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Biological Assessments of *Baccharis dracunculifolia* DC from a Cerrado fragment in Central-West Brazil

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Abstract

Baccharis dracunculifolia is used as an herbal medicine, and green propolis, synthesized by Apis mellifera bees from it, has inflammatory, antioxidant and antimicrobial principles. This bee product is effective in combating a series of microorganisms in folk medicine, so it is essential to develop research to prove this effectiveness. The objectives of the work were to analyze the microbiological profile, dose the flavonoids and verify the plant's antioxidant activity. The aerial parts were collected in a fragment of cerrado sul-mato-grossense and processed, obtaining extracts with hexane, ethyl acetate and ethanol. For microbiological analysis, Staphylococcus aureus bacteria was seeded in agar and filter discs were soaked with the extracts and, together with the control group, were placed in Petri dishes for subsequent measurement of inhibition halos. To measure the flavonoids and evaluate the antioxidant activity,

processed leaves were taken to Soxhlet for extraction in methanol. An aliquot was read in a spectrophotometer with different concentrations of quercetin to build a standard curve. The antioxidant assay was based on the radical reduction and development method using DPPH. As a result, all extracts showed inhibition of bacterial growth, indicating antimicrobial activity of the plant. Flavonoids with satisfactory antioxidant activity were also found. Taking into account that B. dracunculifolia is used as a medicinal plant, studies such as this one can corroborate with such use, since the scientific proof of possible herbal medicines is essential for the pharmaceutical industry and for health professionals, contributing to the evolution of medicine.

Keywords: *antimicrobial activity; antioxidant activity; Baccharis dracunculifolia; flavonoids.*

1. Introduction

The botanical family Asteraceae is one of the largest families of angiosperms and although cosmopolitan, it is generally dominant in arid and temperate vegetation (RIVERA *et al*., 2019). This family is known for its therapeutic, cosmetic and aromatic properties and its medicinal use is anthelmintic, astringent, antihemorrhagic, antimicrobial, diuretic, analgesic and antispasmodic (FABRI *et al*., 2011).

Extracts and essential oils obtained from species of this family have shown, in addition to antioxidant and anticancer activity, antimicrobial activity, and other species of this family have been described as having anti-inflammatory activities (LUCENA *et al.,* 2019). Many specimens of Asteraceae are used in folk medicine because of their important physiological resources, evidenced in several literatures, and thus enthusiasm for the search for extracts with a relevant medicinal profile within this family is established. An example of this is the growing interest in the plant *Baccharis dracunculifolia* DC, in terms of its various biological properties (SOARES *et al*., 2013; DE ABREU; ONOFRE, 2010).

This plant is popularly known as alecrim-do-campo, vassourinha, vassourinha-do-campo (FAGUNDES, 2005) and is used as a medicinal plant, where substances with antimicrobial activity have already been identified in its aerial structures, in addition to flavonoids that, by present antioxidant activity, may contribute to the prevention of cardiovascular diseases, aging and cancers (SALGUEIRO; CASTRO, 2016).

The research on flavonoids is necessary and is stimulated by "having a broad spectrum of biological activities" and these compounds have been highlighted as natural preventive or therapeutic agents (DERAKHSHANIAN *et al*., 2020). At the same time, studies of natural antioxidants have become a trend within the pharmaceutical market, because in addition to being associated with health maintenance, they have great benefits for improving the quality of life, as they protect organisms from damage caused by free radicals, preventing or delaying the onset of several diseases (FERRERA *et al*., 2016).

The search for effective remedies and medicines is constant and the proof of these benefits is of paramount importance, since the use of plants for medicinal purposes is secular and corroborates the need to identify their biological potential, since a large portion of the poorest population makes frequent use of this type of medicine (VEIGA JUNIOR; PINTO; MACIEL, 2005).

Alecrim-do-campo has been used as an anti-inflammatory, antioxidant and bactericidal for many

years and studies that prove these possible attributions are considerable when analyzing individuals in some Brazilian biomes, however, in specimens inhabiting the Cerrado Sul-mato-grossense, such as studies are still lacking (LIMA *et al*., 2019). Faced with the discussion, a more in-depth investigation is needed, which can bring improvements to the medical and scientific field and to the health and economic development of the local population.

The objective of this study was to quantify the total flavonoid content to verify the existence of antioxidant activity of the species *Baccharis dracunculifolia* inhabiting the Cerrado Sul-mato-grossense, in addition to evaluating its antimicrobial effectiveness against bacteria of the species Staphylococcus aureus.

