,2-diphenyl-1 picrylhydrazyl radical (DPPH)

The antioxidant activity of the *C. jamacaru* extract was determined using the *in vitro* photocolorimetric method performed via free radical scavenging using DPPH (2,2-diphenyl-1-picrylhydrazyl). The samples were prepared by adding 3.9 mL of DPPH solution (60 µM) in 100 µl of extract solutions, which were diluted in methanol at µM/mL concentrations, in triplicate. After the reaction time of 30 minutes, the absorbances of the prepared samples were read on the UV-Vis spectrophotometer with the wavelength adjusted to 515 nm. As a negative control, a mixture of 3.9 mL of DPPH solution and 100 µL of control solution (Trolox) was used. The antioxidant activity of the samples was expressed in IC_{50} (inhibitory concentration), which was defined as the mg mL^{-1} of sample required to inhibit the formation of DPPH radicals by 50% (Mensor *et al.* 2001; Sousa *et al.* 2007).

2,2'-Azino-bis-(3-ethylbenzothiazoline)-6-sulfonic radical (ABTS)

The sequestering activity of the ethanolic extract was determined using the methodology described by Rhee *et al.* (2001). The ABTS radical solution (ABTS•+) was prepared by reacting 5 mL of ABTS solution (7 mmol L^{-1}) with 88 µL of K_2SO_4 solution (140 mmol L^{-1}) and allowing the mixture to stand in the dark (at room temperature) for 12-16 hours before use. For the assay, the ABTS+ solution was diluted with 5 mmol L^{-1} PBS buffer solution (pH 7.4) to an absorbance of 0.7 (±0.02) at 734 nm. A sample of 10 µL (500 mg mL^{-1}) was mixed with 1 mL of diluted ABTS+ solution and the absorbance (at

734 nm) was measured after 6 min. The percentage decrease in absorbance was calculated compared to that of the controls. The antioxidant activity value of the extract was compared with the BHT control (synthetic antioxidant). The result was also expressed in milligrams of equivalent.

Total phenolic contents

The total phenolic content was determined using the Folin-Ciocalteau method, modified by Roesler *et al.* (2007), by which an extract was used at a ratio of 1:10 (w/v). The quantification process was minimized to a total volume scale of 1.0 mL. From this solution, 200 µL of the extract were taken, added to 800 µL of distilled water, 1 mL of Folin-Ciocalteau reagent and 2 mL of 20% sodium carbonate. The absorbance (Abs) of the liquid fraction was determined at 760 nm in a spectrophotometer (Biochrom Libra S12). The result was expressed in gallic acid equivalents (mg GAE.100 g^{-1}).

All readings were performed in triplicate and, with the means of the data, the difference in absorbance between the samples and the control was calculated, with the antioxidant activity (AA) percentages determined using Equation (1).

Inhibition of activity (%)= (1-B)/A x 100 (1)

Where:

A = Absorbance of control solution.

B = Absorbance of the solution in the presence of the extract.

Toxicity test using *Artemia salina*

In a round-shaped aquarium that served as an incubator, an artificial saline solution (20 g of sea salt per 1.0 L of distilled water) was added and exposed to the light of a 40 W lamp with aeration, and with a pH of between 8 and 9. After 24 hours, the nauplii (10 units) were placed in test tubes containing the extract of *C. jamacaru* dissolved in DMSO (dimethylsulfoxide) 1% and supplemented with 5 mL of artificial seawater. Concentrations of extracts of 2,250, 2,000, 1,500, 1,250, 1,000, 500, 250 and 125 µg.mL^{-1} were tested in triplicate. DMSO was used as the positive control, and was prepared in a similar way to the samples. After 24 hours, the number of dead and living nauplii was counted and the percentage of mortality calculated (Meyer *et al.* 1982).

Determination of antimicrobial activity

Following the Clinical and Laboratory Standards Institute (CLSI, 2009) the determination of antimicrobial activity and minimum inhibitory concentration (MIC) were performed using the disc diffusion method. For the bacteria, standard strains (ATCC) were used, with Gram-positive: *Staphylococcus aureus* (ATCC25923), *Enterococcus faecalis* (ATCC00531), *Bacillus cereus* (ATCC9634) and *Listeria monocytogenes* (ATCC7644), and Gram-negative: *Escherichia coli* (ATCC10536), *Klebsiella pneumoniae* (ATCC700603) and *Enteric Salmonella* (ATCC13076). For the evaluation of antifungal activity, a standard strain of the American Type Culture Collection (ATCC), *Candida albicans* (ATCC10231), was used. The microorganisms were cultured and maintained in tryptone soy agar (TSA) and incubated at 37 ºC for 24 hours. The density of the microbial suspension was adjusted to approximately 108 CFU/mL by comparing it with the MacFarland scale (Biomerieux, Italy). This

suspension was diluted in sterile saline (0.85%) and, subsequently, the microorganisms were made into a carpet, adding sterile filter paper discs (6 mm Ø) with an aliquot of 20 µL of the extract in certain concentrations, ranging from 500 to 100 µg mL-1 (Rabanal *et al.* 2002; Karaman *et al.* 2003). For the disc diffusion test, halos with a diameter ≥ 6 mm were considered inhibitory. The antimicrobials amoxilin, vancomycin and fluconazole discs were used as the positive control.

