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Article

# Evaluation of the anti-inflammatory effect of plant extracts from *Miconia pseudocentrophora, Brachyotum ledifolium*, and *Fuchsia loxensis* in rats

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## ABSTRACT

*Miconia pseudocentrophora*, *Brachyotum ledifolium*, and *Fuchsia loxensis* are some of the Ecuadorian ancestral medicines, a heritage passed down through generations for treating various ailments, including inflammation. This pioneering study delves into the ethnopharmacological properties of extracts from these plants' leaves, stems, and fruits collected in their native Ecuadorian habitats. The ethanolic and chloroform sub-extracts underwent meticulous quality assessment, with the ethanolic extract efficiency yielding between 78.6-98.5%. Phytochemical screening uncovered various secondary metabolites, encompassing flavonoids, alkaloids, quinones, triterpenes, and reducing sugars. In vivo evaluation at 1, 3, 5, 7, and 8 hours of treatment, utilizing a rat paw-edema model, demonstrated a significant reduction in inflammation volume comparable to naproxen sodium. The maximum effect was observed after 3 hours of treatment. *Miconia*'s chloroform sub-extract exhibited superior performance, achieving a 54% inhibition of inflammation, followed by *Brachyotum* and *Fuchsia*, both with 52%. These findings support the traditional medicinal efficacy of these plants and underscore the need for further exploration, holding considerable promise for the pharmaceutical industry.

**Keywords:** ethnopharmacology, anti-inflammation, percentage inhibition, carrageenan-induced model, phytochemical screening.

## INTRODUCTION

Throughout ancient times, plants have stood as the primary source of medicinal preparations, having cultivated an ancestral medicine that endures to the present day. This legacy remains a focal point in pharmaceutical research, where exploration into conventional medicine relies on the empirical knowledge transmitted across generations. The extensive diversity of plant species worldwide still presents an untapped frontier. Numerous native species from the Andean region of Ecuador captivate attention with their vibrant and intense color palette[1]. Beyond their allure to pollinators, these colors imply the presence of flavonoids, secondary metabolites recognized for their role in enhancing vivid colors and identification in medicine as anti-inflammatory agents[2].

Inflammation is a body's physiological response aimed at protecting and freeing the individual from the initial cause of injury and its consequences. Inflammation is characterized by persistent pain, swelling, and functional impairment of the affected area to facilitate the destruction of pathogenic microorganisms. Subsequently, it promotes the repair and healing of the injured tissue[3]. Without the inflammatory reaction, infections and toxins would spread throughout the body, causing severe injuries and, ultimately, death[4]. Inflammation is recognized to consist primarily of three fundamental components. Firstly, there is a modification in the caliber of vessels, resulting in an increased regional blood flow to the affected area. Simultaneously, alterations in microvascular permeability occur, facilitating the exit of cells and substances into the interstitium. Finally, the migration of leukocytes is observed, leaving the bloodstream to head toward the focus of the lesion, where the inflammatory response is activated and organized[4]. Chemical mediators involved in this process include histamine, serotonin, lysosomal enzymes derived from neutrophils, prostaglandins, leukotrienes, cytokines, nitric oxide, and complement factors[5]. Despite inflammation serving a protective function, it can also be a tissue or systemic injury source. While it eliminates the causal agent, it may simultaneously cause damage to the affected organs and systems, leading to a complex interplay between protective and detrimental effects. Inflammation can manifest acutely or be part of a chronic disease that affects one or more organs in the body[6].

The metabolism of arachidonic acid, a component of the lipid portion of the cell membrane, gives rise to inflammatory mediators of significant biochemical and pharmacological importance. Different enzymes act on this compound: cyclooxygenase produces prostaglandins and thromboxanes, while lipoxygenase produces leukotrienes[7]. Prostaglandins can modify the deformation and passage of leukocytes through capillary walls, reduce gastric acid secretion, alter pituitary functions, stimulate renin secretion, and, most importantly, are essential for maintaining both macro and microvascular circulation[7].

Compounds derived from plants, encompassing various chemical classes, have shown established anti-inflammatory properties. The utilization of medicinal plants and their secondary metabolites is increasingly prevalent in treating diseases as a form of complementary medicine[8]. Notably, alkaloids, terpenes, and phenolic compounds like tannins, lignans, coumarins, and saponins, with a particular emphasis on flavonoids, are prominent among them[9–12]. Flavonoids stand by exhibiting a high affinity for binding to proteins and other biological macromolecules andetallic anions[13,14]. Additionally, they possess a significant ability to catalyze electron transport and capture free radicals[13]. Recently, there has been a renewed interest in ethnopharmacology in Ecuador, addressing diversepecies[15–20]. In the present study, we have undertaken the characterization for the first time of the plants *Miconia pseudocentrophora, Brachyotum ledifolium,* and *Fuchsia loxensis*, in response to their significance in traditional medicine.

