



## **Baccharis dracunculifolia extract as a promising active for healing chronic wounds**

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**Abstract:** Chronic wounds are all wounds that have a difficult healing process and a delay in physiological healing repair. Such wounds are considered a public health problem, which generate high costs for health services. The *Baccharis dracunculifolia* (BD) plant, popularly known as “alecrim do campo” or “vassourinha” and widely used in folk medicine, has several biological activities, such as antibacterial and immunomodulatory activities, which may favor the wound repair process. In this context, the objective of this study is to analyze the biological activity of *Baccharis dracunculifolia* extracts regarding cytotoxicity for subsequent production of a dressing with future applications in tissue repair of chronic wounds. FTIR and TG analyzes were performed to identify functional groups and HPLC analysis to quantify certain flavonoids. The cytotoxicity capacity of the extracts was evaluated by the MTT test on fibroblasts of animal origin (L929) and human keratinocytes (Nok-1). Cytotoxicity tests revealed that the ethanolic extract of *Baccharis dracunculifolia* (EEBD) showed lower cytotoxicity for most of the concentrations tested.

**Keywords:** Wound healing. *Baccharis dracunculifolia*. Dosage biologique. Tissue engineering.

### **Introduction**

Chronic wounds are all wounds that have a difficult healing process and a delay in the physiological repair of healing, lasting longer than six weeks<sup>[1,2]</sup>. These wounds can last for a long period of time and cause harm to the individual. The disabilities generated by chronic injuries to the subjects go beyond work issues, including physical limitations that are painful and make it difficult to walk<sup>[3]</sup>.

One should also consider the degree of psychic suffering of people with these injuries, changes in self-image and self-concept are common, leading to chronic processes of low self-esteem, in addition to the development of mood disorders and social isolation<sup>[3]</sup>. In this way, such wounds create embarrassment for the patient and interfere with social relationships, thus affecting the quality of life<sup>[2]</sup>.

Chronic wounds are considered a public health problem, which affect 5% of the adult population in the western world and generate high costs for health services, since they involve home care, prolonged hospitalization, complex treatments, and the use of adjuvant therapies, in addition to of being associated with high rates of recurrence<sup>[1]</sup>.

The dressing is one of the main resources for the treatment of chronic wounds. A good dressing should be able to interact effectively with the wound and facilitate the healing process by offering protection against bacterial infection, maintaining a hydrated environment, providing thermal insulation, promoting sufficient oxygen circulation,

and allowing cell migration and exudate drainage. With the advent of tissue engineering, it was possible to discover biomaterials that play a key role in creating this environment<sup>[4]</sup>. During tissue repair, a biomaterial alone or with active biomolecules can be implanted in the repair region to provide a favorable microenvironment for repair<sup>[5]</sup>.

The *Baccharis dracunculifolia* (BD) plant, popularly known as “alecrim do campo” or “vassourinha”, is a native shrub widely found in southern Brazil<sup>[6-8]</sup>. In popular medicine, wild rosemary is used as a tea to treat fever, diabetes, detoxification of the body, cold, fatigue, intestinal problems, gastric ulcers, anemia, liver problems, improve blood circulation, among others<sup>[9]</sup>.

BD has already been identified as the botanical source for the production of Brazilian green propolis by *Apis mellifera* bees, through studies such as those by Park et al. (2004)<sup>[10]</sup>, Alencar et al. (2005)<sup>[11]</sup> and Rodrigues et al. (2020)<sup>[12]</sup> who analyzed the chemical composition of Brazilian green propolis with its probable plant source – *Baccharis dracunculifolia*. Although there are some differences between the chemical compositions of the two matrices, the plant has similar biological and medical properties to Brazilian green propolis<sup>[13]</sup>.

In a review study carried out in 2016 by Ramos Campos and collaborators<sup>[14]</sup>, 139 compounds isolated from the genus *Baccharis* (33 species) were described in studies reported in the last eleven years before publication. Among the substances found in a greater number of species are the

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Received 10 July 2023; Accepted 25 September 2023.