2. Methodology

This is an exploratory, field and experimental study whose approach was quantitative.

2.1 Collection of material

In a preserved Cerrado fragment, located in the peripheral region of Campo Grande, Mato Grosso do Sul, new leaves and in the process of senescence of three adult individuals of *Baccharis dracunculifolia* were randomly collected with the aid of pruning shears. After the leaves were dried in an oven with air circulation (40°C) and crushed in a cup grinder, the powder was sieved (ABNT 60 sieve), mixed and stored in amber flasks in which particles with a granulometry less than or equal to 0.250 mm of size were used in all experiments.

2.2 Preparation of extracts

600g of the dry material was submitted, in triplicate, for individual sequential extraction with 1000 ml of three extracting agents (hexane, ethyl acetate and ethanol – Dinâmica® brand). To ensure adequate extraction of the extracts, the mixtures were taken to a sonicator (Unique 1450®, Model Ultrasonic Cleaner®) and subjected to agitation for 10 minutes and rest for five minutes; the present cycle was repeated three times, until the depletion of the desired substances found in the plant (vegetable drug).

After extraction, the products were filtered, rotoevaporated and hexane extracts (ExtHex) were obtained; ethyl acetate (ExAcet) and ethanolic (ExtEtan). These were placed in amber containers until use, according to the methodology by Beraldo *et al.* (2015).

2.3 Antimicrobian activity

The strain of bacteria chosen for the antimicrobial test of Baccharis dracunculifolia extracts was Staphylococcus aureus ATCC 25923. The antimicrobial evaluation was performed according to De Abreu; Onofre (2010), using the Disc Diffusion method, using Whatman n°1 filter discs (6 mm in diameter) impregnated with the extracts obtained and distributed in Petri dishes containing previously seeded Müller-Hinton agar (Merck®) on the surface with the strain; controls (Gentamycin discs, Newprov®) were run in parallel. After the previous sowing, each plate received disks containing the three extracts produced, with their appropriate concentrations (10 uL) and identifications on the plates. After inoculation, they were

incubated for 24 hours at a temperature of 37ºC.

The tests were performed in triplicate and the results expressed in mm by the arithmetic mean of the diameter of the inhibition halos formed around the discs in the 3 repetitions. The diameters of the inhibition halos should be interpreted according to the criteria recommended by the Committee for Clinical Laboratory Standards International – CLSI.

2.3 Determination of total flavonoids

To determine total flavonoids, a methodology adapted from PELOI *et al*. (2016) and for this, 2g of dried and powdered leaves were extracted with 150 mL of 70% methanol (MeOH) in a Soxhlet® device for three hours. The extract was filtered and the volume made up to 250 ml.

A 15 ml aliquot was placed in a volumetric flask plus 1 ml of aluminum chloride solution (5 g of aluminum chloride in 100 ml of MeOH, the volume being made up to 50 ml). After resting for 30 minutes, readings were taken at 425 nm in a UV/visible spectrophotometer, and the analysis was performed in triplicate. The absorbance data of the samples were compared with a standard curve constructed from solutions with increasing concentrations of quercetin at concentrations of 0.2; 0.4; 0.6; 0.8; 1.0; 1.2; 1.4; 1.6; 1.8 and 2.0 mg/ml.

Quercetin 3,3',4',5,7-pentahydroxyflavone, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one, is a flavonoid known for its antioxidant and anti-inflammatory properties and used in comparative analyzes with flavonoid assays (DERAKHSHANIAN *et al*., 2020). The absorbance readings of the samples of the three extracts produced and the blank (MeOH + DPPH) were performed in a spectrophotometer.