Regarding the *Brachyotum* genus, it has been reported that several species are used as medicinal plants for treating chicken colds, dyeing, and construction, as well as for firewood and brooms. In the specific case of *Brachyotum ledifolium*, it is used medicinally by the Andean Kichwas[21]. For *Miconia pseudocentrophora*, there are no reports of its medicinal application; however, species of the genus *Miconia* have been known to have applications as a vermifuge, to stimulate dilation during childbirth, cure throat and neck pain, treat tuberculosis, toothaches, oral infections, mycosis, scabies, and to cure diarrhea in newborns. They can even be an antidote for ant bites and abscesses [21]. Regarding *Fuchsia loxensis*, there are no specific studies concerning its medicinal properties. However, it has been observed that species from the Onagraceae family exhibits pharmacological activities, including anti-inflammatory, antiarthritic, analgesic, antioxidant, cytotoxic, antidiabetic, and antimicrobial effects[22].

*Miconia pseudocentrophora* (Melastomataceae), or "colca", is a shrub or small tree with simple and opposite leaves. The leaves have entire margins and a brownish pubescent underside, displaying main veins extending from the base to the apex. The flowers are arranged in terminal panicles, showcasing five white petals, white filaments, and purple anthers. The fruits are green berries in their immature state, turning black upon maturation, and contain numerous seeds[23].

*Brachyotum ledifolium* (Melastomataceae), commonly known as "llumbre", "puka chaklla", or "inchichaklla" from the Kichwa language, and is also known as "arete de inca" or Inca earrings. This shrub

has peeling bark, and ovate leaves covered with tiny hairs. The hanging flowers have a red calyx with yellow hairs, and the pale-yellow petals form a tube. The fruits are dry with tiny seeds[24]. The flowers are edible and sweet, and their stems serve multiple purposes, such as brooms, beams and arches for building and decorations for festivities. The juice is utilized for extracting indelible dyes and treating poultry colds. Additionally, it finds application in agroforestry as a live fence[24].

*Fuchsia loxensis* (Onagraceae), also known as "pena pena". Is a climber shrub with opposite leaves. The leaves are lanceolate and simple, although they typically have serrated margins and are evergreen. The flowers are pendulous, hanging from long peduncles that give them a downward-facing appearance. The characteristic intense fuchsia calyx is cylindrical, with four lobes, and the corolla consists of four petals, resembling decorative earrings. The plant has four elongated, narrow sepals and four short and wide petals. The fruit is a small berry, ranging from dark red-green to intense red, it is edible and contains numerous small seeds. *Some indigenous groups use Fuchsia loxensis as medicine and has*igh potential as an ornamental[25,26].

The present research aims to characterize the native plant species *Miconia pseudocentrophora, Brachyotum ledifolium,* and *Fuchsia loxensis* and evaluate their potential as anti-inflammatory agents. Our study started by collecting plant material from the species for taxonomic identification. Subsequently, the preparation of ethanolic extracts and chloroform sub-extracts from this material occurred, followed by a comprehensive assessment of their physical and chemical properties. The evaluation involved determining the humidity content through the gravimetric method, ensuring avoidance of enzymatic procedures. Additionally, total ashes were quantified for quality assessment. Then, the anti-inflammatory effect was evaluated by using the ethanolic extracts and chloroform sub-extracts from the mentioned plant species, followed by *in vivo* tests conducted on Winstar rats. The carrageenan-induced paw edema model was employed for screening the anti-inflammatory efficacy in the acute phase of inflammation. Finally, for a more rigorous analysis of the observed anti-inflammatory effect, a statistical analysis was applied to ensure the validity and reliability of the results obtained at each stage of the research.

## MATERIALS AND METHODS

### Plant materials

The leaves stems, and fruits of *Miconia pseudocentrophora*, *Brachyotum ledifolium,* and *Fuchsia loxensis* were collected in a rainy season (february) in the native forest of San Francisco de Guayllabamba, Chambo, Chimborazo Province (Ecuador) and identified in the herbarium of the Escuela Superior Politécnica de Chimborazo (ESPOCH) by the curator Jorge Caranqui.