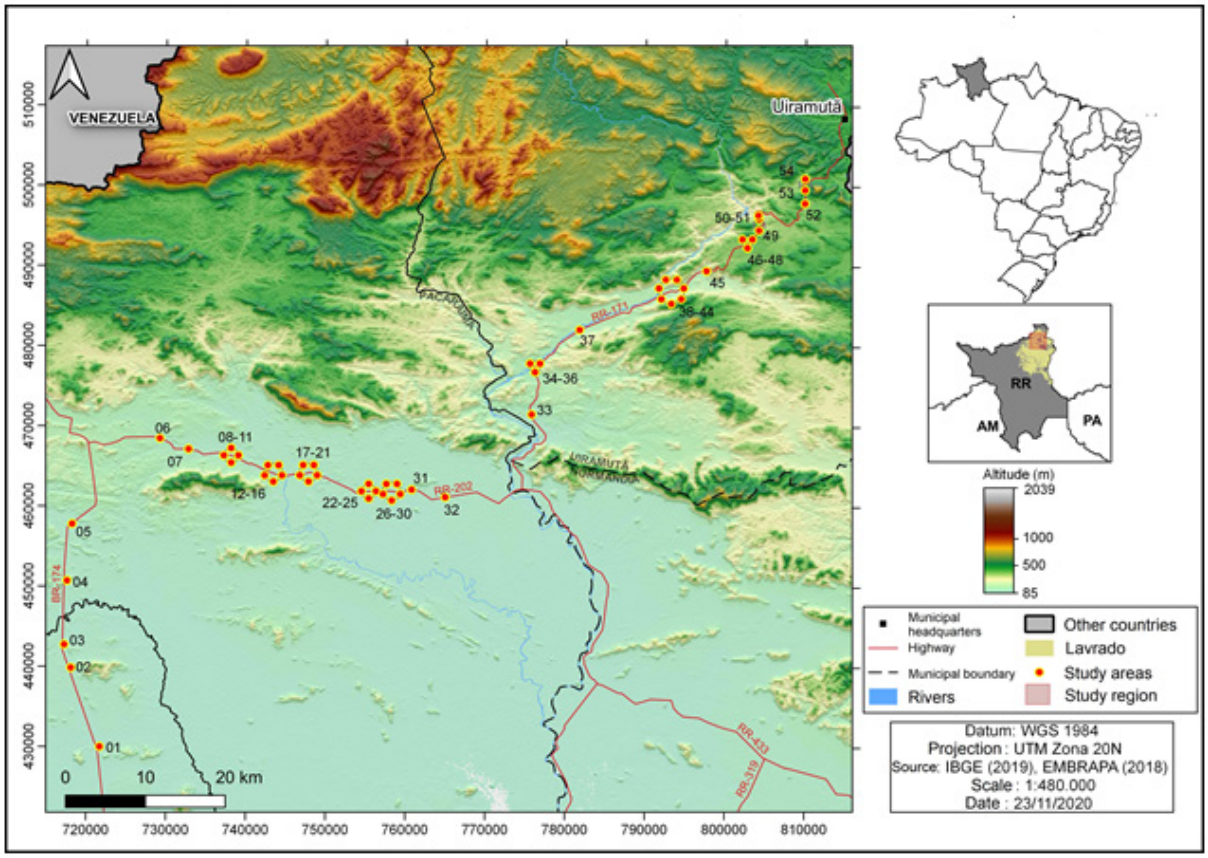
Results and discussion

Below we present the results and discussions regarding the phytochemical prospection, chemical profile and biological activities of the ethanolic extract of *C. jamacaru* from the "lavrado" in Roraima.

Mapping the occurrence of cacti in the lavrado

The occurrences of cacti were recorded along a route of 290 km, within the five-meter limit of the verges, on the BR-174, RR-202 and RR-171 highways, totaling 54 points (Figure 3). The mandacaru (*C. jamacaru*) proved to be the most abundant in this study area, as its occurrence was recorded at all points. Only at point 2 of the route were two other species found, namely *Melocactus neryi* and *Cereus paraensis*.

Figure 3. Location of occurrence points of cacti on the verges of the highway between Boa Vista and Uiramutã, Roraima.