2.3 Antioxidant activity

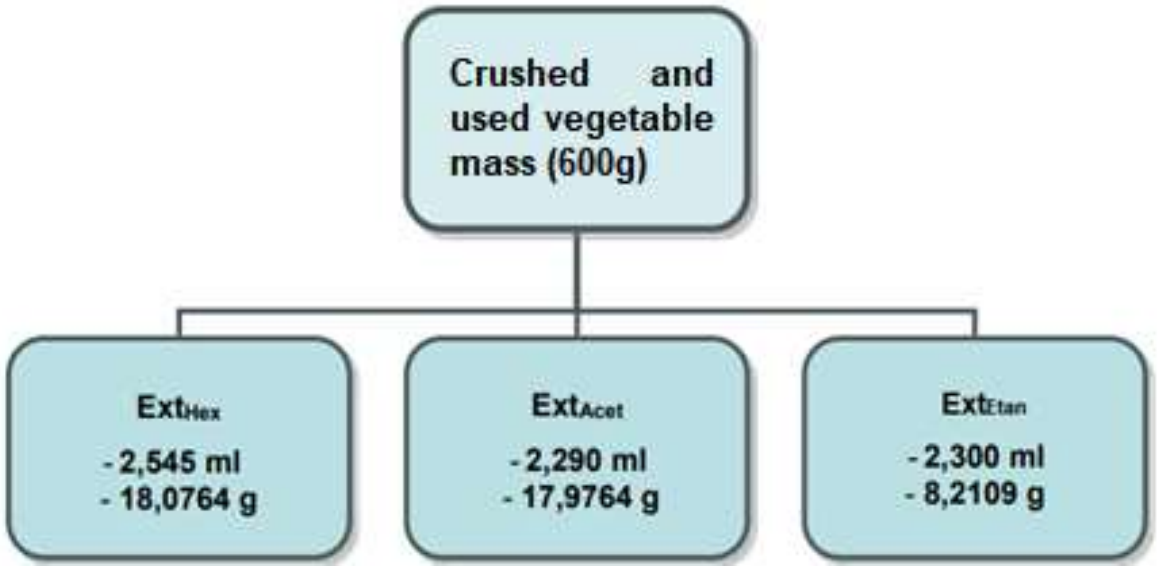
The antioxidant assay was based on the 2,2'-diphenyl-1-picryhydrazyl (DPPH) radical reduction method. To carry out this test, 10 µL of extract solution added to 1 mg/mL of methanol was applied to chromatosheets, which were revealed using 0.2% methanolic DPPH solution, by spraying (FABRI *et al*., 2011). The antioxidant activity is evidenced by the presence of white or yellow spots resulting from the reduction of DPPH, against the purple background color (SILVA, 2005). The oxidation that causes the aging of living tissue is directly related to potent oxidants present in the body, which are known as free radicals.

4. Results and discussion

4.1 Performance

At the end of the extractions, after filtration and evaporation of the solvents, three different extracts were obtained and their dry weights served as a basis for calculating the yield. The result of the extraction from the solvents Hexane, Ethyl Acetate and Ethanol were pooled for yield sampling (Flowchart 1).

Flowchart 1: Sampling yield of plant mass and extracts produced.



Source: Research data.

4.2 Sensitivity test

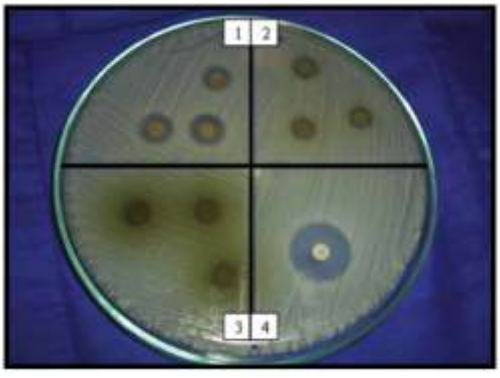
Different measures of inhibition halos were observed (Table 1) during the agar diffusion test (Figure 1) in all analyzed extracts of *B. dracunculifolia*. The control with Gentamicin showed an inhibition halo of 20 mm, indicating the sensitivity of the S. aureus test against the antibiotic. The microbial growth inhibition halos were measured in millimeters, with the aid of a millimeter ruler.

Table 1: Measurement of bacterial growth inhibition halos using ExtHex, ExAcet, ExtEtOH extracts from *B. dracunculifolia*.

Extracts (mg/ml)	Concentration used of extracts (µL)	Inhibition halos (mm)
Ext_{Hex}	10	14
Ex_{Acet}	10	10
Ext_{Etan}	10	9
Gentamicin Control	10	20

Source: Research data.

Figure 1: *S. aureus* growth-inhibiting halos and discs impregnated with 10 µL dilutions of ExtHex (1) extracts; ExAcet (2) and ExtEtan; (3) from *B. dracunculifolia*; standard – Gentamicin (4).



Source: Research data

The measurements of the halos were carried out, and as results were obtained: three halos of the hexane extract equal to 14 mm; three halos of the ethyl acetate extract equal to 10 mm and three halos of the ethanol extract equal to 9 mm. Such results are in agreement with Bona *et al*. (2014) who propose that antimicrobial activity is evidenced when the inhibition halo is equal to or greater than 6 mm and in the case of plant extracts, halos greater than 7 mm characterize antimicrobial activity.