 <https://doi.org/10.52466/ijamb.v5i2.114>

flavonoids (quercetin, kaempferol, apigenin, naringenin and aromadendrin); phenolic acids (artepillin C, drupanin, ferulic acid, caffeic acid and dicaffeoylquinic acid); and terpenoids (oleanolic acid and  $\alpha$ -spinasterol). Of the phenolic compounds found in *Baccharis dracunculifolia*, artepillin C, kaempferol, *p*-coumaric acid and baccarin stand out<sup>[15]</sup>, with *p*-coumaric acid, artepillin C and baccarin being the three compounds considered chemical markers for BD as well as for Brazilian green propolis<sup>[12,16]</sup>.

*Baccharis dracunculifolia* extract has several biological activities, such as anti-inflammatory<sup>[17]</sup>, antibacterial<sup>[18]</sup>, immunomodulatory<sup>[19]</sup>, antigenotoxic and antimutagenic<sup>[20]</sup>. The biological properties of the extract are often attributed to its chemical compounds, such as artepelin C, which has antimutagenic action<sup>[21]</sup>.

Studies on the biological activities of plant extracts must be complemented with information on their chemical compositions. In this context, the objective of this study is to analyze the biological activity of *Baccharis dracunculifolia* extracts regarding cytotoxicity for subsequent production of a dressing with possible future applications in tissue repair of chronic wounds.

## Materials and Methods

### Preparation of Baccharis dracunculifolia extract

The plant extract of *Baccharis dracunculifolia* (EVBD) was obtained from Ciclo Farma Indústria Química Eireli company, using 96% ethyl alcohol as extractor vehicle, obtaining an extract at a concentration of 11%. The EVBD was concentrated under vacuum in a rotary evaporator (Eppendorf Concentrator), providing 0.28 g of wet crude extract. Afterwards, the EVBD was solubilized in 100% ethyl alcohol, obtaining the ethanolic extract of *Baccharis dracunculifolia* (EEBD) at a concentration of 28 mg/mL.

### Vibrational spectroscopy in the infrared region

The dry ethanolic extract of *Baccharis dracunculifolia* were structurally characterized by Attenuated Total Reflectance. Fourier Transform Infrared Spectroscopy (ATR-FTIR). Infrared spectra were obtained on a Thermo Scientific NICOLET IS5 spectrometer with iD3 ATR transmission module with germanium crystal under the following conditions: 32 background scans, 32 sample analysis scans, 2 cm<sup>-1</sup> resolution and absorption range between 4000 and 650 cm<sup>-1</sup>.

### Thermogravimetric analysis

The thermal properties of the dry ethanolic extract of *Baccharis dracunculifolia* were determined by Thermogravimetric Analysis (TGA) and Derived Thermogravimetry (DTG). TGA/DTG curves were obtained on a TA Instruments SDT Q600 under the following conditions: synthetic air atmosphere with continuous flow of 100 mL/min and heating rate of 10 °C/min over temperature range of 30–600 °C using a sample mass of about 5 mg. Alumina pan was used as reference.

## Cell culture and cell viability assessment

Nok-1 (human keratinocytes) and L929 (animal fibroblasts) cells were cultured in DMEM medium supplemented with 10% fetal bovine serum and cultivated in an humidified incubator at 37° C with 5% CO<sub>2</sub> saturation. The experiments were performed only when the cells were 90% confluent.