Samples of mandacaru cladodes were collected on the banks of the BR-171 in the municipality of Uiramutã, Roraima, at point 33 of the map at 04°15' 32.01" N 60° 30' 51.12" W. After collection, the plant material was sent to the herbarium at the Federal University of Roraima in order to obtain botanical identification and the ethanol extract in the laboratory. Collection Request – SISBIO - Number: 87135

Extract yield

From 320 grams of powdered cladodes of *C. jamacaru*, we obtained 1,324 grams of ethanolic extract, which is equal to a yield of approximately 0.41%.

Phytochemical prospection

The metabolites found in the crude ethanolic extract of cladodes were phenols, tannins, alkaloids, flavonoids, sesquiterpene lactones saponins, steroids, triterpenoids and flavones (Table 2).

Table 2. Results of phytochemical prospecting for secondary metabolites in the ethanolic extract of *C. jamacaru* cladodes.

Secondary metabolites	Results	Secondary metabolites	Results
Phenols	+	*Saponins*	+
Tannins	+	*Steroids*	+
Alkaloids	+	*Triterpenoids*	+
Flavonoids	+	*Flavones, lavonols, chalcones, aurones and isoflavones*	+
Sesquiterpene lactones and other lactones	+		

(-) Absence; (+) Presence

The results obtained in the prospection provide information on the chemical classes that make up the extracts. However, it is worth mentioning that there are variations in the profile of secondary metabolites of plant species that have been attributed to biotic and abiotic factors (Hernandez *et al.* 2022) The presence of tannins and phenolic compounds in the present study corroborate the studies of Dias, Simão, Verib and Carasek (2013) who detected these two substances in the aqueous methanolic extract of the peel of manacaru fruits (*Cereus fernambucenses*), a species belonging to the same family as *C. jamacaru*. The results are also similar to the studies conducted by Felker *et al.* (2005), Davet *et al.* (2009) and Medeiros *et al.* (2019), who detected the presence of tannins, alkaloids, anthraquionones, phenol and flavonoids in the cladodes of *C. jamacaru*.

The results of this prospection corroborate the studies of De Almeida *et al.* (2022), who found the presence of phenols, flavones, flavonols, xanthones and saponins in the ethanolic extract of *C. jamacaru* cladodes. Unlike in the present study, terpenoids were only found in the peel and pulp of ripe and semi-ripe fruits. The authors also detected tannins in their samples.

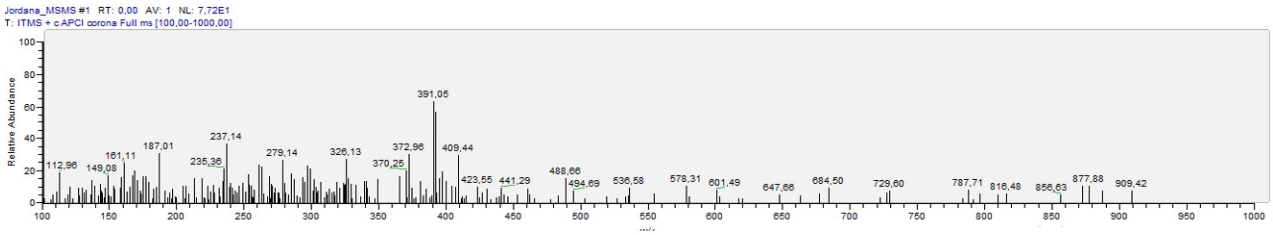
Phytochemicals such as flavonoids, tannins and alkaloids have anti-inflammatory properties. El-Beltagi *et al.* (2019) attributed this good antibacterial activity to the tannins present in the cactus

Opuntia ficus-indica. Alkaloids have been reported to be responsible for antibacterial activity in some plants. This probably explains the reason why plants containing these basic alkaloids and alkaloid salts have good antibacterial properties (El-beltagi *et al.* 2019).

Chemical profile

The spectrum of the chemical profile of the ethanolic extract of *C. jamacaru* obtained by APCI-MS is represented in Figure 4.

Figure 4. APCI-MS mass spectra of the ethanolic extract of *C. jamacaru.*



The possible compounds identified in the positive and negative ionization modes in the fragments are described in Table 3.