In studies by Garcia, Ueda and Mimica, (2011) ethanolic extracts from the Asteraceae family, in this case the species *Bidens pilosa*, showed antibacterial activity against strains of S. aureus. Fabri *et al*. (2011) also corroborate this study, since in their research, *B. dracunculifolia* showed, through methanolic extracts, antimicrobial activity against *Pseudomonas aeruginosa* and *Bacillus cereus* and *Cryptococcus neoformans*, indicating that "the percentage of activity that evaluates the antimicrobial potential of the tested extracts showed that the extract of *B. dracunculifolia* was the most active, followed by the extract of *Taraxacum officinale*", showing that rosemary-do-campo has a much higher percentage of bactericidal activity than in comparison with other species of the Asteraceae family.

The antimicrobial result obtained in this study confirms what Soares *et al*. (2007) determined in their study on the acidogenic potential of *Staphylococcus mutans*, and the hexane fraction of *B. dracunculifolia* was the most active in the antimicrobial assay against the pathogen. Based on the results obtained, these authors suggest that B. dracunculifolia is also capable of acting on the glycolytic pathway of *S. mutans*, inhibiting the production of nucleic acids, also confirming the study by Ferronatto *et al*. (2007), which reported the antimicrobial efficacy of *B. dracunculifolia* oil against *S. aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Nader *et al.* (2010) presented data showing that the chloroform extract of *B. dracunculifolia* had important in vitro activity on *S. aureus* strains. Their data state that the genus Baccharis as well as the species *B. dracunculifolia* "are promising for the development of an antimicrobial herbal medicine and that several substances are responsible for this activity".

Miranda *et al.* (2016) found that essential oils of alecrim-do-campo associated with monoterpenes were efficient in inhibiting the growth of bacteria. The essential oil of *B. dracunculifolia* was bactericidal on Gram-negative bacteria and on Gram-positive bacteria, in which the inhibited strains were *Listeria monocytogenes* and *Salmonella choleraesuis*. Also, according to this study, antimicrobial activities may vary according to "place and time of collection of the plant species, vegetative cycle and edaphoclimatic factors". Salazar *et al.* (2018) also corroborate when the study focuses on the pure essential oils of the studied plant, as they evaluated the antibacterial activity of *B. dracunculifolia* oil also against *S. aureus* and report that the oil is a promising natural product with antimicrobial potential for use. clinical.

According to Machado *et al*. (2015) the essential oil of *B. dracunculifolia* was investigated against *Mycobacterium* species. According to the products produced, the oil did not present itself as a promising source of antimycobacterial drugs. Therefore, as for folk medicine for herbal medicines, folks and/or health professionals should pay attention to the use of the plant against possible mycobacterial infections, which may not have the expected results.

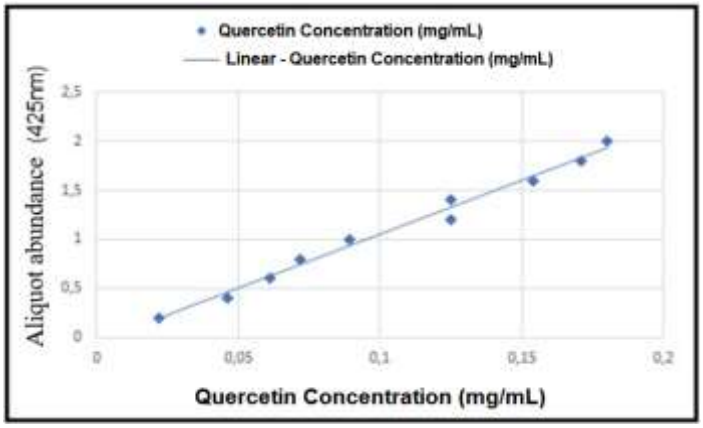
Unlike the present study, in which there was a bactericidal action against *S. aureus* strains in the ethanol extract, Lima *et al*. (2019) did not find the same bactericidal action of the same type of *B. dracunculifolia*

extract in their study. It is likely that the extract obtained in their work contains mostly the apolar fraction, which is not capable of inhibiting bacterial growth.

4.3 Total flavonoids and antioxidant activity

About 120 species of the genus Baccharis were chemically studied, and, in general, the compounds that stand out are flavonoids and terpenoids (AGOSTINI et al., 2005). The results of the absorbance reading of the extracted aliquot for determination of total flavonoids in this study were gathered to construct the standard curve of antioxidant activity (Graph 1) from solutions with increasing concentrations of quercetin.