Quality assessment was based on the determination of the humidity content (< 10%) to avoid enzymatic procedures and it was performed through the gravimetric method. Quality was also assessed employing the determination of total ashes.

### Preparation of plant extracts

The preparation of the alcoholic extract involved the processing of plants by cutting and triturating the leaves, stems, and fruits. Ethanol 96% is added, and the mixture is left to macerate for 2 to 3 days at room temperature. After maceration, the solution is decanted, and the filtrate is concentrated to one-eighth of the initial volume. The resulting solution is then refrigerated to eliminate chlorophyll, yielding the alcoholic extract alone.

To prepare the alcoholic sub-extracts, chloroform was used to acquire the secondary metabolites. An aliquot of 25 ml is taken from the alcoholic extract, and placed in a separation funnel, and an equal volume of chloroform is added. The mixture is stirred, and allowed to settle. Once two phases are formed, the upper phase corresponding to the ethanolic extract is separated, and the lower phase containing chloroform is

collected in a previously tared container. This process is repeated until the solution is transparent. Subsequently, all chloroform phases are combined, the solvent is removed, and the sub-extract is obtained. To reconstitute the extract and achieve the required volume for application in rats, a liquid-liquid emulsion is prepared using water, sub-extract, and alcohol, followed by alcohol evaporation.

Quality control of plant extracts was performed to ensure purity, health, and interference-free analysis. Organoleptic properties were tested by checking odor, color, taste, and appearance. Properties like pH, refraction index, and relative density were also determined.

**Phytochemical screening**

Phytochemical screening allowed us to qualitatively determine plants' main chemical group constituents as secondary metabolites. Different approaches were used for the phytochemical screening in this research: Dragendorff assay, Mayer assay, Wagner assay, Baljet assay, Ferrum chloride assay, Foam assay, Shinoda assay, and Bitter principles assay.

**Animals**

Twenty adult (8 weeks-old) Wistar rats (180 -200 g) were obtained from the animal facility of the Faculty of Sciences (ESPOCH), 13 females and 7 male rats. Three repetitions were performed per batch (alcoholic extract: L1, L2, L3); (chloroform sub-extract: Ls1, Ls2, Ls3). Animals were provided with water and diet *ad libitum*.

**Carrageenan-induced rat paw edema model**

Subcutaneous administration of 0.5 ml of carrageenan solution at the rat's plantar aponeurosis level was inoculated to induce an inflammatory reaction in the posterior legs. The assays were performed in batches of three rats. The normal volumes of the rats' right hind legs were measured. The formulations were administered orally. The control group received only the vehicle (distilled water), while the experimental group received a Naproxen sodium 550mg (7.86 mg/kg). The experimental group received 0.1 ml of Miconia pseudocentrophora, Brachyotum ledifolium, and Fuchsia loxensis extracts and subtracted at concentrations of 100% via oral.

Thirty minutes after administering the test substances, edema was induced by injecting 0.1 ml of a 0.5% aqueous carrageenan solution into the right plantar aponeurosis of the rats. The volume of the inflamed right leg (length and diameter) was measured. Inflammation was quantified by measuring the volume of the legs at 0, 1, 3, 5, 7, and 8 hours after carrageenan injection. The difference in volume between the inflamed right leg and the same normal right leg before the carrageenan injection indicates the degree of inflammation.

Induced inflammation volume was computed with the formula.

$$VI = \frac{\pi}{4} d^2 h, \quad (1)$$

where $d$ represents the diameter of the leg and $h$ is the length. The results were determined as inflammation percentage applying the formula

$$Inflammation\ \% = \frac{V_{positive\ control} - V_{normal}}{V_{normal}} \times 100, \quad (2)$$

$V_{poxitive\ control}$ is the volume of the inflamed leg at time $X$, and $V_{normal}$ is the length before the application of the carrageenan.

The inhibition percentage of the inflammatory reaction induced by carrageenan is computed in acute phase at 1, 3, 5, 7, and 8 hours after the inoculation and is calculated through:

$$acute\ phase\ inhibition\ \% = \frac{V_{problem\ control} - V_{positive\ control}}{V_{problem\ control}} \times 100 \qquad (3)$$

Where $V_{problem\ control}$ is the volume of the inflamed leg without anti-inflammatory, $V_{positive\ control}$ represents the volume of the inflamed leg with anti-inflammatory, and $V_{normal}$ is the volume before the application of the carrageenan.

**Statistical analysis**

Statistical analysis was performed using R software. One-way analysis of variance (ANOVA) and multiple Tukey comparison tests were carried out. A $p$-value < 0.05 was considered statistically significant.