Table 3. Compounds identified in the ethanolic extract of *C. jamacaru.*

Possible identification	m/z	MS/MS	Reference
Quercetin	301	179; 151	Campelo *et al.* (2021)
Quercertin hexoside	463	301	Campelo *et al.* (2021)
Quercetin-3-O-arabinoyl glycoside	595	301	Campelo *et al.* (2021)
Quercetin di-deoxyhexose	755	446	Campelo *et al.* (2021)
(Epi) catechin - (Epi) catechin (procyanidin B IV)	577	451; 425; 407; 289; 287	Campelo *et al.* (2021)
Catechin	289	125; 179; 205; 231; 245	Campelo *et al.* (2021)
P-coumaric acid	165	119; 147	Campelo *et al.* (2021)
Caffeic acid	179	135	Campelo *et al.* (2021)
Ferulic acid	193	134; 149; 178	Campelo *et al.* (2021)
Apigenin-6-C-glucoside (isovitexin)	431	269; 341	Campelo *et al.* (2021)
Taxifolin hexoside	435	285; 303; 399	Campelo *et al.* (2021)
Naringenin hexoside II	433	271; 313; 415	Campelo *et al.* (2021)
Kaempferol 3-rutinoside-7-rhamnoside	739	593	Campelo *et al.* (2021)
Kaempferol-3-O-rutinoside	593	285, 255	Campelo *et al.* (2021)
Benzoic acid derivative	205	143, 125, 81	Campelo *et al.* (2021)
Benzoic acid derivative	411	205, 143, 125, 81	Campelo *et al.* (2021)

Benzoic acid derivative	369	205, 125	Campelo *et al.* (2021)
Saccharide	371	249, 231, 175, 113	Campelo *et al.* (2021)
Isorhamnetin-3-O-dirhamnosyl glucoside	769	623, 315	Campelo *et al.* (2021)
Isorhamnetin-3-O-rutinoside	623	315, 300	Campelo *et al.* (2021)
Oxo-dihydroxy-octadecenoic acid	327	229, 211	Campelo *et al.* (2021)
L-arginine	175	116	Campelo *et al.* (2021)
3-O-methylquercetin	317	301; 274; 273	Campelo *et al.* (2021)
Sucrose	381	201; 219	Campelo *et al.* (2021)
Apigenin mono-C-glycosidic	433	361; 349; 337; 323	Campelo *et al.* (2021)
Myricetin-hexose	743	431; 611; 743	Campelo *et al.* (2021)
D-tyramine	137.2		Schwartz *et al.* (2010)
N-methyltyramine	151		Schwartz *et al.* (2010)
Hordenine	165.2		Schwartz *et al.* (2010)
D-tyrosine	181.2		Schwartz *et al.* (2010)
Oleic acid	282.4		Schwartz *et al.* (2010)
Acetic acid	60.1		Schwartz *et al.* (2010)
Camphor	152.2		Schwartz *et al.* (2010)
Cysteine	121.2		Schwartz *et al.* (2010)
Geranylacetone	194		Schwartz *et al.* (2010)

In the class of flavonols, the presence of quercetin glycosylates, kaempferol, myricetin and isorhamnetin is widely associated with several beneficial health effects, as they present antioxidant, anticancer, anti-inflammatory, antiviral, cardioprotective, and other properties (Wang *et al.* 2018). Phenolic acids, among them caffeic acid, ρ-coumaric acid and ferulic acid, stand out for being some of the most studied phenolic compounds in cactus species due to their antioxidant action and potential to prevent or delay the appearance of symptoms of transmissible diseases (Del Socorro and Camarena 2019).

The main compounds that are produced by cacti and which have nutritional or pharmacological properties are polyphenols, alkaloids, betalains, terpenes and fatty acids (DAS *et al.* 2020). Hordenine, N-methyltyramine and tyrosine are some of the most commonly detected alkaloids in cacti. According to Vencioneck Dutra *et al.* (2018), the alkaloids tyramine and N-methyltyramine are compounds produced by *C. jamacaru* that are chemical markers of this species. Such compounds were identified in the present study with an m/z 137.2 and 151, respectively.

Oleic acid is known for its beneficial health effects, and is an unsaturated fatty acid that can prevent cardiovascular disease (Bakari *et al.* 2017). Vitamins can be found in both the pulp and skin of cacti (El-beltagi *et al.* 2019). Previous studies have indicated that there is a higher concentration of vitamin C (ascorbic acid) in the fruit of cacti (*Opuntia ficus-indica*) than that found in common fruits such as apple, banana or grape. Vitamin C is an important antioxidant and reduces the adverse effects of reactive oxygen species that cause damage to macromolecules, such as lipids, DNA and proteins, which are related to cardiovascular disease, cancer and neurodegenerative diseases.

Antioxidant activity

The values obtained in the radical sequestration methods (DPPH and ABTS) and total phenolic contents are presented in Table 4.