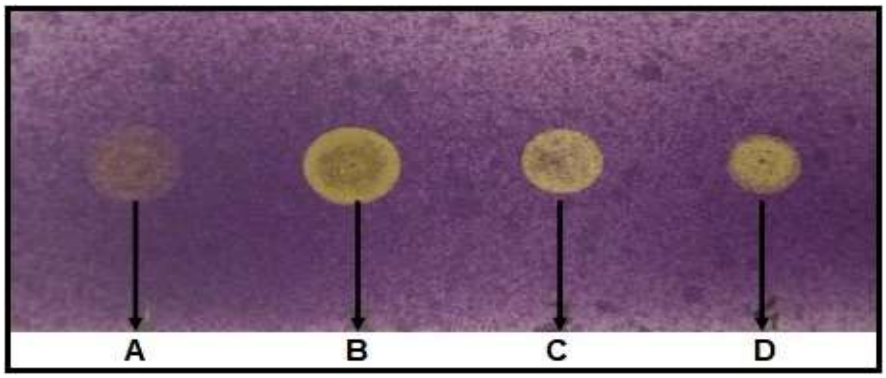
Graph 1: Calibration curve of the antioxidant activity of *B. dracunculifolia*.



Source: Research data

Of the extracts analyzed in comparison with quercetin (one of the flavonoids present in the genus *Baccharis* and used as a standard), the extract that showed the highest activity was ExAcet followed by ExtEtan and ExtHex showed no evidence of activity (Figure 2). In this context, it was detected that the extracts obtained from the species under study from the Brazilian cerrado showed antioxidant activity.

Figure 2: Antioxidant evaluation of extracts and quercetin pattern.



Source: Research data. A: ExtHex; B: ExAcet; C: ExtEtan; D: Quercetin standard.

According to Marques *et al.* (2016), antioxidant activity corresponds to the amount of DPPH consumed by the antioxidant, and the amount of antioxidant needed to decrease the initial concentration of DPPH by 50% is called efficient concentration (EC50), also called inhibitory concentration (IC50). The greater the

consumption of DPPH by a sample, the lower its EC50 and the greater its antioxidant activity and the activity evidenced with the presence of white or yellow spots resulting from the reduction of DPPH against the purple background color.

According to Dias *et al*. (2009) an antioxidant substance is one that tries to inhibit the oxidation process, and thus protect biological systems against damage from processes or reactions that can promote the oxidation of cellular structures. The antioxidant activity of the species *B. dracunculifolia* has been attributed to its different extracts (RODRIGUES, 2017), confirmed in this study, strongly evidenced in ExAcet. Likewise, Ferronatto *et al*. (2007), in their study on the antioxidant activity of essential oils produced by *B. dracunculifolia*, present as results the confirmation that there is antioxidant activity in the oil of this plant.

According to Sforcin *et al*. (2012), the phytochemical analysis of the extracts and oils from their work suggests that the results presented are related to the presence of flavonoids, cinnamic acid and triterpenes present in *B. dracunculifolia*, and that their results corroborate the popular use of the plant in the treatment of various inflammatory causes, which "contributes to the pharmacological validation of this important medicinal species".

Tests performed on crude extracts and fractions of plants of the genus *Baccharis* showed that most biological activities, such as antioxidant, are not only related to flavonoids, but also to terpenes and trichothecenes (VERDI; BRIGHENTE; PIZZOLATTI, 2005). These authors confirm that antioxidant activity was observed in several species of *Baccharis*, including *B. trinervis* and *B. coridifolia*, where such activities were associated with the presence of flavonoids.

Paroul *et al*. (2016) indicate that the antioxidant properties found in aqueous extracts of *Baccharis*, from the Pampa Sulista biome, show similar effects for different extracts of this genus, found in other literatures. The authors explain that this antioxidant effect is due to the presence of flavonoids and that these results in aqueous extracts of the genus were approximately 100 times higher than those obtained with essential oils from the same plants, evidencing antioxidant actions in compounds of the most varied types of extracts, which corroborates the present study, in which three different types of extractors were used for the production of extracts.

5. Conclusion

Taking into account the well-known application of alecrim-do-campo as a medicinal plant by the simpler population, studies such as this one can support such use, since the scientific proof of phytotherapic profiles is essential for a possible indication by health professionals, especially those of the Brazilian Unified Health System (SUS).

Based on this study and on other research carried out with the same species, it is possible to provide data on its antibacterial and free radical fighting efficacy. Such results can serve as a subsidy for other studies involving the Asteraceae family, since they inhabit the most different biomes in Brazil, and, thus, it is possible to find the most distinct physiological and chemical characteristics of these plants, with great potential. of improving the health of the population, according to the environmental pressure to which they are subjected.

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