**Ethical considerations**

Experimental procedures and protocols used in this study were approved by the animal facility committee of the Escuela Superior Politécnica de Chimborazo (ESPOCH).

## RESULTS AND DISCUSSION

### Quality assessment of the *Miconia pseudocentrophora, Brachyotum ledifolium,* and *Fuchsia loxensis* extracts.

To perform quality control, leaves, stems, and fruits of *Miconia pseudocentrophora, Brachyotum ledifolium,* and *Fuchsia loxensis* were collected. The samples were analyzed in triplicate for each test. The physical and chemical properties obtained results are presented in Table 1. The percentage of humidity in the fresh plant can be observed, yielding a value of 79.48% for *Fuchsia loxensis*, 49.49% for *Brachyotum ledifolium*, and 48.90% for *Miconia pseudocentrophora*. These values reflect the high amount of free water present in the plant material. Newly harvested plants contain significant water, varying in different organs. Therefore, the moisture content is higher for *Fuchsia loxensis*, possibly due to the high humidity in the area where the plant material was collected, characterized by fog[27]. Another contributing factor could be that it is a less woody shrub, retaining more water.

Additionally, Table 1 shows the total ash content in the fresh plant, which is 4.07% for *Brachyotum ledifolium*, and 3.70% for *Fuchsia loxensis*, which may be attributed to its thick and deep roots that absorb a higher number of organic compounds. For *Miconia pseudocentrophora*, the ash content is 2.91%, indicating that this plant belongs to materials less submerged underground. These results align with established limits, as traditional medicinal plants typically exhibit ash contents up to 12%. Exceeding this threshold would warrant excluding the plant material, indicating potential contamination with earthy materials such as salts, sand, or heavy metals [28].

| Parameter | Humidity (%) | Ashes (%) |
|---|---|---|
| *Brachyotum ledifolium* | 49.49 | 4.07 |
| *Fuchsia loxensis* | 79.48 | 3.70 |
| *Miconia pseudocentrophora* | 48.90 | 2.91 |

**Table 1. Results of the determination of the humidity and ashes content of the studied plants.**

### Characterization of the extracts from *Miconia pseudocentrophora, Brachyotum ledifolium,* and *Fuchsia loxensis*

The extraction of active principles is owed to the difference in osmotic pressure within the plant cell. This process occurs as the solvent (ethanol) fills the space between intracellular and extracellular fluids. Eventually,

the cell undergoes lysis, releasing its contents into the solvent. A portion of this content constitutes the active principles responsible for the anti-inflammatory activity.

Table 2 presents the physicochemical properties of the plant extracts. Notably, for *Brachyotum ledifolium*, the concentrated extract has a yield percentage of 78.6%, implying its origin from a woody shrub with shallow roots, resulting in reduced water absorption. The organoleptic characteristics of the ethanolic extract include a liquid consistency, yellowish-green color, and a slightly sweet taste, potentially attributed to the presence of sugars like glucose in the plant, accompanied by a robust herbal aroma. The ethanolic extract exhibits a pH of 4.18, indicating the presence of weakly acidic chemical compounds. In comparison to the density of the solvent used (ethanol, 0.91 g/ml), this divergence implies the existence of dissolved substances responsible for the observed activity. The refractive index, measuring 1.355, further supports the presence of dissolved substances and hints at the potentially lowest extract viscosity.

The concentrated extract from *Fuchsia loxensis* shows a yield of 89.2% (Table 2), considered optimal due to the woody nature of the plant, concentrating moisture in leaves, flowers, and fruits. The ethanolic extract exhibits liquid organoleptic characteristics, with a yellowish-brown hue, a possible sweet taste attributed to glucose esters, and a distinctive odor. With a pH of 4.18, it suggests the presence of weakly acidic compounds such as phenols, tannins, and flavonoids. Comparing its density (0.91 g/ml) to that of the solvent (ethanol) indicates the existence of dissolved substances. The refractive index, 1.355, denotes the presence of dissolved substances, slightly higher than that of water. It is essential to note that quality parameters lack specific reference standards, as each extract species possesses its own values and characteristics.