Table 4 - Antioxidant activity of the ethanolic extract of cladodes from *C. jamacaru.*

	Phenolic Compounds Total (mg GAE 100 g^{1})	DPPH Radical IC_{50} (mg mL^{-1})	ABTS Radical (µMTrolox g^{-1})
Ethanolic Extract	102.4 ± 0.05	69.8 ± 0.2	1.843,4 ± 5,74
Gallic Acid	2,931.04 ± 8.8	-	-
Ascorbic Acid	-	100.2 ± 2.26	1.978,4 ± 7,12
BHT	-	94.5 ± 3.4	1.368,7 ± 4,98

(-) Absence
BHT: butylated hydroxytoluene

DPPH and ABTS assays are methods used to measure the antioxidant's ability to scavenge free radicals, which are the main factor in biological damage caused by stress. In studies conducted by Vencioneck Dutra *et al.* (2018), the hydroalcoholic extract of *C. jamacaru* was able to inhibit the activity of DPPH radicals by up to 57.36% and ABTS antioxidant activity by up to 65.76% when compared to the standards. The results of the present study were even more satisfactory, the ethanolic extract of the cladodes from *C. jamacaru* showed antioxidant activity of 92.3% for the ABTS method and 63.8% for the DPPH method. El-beltagi *et al.* (2019) attributed the excellent antioxidizing activity of cactus extract to flavonoids. The authors claim that flavonoids are more efficient antioxidants than vitamins, since phenolic compounds are able to slow down pro-oxidative effects on proteins, DNA and lipids via the generation of stable radicals.

A study conducted with the alkaloid tyramine, a compound detected in the extract from *C. jamacaru*, showed strong sequestering activity in the DPPH assay, as well as reducing power, reaching an 86.34% inhibition of the DPPH radical, which may have contributed to the overall antioxidant activity (Yen and Hsieh 1997). The phenolic levels of the cladodes from *C. jamacaru* were lower than the results obtained by Santos-Zea *et al.* (2011) who found a minimum of 318.1 mg kg^{-1} and 1 g kg^{-1}, and Medina-Torres *et al.* (2013) who found 1 g kg^{-1} in the cladodes of Mexican cacti using methanol as an extractive vehicle. De Wit *et al.* (2019) observed that the total phenolic contents are always higher in the stem and seeds of cacti than in their fruits. In this study, levels of phenolic compounds in the cladodes were 102.4 ± 0.05 mg GAE.100 g^{-1}. A possible explanation for obtaining lower levels is the use of ethanol as an extractive vehicle. Methanol has a higher polarity than ethanol due to its hydrocarbon chain being smaller than that of ethanol.

Determination of antimicrobial activity (AMA)

There was no inhibition of microbial activity with the *C. jamacaru* ethanolic extract at the concentrations tested. Unlike the present study, Davet *et al.* (2009) demonstrated the antibacterial potential of the ethanolic extract of mandacaru stems against *Streptococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The reason why the extract did not show halo

inhibition can be attributed to the concentration tested. Rabe and Staden (1997) relate the absence or slight activity in some extracts to the low concentrations of antibacterial compounds in the extracts.

Leyva-Jimenez *et al.* (2018) tested the 80% ethanol extract of the peel of the fruit of *C. jamacaru* and the best inhibition halo obtained was of 10 mm and, of the eight bacteria tested, the extract inhibited only three. The authors attributed to the inhibition of microbial growth to the presence of certain flavonoids such as rutin or chrysin. Flavonoids are effective against microbial pathogens, since they are a phenolic hydroxyl compound; as the number of -OH groups increase in the natural structure of the compound, toxicity to microorganisms increases (Bardakci *et al.* 2019).

Toxicity test

In the toxicity tests, the number of dead nauplii was confirmed by visualization with the aid of a magnifying glass after 24 hours, and it was found that the nauplii still showed movement.

The toxicity test against *A. salina* was performed with the ethanolic extract. The bioassay was performed in triplicate, and live, dead, or paralyzed nauplii were counted, and then mortality was determined at a concentration between 2,250 μg mL^{-1} and 125 μg mL^{-1}. Once the mortality count is determined, toxicity is considered low when the 50% lethal dose (LD_{50}) is greater than 1,000 μg mL^{-1} and high when the LD_{50} is less than 1,000 μg.mL^{-1} (Meyer *et al.* 1982). With the determination of the data, it was observed that the ethanolic extract does not present lethality against *A. salina*, as there was no mortality in the lethal dose range.

This result demonstrates that the *C. jamacaru* extract has no toxicity. Unlike the study by Sánchez *et al.* (2016), who evaluated the toxicity of the extract of *O. ficus-indica*, also a member of the *Cactaceae* family and popularly known as the "prickly pear", and found that a concentration of 100 μg/mL was able to eliminate about 60% of the microcrustaceans.