To evaluate the cytotoxicity of EBD, L929 cells were cultured in 96-well plates (1x10<sup>4</sup>/well), in triplicate, in the presence of EVBD in different concentrations (17, 8, 4, 2 and 1%) for 24h. To evaluate the EEBD cytotoxicity, Nok-1 cells were cultured in 96-well plates (1x10<sup>4</sup>/well), in triplicate, in the presence of different concentrations (28, 14, 7, 3.5, 1.75, 0.87, 0.43, 0.21, 0.11, 0.054 and 0.027 mg/mL) for 24h. After the incubation time, the medium was removed and a volume of 50  $\mu$ L/well of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) 5 mg/mL was added and the plates were incubated for 4 h at 37°C. After MTT reduction and formazan crystals solubilization by DMSO, the absorbance was measured at 570 nm in a plate reader (Spectra Max, Molecular Devices) and the results were represented as percentage of cell viability.

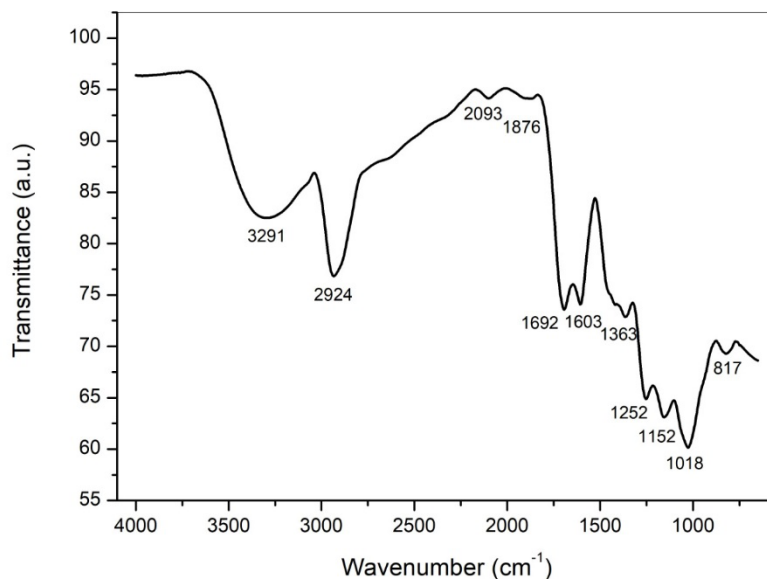
## Results and Discussion

### Vibrational spectroscopy in the infrared region

The FTIR spectrum can be used to identify functional groups of active components present in the extract. In this way, the ATR-FTIR spectrum of the ethanolic extract of *Baccharis dracunculifolia* was determined and the data were interpreted from the infrared spectral pattern (Figure 1). The analysis of the ATR-FTIR spectrum shows bands positioned at 3291 cm<sup>-1</sup>, referring to the stretching of the OH bonds present in alcohols and phenolic compounds<sup>(22-24)</sup>; in 2924 cm<sup>-1</sup> referring to the stretches of the C-H bonds of hydrocarbons linked to oxygen (24–26); at 1600 cm<sup>-1</sup>, which can be attributed to the stretching vibration of C=C groups in aromatic compounds<sup>(23,25,26)</sup> and finally, the band at 1252 cm<sup>-1</sup>, corresponding to the vibration of the C-O group<sup>(23)</sup>.

The FTIR results indicated that the ethanolic extract of *Baccharis dracunculifolia* consists of a mixture of several unseparated compounds, showing the bands assigned as described above.