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Conclusions

The phytochemical prospecting of *C. jamacaru* cladodes revealed the presence of various classes of compounds, including phenols, tannins, alkaloids, flavonoids, sesquiterpene lactones, and saponins. Significant antioxidant properties were highlighted, especially concerning the ABTS and DPPH methods. Mass spectrometer analysis confirmed the presence of compounds such as hordenine and N-methyltyramine, chemical markers of the species, in addition to identifying oleic acid, vitamin C, and other health-beneficial compounds. Despite the absence of antimicrobial activity at the tested concentrations, the ethanolic extract demonstrated safety concerning A. salina, indicating promising potential for use as a source of natural antioxidants in food and pharmaceutical applications.

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Data availability: The manuscript was based on data from the thesis of the first author, which after the defense will be made available on the page of the Graduate Program in Natural Resources of the Federal University of Roraima (Teses (ufrr.br)) and in the Catalog of Capes theses (Catálogo de Teses & Dissertações - CAPES)

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References

Bakari S, Daoud A, Felhi S, Smaoui S, Gharsallah N, Kadri A. 2017. Análise aproximada, composição mineral, conteúdo fitoquímico, atividades antioxidantes e antimicrobianas e GC- Investigação de MS de vários extratos de solventes de cladódio de cacto. Ciência Alimentar. Tecnol. 37(2): 286-293. https://doi.org/10.1590/1678-457X.20116

Barbosa WLR, Quinard E, Tavares, ICC, Pinto LN, Oliveira, FQ, Oliveira RM. 2004. Manual para análise fitoquímica e cromatográfica de extratos vegetais (Edição Revisada). Revista Científica da UFPA, 4.

Barbosa RI, Campos C.2011. Detection and geographical distribution of clearing areas in the savannas ('lavrado') of Roraima using Google Earth web tool. Journal of Geography and Regional Planning 4(3): 122.

Bardakci H, Celep E, Gözet T, Kurt-Celep I, Deniz I, Şen-Utsukarci B, Akaydin, GALİP. 2019. A comparative investigation on phenolic composition, antioxidant and antimicrobial potentials of Salvia heldreichiana Boiss. ex Bentham extracts. South African Journal of Botany 125: 72-80. https://doi.org/10.1016/j.sajb.2019.07.010

Barthlott W, Hunt DR, 1993 Cactaceae. In: Kubiztki, K; Rohwer, JG; Bittrich, V (Ed.). The families and genera of vascular plants. Berlin: Springer-Verlag, 161-197.

Brasil. Departamento de Infraestrutura de Transportes. Lei nº 13.913, de 25 de novembro de 2019. Altera a Lei nº 6.766, de 19 de dezembro de 1979, para assegurar o direito de permanência de edificações na faixa não edificável contígua às faixas de domínio público de rodovias e para possibilitar a redução da extensão dessa faixa não edificável por lei municipal ou distrital. Diário Oficial da União, Brasília, DF. 2019. Available at: <http://www.planalto.gov.br/ccivil_03/_ato2019-2022/2019/lei/L13913.htm>. Accessed on: 26 Nov. 2019.

Brasil. Departamento Nacional de Infraestrutura de Transportes. Resolução nº 9, de 12 de agosto de 2020. Dispõe sobre o uso das faixas de domínio de rodovias federais sob circunscrição do departamento nacional de infraestrutura de transportes. Diário Oficial da União, Brasília, DF. 2020. Available at: http://www.planalto.gov.br/ccivil_03/_ato2019-2022/2019/lei/L13913.htm. Accessed on: 26 Nov. 2019.

Campelo FA, Henriques G, Simeone M, Queiroz V, Ramos A, Silva M, Araújo RLM. 2021. Características químicas e nutricionais de dois genótipos de sorgo, depois da extrusão termoplástica. In: MELO, J. O. F. (org.). Ciências Agrárias: o avanço da ciência no Brasil. Guarujá: Científica Digital 1: 234-260.

CLSI, Clinical and Laboratory Standards Institute, 2009. Performance Standards for Antimicrobial Disk Susceptibility Test; Approved Standard-Tenth Edition. Wayne, CLSI document M02-A10.

Da Silva AB, De Brito JM, De Araújo Silva R, Braz AS, Da Silva Filho ED. 2017. Parâmetros físico-químicos da água utilizada para consumo em poços artesianos na cidade de Remigio-PB 31(2): 109-118. https://doi.org/10.14295/ras.v31i2.28807

Das G, Lim KJ, Tantengco OAG, Carag H M, Gonçalves S, Romano A, Das SK, Coy-Barrera E, Shin HS, Gutiérrez-Grijalva EP, Heredia J B, Patra J K.2021. Cactus: Chemical, nutraceutical composition and potential bio-pharmacological properties. Phytotherapy Research: PTR 35(3): 1248–1283. https://doi.org/10.1002/ptr.6889