**Figure 1** – FTIR spectra of dry extract of ethanolic extract of *Baccharis dracunculifolia* (EEBD).



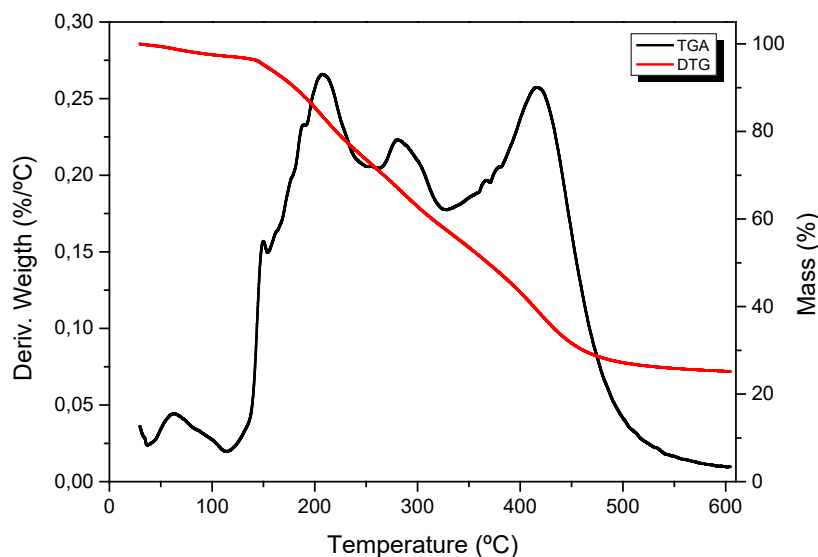
Source: Own authorship, 2023.

### Thermogravimetric analysis

The thermal behavior of the dry extract of *Baccharis dracunculifolia* was evaluated by thermogravimetry (Figure 2). The dry extract sample showed three distinct stages of mass loss. In the range of 36 to 112 °C, the first stage of mass loss occurs, which can be attributed to the loss of ethanol and water (4%). Between 137 and 190 °C there is a peak in the TG curve and a small mass loss

detected (9%), which may be associated with the loss of other volatile compounds. The greatest degradation and loss of extract components occurs between 191 and 476 °C, with a thermal decomposition of approximately 59%, confirmed by the DTG curve. Above 476 °C, the TG curve is flat and there is no mass loss detected, leaving about 28% of components that have not been degraded.

**Figure 2** – TG thermograms and diferential thermogram of dry ethanolic extract of *Baccharis dracunculifolia* (EEBD).



Source: Own authorship, 2023.

**High Performance Liquid Chromatography (HPLC)**

The *Baccharis dracunculifolia* extract provided by the company Ciclo Farma Indústria Química Eireli, from batch number 0062017, was submitted to HPLC analysis for quantification of certain flavonoids present in the sample, namely: ferulic acid, coumaric acid, cinnamic acid, baccarin and artepelin C. As a result of the collaboration for this work, the results were provided by the company and are described in Table 1.

From a qualitative point of view, it was possible to identify all chemical compounds that one would like to find in the BD extract sample (ferulic acid, coumaric acid, cinnamic acid, baccarin and artepelin C). For these chemical compounds, also commonly found in Brazilian green propolis, different biological activities were found.

Caffeic, ferulic and p-coumaric acids are trans-cinnamic acids found naturally in their free forms

or as a family of mono- or diesters with (-)-quinic acid, known as chlorogenic acids (CGAs)<sup>[27]</sup>. CGAs are important antioxidant components produced by plants in response to environmental stress conditions. The antioxidant action is a biological activity that can contribute in several ways to the health of an organism. Studies demonstrate biological activities of *B. dracunculifolia* being, at least in part, attributed to the antioxidant capacity of its active components. The study by<sup>[28]</sup> demonstrates that coumaric acid is a key bioactive compound in the gastric healing process. Other compounds such as artepelin C and baccarin are revealed as potential antigenotoxic agents<sup>[29,30]</sup>. In addition, artepelin C also has antitumor<sup>[31]</sup>, anti-inflammatory<sup>[32]</sup> and antimicrobial<sup>[33]</sup> action.

Therefore, knowledge of the chemical variations of phenolic compounds in *B. dracunculifolia* becomes essential for the development of new products.