Davet A, Virtuoso S, Dias JF, Miguel, MD, Oliveira AB, Miguel OG. 2009. Atividade antibacteriana de *Cereus jamacaru* DC, Cactaceae. Revista Brasileira de Farmacognosia 19: 561-564. https://doi.org/10.1590/S0102-695X2009000400009

De Almeida MMV, Araújo FDS, Andrade AP, Aquino PGV, Valença RDL, Bruno RDLA. 2022. Nutritional Potential and Bioactive Compounds in *Cereus jamacaru* DC: An Multifaceted Cactus From Brazilian Semiarid. Journal of Agricultural Studies 10(1): 68-81. https://doi.org/10.5296/jas.v10i1.19006

De Medeiros IU, De Medeiros RA, Bortolin RH, De Queiroz FM, Silbiger V N, Pflugmacher S, Schwarz A. 2019. Genotoxicity and pharmacokinetic characterization of *Cereus jamacaru* ethanolic extract in rats. Bioscience Reports 39(1). https://doi.org/10.1042/BSR20180672

De Wit M, Du Toit A, Osthoff G, Hugo A. 2019. Cactus pear antioxidants: A comparison between fruit pulp, fruit peel, fruit seeds and cladodes of eight different cactus pear cultivars (*Opuntia ficus-indica* and *Opuntia robusta*). Journal of Food Measurement and Characterization 13: 2347-2356. https://doi.org/10.1007/s11694-019-00154-z

Dias AN, Simão V, Merib J, Carasek E. 2013. Cork as a new (green) coating for solid-phase microextraction: Determination of polycyclic aromatic hydrocarbons in water samples by gas chromatography–mass spectrometry. Analytica Chimica Acta 772: 33-39. https://doi.org/10.1016/j.aca.2013.02.021

El-Beltagi HS, Mohamed HI, Elmelegy A A, Eldesoky SE, Safwat G. 2019. Phytochemical screening, antimicrobial, antiaxidant, anticancer activities and nutritional values of cactus (*Opuntia ficus indicia*) pulp and peel. Fresenius environmental bulletin 28(2A):1534-1551.

Felker P, Rodriguez SDC, Casoliba RM, Filippini R, Medina D, Zapata R. 2005. Comparison of *Opuntia ficus indica* varieties of Mexican and Argentine origin for fruit yield and quality in Argentina. Journal of Arid Environments 60(3): 405-422.

Hernández F, Andreu-Coll L, Bento-Silva A, Serra A T, Mena P, Legua P, Bronze MR. 2022. Phytochemical Profile of *Opuntia ficus-indica* (L.) Mill Fruits (cv. 'Orito') Stored at Different Conditions. Foods 11(2): 160. https://doi.org/10.3390/foods11020160

Karaman I, Şahin F, Güllüce M, Ögütçü H, Şengül M, Adıgüzel A. 2003. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. Journal of Ethnopharmacology 85(2-3): 231-235.

Leyva-Jimenez FJ, Lozano-Sanchez J, Borras-Linares I, de la Luz Cadiz-Gurrea M, Mahmoodi-Khaledi E. 2019. Potential antimicrobial activity of honey phenolic compounds against Gram-positive and Gram-negative bacteria. LWT 101: 236-245. https://doi.org/10.1016/j.lwt.2018.11.015

Matos, FJA. Introduction to Experimental Phytochemistry. 2nd ed. Fortaleza: Edições UFC, 2001.

Medina-Torres L, García-Cruz E E, Calderas F, Laredo RG, Sánchez-Olivares G, Gallegos-Infante JÁ, Rodriguez-Ramirez J. 2013. Microencapsulation by spray drying of gallic acid with nopal mucilage (*Opuntia ficus indica*). LWT-Food Science and Technology 50(2): 642-650. https://doi.org/10.1016/j.lwt.2012.07.038

Martins SA, Rodrigues NRDS, Santos RVM, Moraes CM, Melo JA, Nascimento FC. 2022. Phytochemical prospection and biological activity of the ethanolic extract of the leaves of *Curatella americana* L. (dilleniaceae).International Journal of Development Research 12(05): 55735-55738. https://doi.org/10.37118/ijdr.24411.05.2022

Mensor LL, Menezes FS, Leitão GG, Reis AS, Santos TCD, Coube CS, Leitão SG. 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy Research 15(2): 127-130. https://doi.org/10.1002/ptr.687

Miranda, IS, Absy, ML. 2000. Fisionomia das savanas de Roraima, Brasil. Acta Amazonica 30: 423-423. https://doi.org/10.1590/1809-43922000303440

Meyer BN, Ferrigni, N R, Putnam JE, Jacobsen LB, Nichols DJ, McLaughlin JL. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica 45(05): 31-34.

Necchi RM, Alves IA, Alves S H, Manfron MP. 2012. In vitro antimicrobial activity, total polyphenols and flavonoids contents of *Nopalea cochenillifera* (L.) Salm-Dyck (Cactaceae). Research in Pharmacy 2(3): 01-07.

Oliveira RLC. 2016. Uso e conhecimento das espécies lenhosas em uma Comunidade Indígena na savana de Roraima. Tese de Doutorado. Programa de Pós-Graduação em Botânica. Instituto Nacional de Pesquisas da Amazônia - INPA. Manaus, AM, 123 p.

Passos MAB. 2019. Plantas alimentícias nío convencionais (PANC) ocorrentes em roraima. Revista Eletrônica Científica Ensino Interdisciplinar 5(14): 388-404.

Rabanal RM, Arias A, Prado B, Hernández-Pérez M, Sánchez-Mateo CC. 2002. Antimicrobial studies on three species of Hypericum from the Canary Islands. Journal of Ethnopharmacology 81(2): 287-292.

Rabe T, Van Staden J. 1997. Antibacterial activity of South African plants used for medicinal purposes. Journal of Ethnopharmacology 56(1): 81-87. https://doi.org/10.1016/S0378-8741(96)01515-2

Rebah FB, Siddeeg SM. 2017. Cactus an eco-friendly material for wastewater treatment: A review. Journal of Materials and Environmental Sciences 8(5): 1770-1782.

Rhee IK, Van de Meent M, Ingkaninan K, Verpoorte R. 2001. Screening for acetylcholinesterase inhibitors from Amaryllidaceae using silica gel thin-layer chromatography in combination with bioactivity staining. Journal of Chromatography A 915(1-2): 217-223. https://doi.org/10.1016/S0021-9673(01)00624-0

Roesler R, Malta LG, Carrasco LC, Holanda RB, Sousa CAS, Pastore GM. 2007. Atividade antioxidante de frutas do cerrado. Food Science and Technology 27: 53-60. https://doi.org/10.1590/S0101-20612007000100010

Santos-Díaz MDS, Camarena-Rangel NG. 2019. Cacti for production of metabolites: current state and perspectives. Applied Microbiology and Biotechnology 103: 8657-8667. https://doi.org/10.1007/s00253-019-10125-5

Santos-Zea L, Gutiérrez-Uribe JA, Serna-Saldivar SO. 2011. Comparative analyses of total phenols, antioxidant activity, and flavonol glycoside profile of cladode flours from different varieties of *Opuntia spp.* Journal of Agricultural and Food Chemistry 59(13): 7054-7061. https://doi.org/10.1021/jf200944y

Schwarz A, Medeiros I, Mourão C, Queiroz F, Pflugmacher S. 2010. Phytochemical and toxic analysis of an ethanol extract from *Cereus jamacaru*. Toxicology Letters (196): S344.

Sousa CMDM, Silva H R, Ayres MCC, Costa CLSD, Araújo DS, Cavalcante LCD, Chaves MH. 2007. Fenóis totais e atividade antioxidante de cinco plantas medicinais. Química Nova 30: 351-355. https://doi.org/10.1590/S0100-40422007000200021

Vencioneck Dutra JC, Moisés Ferreira J, Costalonga Pereira PR., Ben-Hur de Oliveira J, Vitorino Gervásio S, Bernardes Xavier M, Pimentel Batitucci MDC. 2018. *Cereus jamacaru* DC hydroalcoholic extract promotes anti-cytotoxic and antitumor activity. Pharmaceuticals 11(4): 130. https://doi.org/10.3390/ph11040130

Wang TY, Li Q, Bi KS. 2018. Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. Asian Journal of Pharmaceutical Sciences 13(1): 12-23. https://doi.org/10.1016/j.ajps.2017.08.004

Yen GC, Hsieh CL. 1997. Antioxidant effects of dopamine and related compounds. Bioscience, Biotechnology, and Biochemistry 61(10): 1646-1649. https://doi.org/10.1271/bbb.61.1646

Yin C, 2010. Emerging usage of plant-based coagulants for water and wastewater treatment. Process Biochemistry 45(9): 1437-1444. https://doi.org/10.1016/j.procbio.2010.05.030

Zappi D, Taylor NP. 2020.Cactaceae in Flora do Brasil 2020. Jardim Botânico do Rio de Janeiro. Available at: <http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB70>. Accessed on: 12 Mar. 2021.

Zara RF, Thomazini MH, Lenz GF. 2012.Estudo da eficiência de polímero natural extraído do cacto mandacaru (*Cereus jamacaru*) como auxiliar nos processos de coagulação e floculação no tratamento de água. Revista de Estudos Ambientais 14(2): 75-83. http://dx.doi.org/10.7867/1983-1501.2012v14n2p75-83