**Table 1** – Mean values of the concentrations of flavonoids analyzed for the plant extract of *Baccharis dracunculifolia*, expressed in  $\mu\text{g}\cdot\text{mL}^{-1}$

Ferulic Acid	Coumaric Acid	Cinnamic Acid	Baccarin	Artepelin C
36,72	165,89	54,18	411,08	750,06

Source: Ciclo Farma Indústria Química Eireli

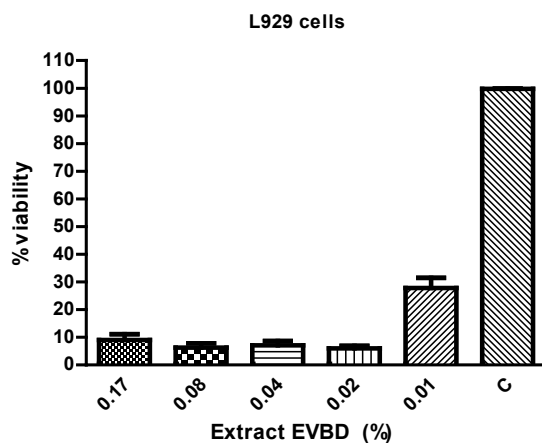
**Cell culture and cell viability assessment**

Considering that toxic reactions trigger inflammatory processes and new lesions, the importance of inserting methodologies for evaluating cytotoxicity is understood as one of the minimum requirements to guarantee the safe use of a product. It should be noted that with the increasingly strict control over the use of laboratory animals, it is necessary to use in vitro tests that can detect the toxicity of medical devices, as described in ISO

10993-5:2009.

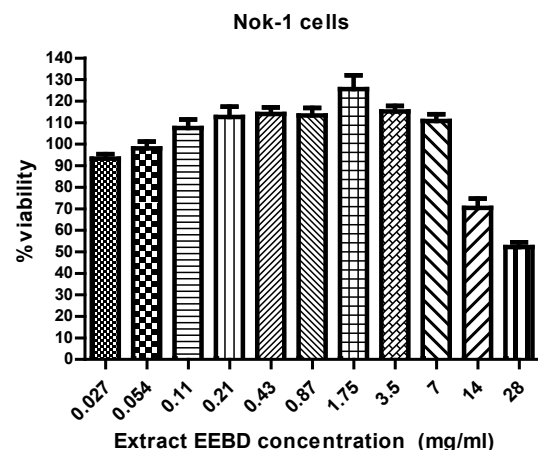
EBD and EEBD were evaluated for their ability to cytotoxicity in the MTT test on L929 and Nok-1 cells, respectively, in which cell viability is characterized according to the potential to reduce MTT to crystals of formazan, an insoluble compound purple in color. Figure 3 and 4 show the percentage of viability after incubation in different concentrations of the two extracts.

**Figure 3** – Cell viability of fibroblasts (L929) in the presence of *Baccharis dracunculifolia* plant extract (EVBD).



Source: Own authorship, 2023.

**Figure 4** – Cell viability of keratinocytes (Nok-1) in the presence of *Baccharis dracunculifolia* ethanolic extract (EEBD).



Source: Own authorship, 2023.

MTT analysis was performed to establish the best concentrations of the extract for incorporation in the development of a new dressing. This analysis is based on >70% cell viability. Our data show that in all tested concentrations EEBD was cytotoxic, while only in the two highest concentrations (28 and 14 mg/mL) of EVBD there was toxicity for the cells. The results obtained with the EEBD agree with the work of Costa et al. (2019) (34) who observed similar effects.

During cell cultivation of L929 cells treated with EVBD, the presence of a precipitate was observed (data not shown). The precipitation of EVBD components may have caused the cytotoxic effect since such precipitation may have prevented the interaction of cells with the medium.

### Conclusion

In this study, the extracts tested showed different effects on cell viability. While EVBD was shown to be extremely toxic to cells, EEBD showed non-cytotoxicity for most tested concentrations. These results suggest a safe use of EEBD in terms of cytotoxicity, thus potentially being an asset for the development of new dressing formulations with applications for wound treatment. However, more advanced studies are encouraged and necessary to better evaluate the use of EEBD in wound healing.

### Acknowledgment

CAPES and Ciclo Farma Indústria Química Eireli

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