In the case of *Miconia pseudocentrophora* (Table 2), the extract yield percentage is 97.5%, with potential variability based on the solvent recovery method, in this case, the rotavapor. The direct distillation method was employed, and the plant-to-70% ethanol ratio increased with maceration time, influencing the overall yield. The ethanolic extract exhibited organoleptic characteristics of a liquid with a brown color and a bitter taste typical of young plants. The pH of the ethanolic extract was 3.60, indicating the presence of acidic compounds (phenols, tannins, flavonoids, etc.) with OH characteristics. Notably, the extract's density was higher than the solvent density (ethanol, 0.97 g/ml), suggesting the presence of dissolved substances. The refractive index, at 1.363, further supported the presence of dissolved substances, slightly surpassing the index of water (1.333). This finding emphasizes the extract's purity relative to water density.

| **Parameter** | *Brachyotum ledifolium* | *Fuchsia loxensis* | *Miconia pseudocentrophora* |
|---|---|---|---|
| **Sample weight (g)** | 2000 | 2000 | 2000 |
| **Yield (%)** | 78.60 | 89.20 | 97.50 |
| **Aspect** | Liquid | Liquid | Liquid |
| **Consistency** | Transparent | Slightly turbid | Turbid |
| **Color** | Green yellowish | Brown yellowish | Brown |
| **Odor** | Strong herbal | Characteristic odor | Sweety |
| **Flavor** | Slightly sweet | Sweet | Bitter |
| **pH** | 4.18 | 3.77 | 3.60 |
| **Refraction index** | 1.355 | 1.358 | 1.363 |
| **Relative density (g/ml)** | 0.91 | 0.75 | 0.97 |
| **Sub-extracts yield (%)** | 3.50 | 1.80 | 1.00 |

**Table 2. Results of the determination of the physicochemical properties of the plant extracts.**

## Phytochemical screening

Qualitative assays, employing precipitation and coloration changes, were conducted to determine the presence of secondary metabolites. The results, as depicted in Table 3, encompass the screening outcomes of eight

distinct techniques aimed at identifying various compound types. Specifically, for *Brachyotum ledifolium* and *Miconia pseudocentrophora,* both belonging to the Melastomataceae family, the assays confirmed the presence of tannins, flavonoids, alkaloids, quinones, triterpenes, steroids, and reducing sugars. Meanwhile, the extract from *Fuchsia loxensis*, of the Onagraceae family, not only exhibited positive results for the aforementioned secondary metabolites shared with the Melastomataceae family but also tested positive for coumarins.

Our findings from *Miconia pseudocentrophora* align with those reported by Mencías Paredes[29] conducted similar research in a comparable region, supporting their screening outcomes through chromatographic assays and the identification of purified terpenoids and flavonoids as secondary metabolites. Regarding *Fuchsia loxensis* and *Brachyotum ledifolium*, no prior characterizations exist, although other members of the Melastomataceae[30,31] and Onagraceae[32] families have undergone similar studies and align with our results. Hydroalcoholic extracts from the Onagraceae family demonstrated anti-inflammatory, antimicrobial, antiproliferative, and anti-angiogenic properties attributed to polyphenols and flavonoids (gallic acid, caffeic acid, epicatechin, coumaric acid, ferulic acid, rutin, and rosmarinic acid), as determined by Shimadzu Chromatograph[32]. In the case of the Melastomataceae family, antioxidant properties have been observed, linked to the presence of phenolic compounds, flavonoids, and fatty acids alongside topical anti-inflammatory activity with low toxicity[30].

| Assay | Metabolite | Description | *Brachyotum ledifolium* | *Fuchsia loxensis* | *Miconia pseudocentrophora* |
|---|---|---|---|---|---|
| **$FeCl_3$** | Fenolic compound/tannins | Red-wine staining (phenolic compounds)<br>Intense green coloring (pyrocatecholic tannins).<br>Blue staining (pyrogallotannic tannins). | Blue coloration, (pyrogallol-type tannins) | Blue coloration, (pyrogallol-type tannins) | Blue coloration, (pyrogallol-type tannins) |
| **Shinoda** | Flavonoids | Intense yellow, orange, light brown, or deep red coloring in all cases. | Brown color (++) | Brown color (++) | Brown color (++) |
| **Dragendorff** | Alcaloids | Opalescence (+)<br>Turbidity (++)<br>Precipitate (+++) | Precipitate (+++) | Precipitate (+++) | Precipitate (+++) |
| **Baljet** | Compounds with lactonic grouping (coumarins). | Red color (++)<br>Red precipitate (+++) | Rusty brown (-) | Red precipitate (+++) | Yellowish-brown (-) |
| **Borntrager** | Quinones | Pink color (++)<br>Red color (+++) | Red color (+++) | Red color (+++) | Red color (+++) |
| **Liebermann-Burchard** | Triterpenes/steroids | Pink-blue<br>Intense green-visible<br>Dark green-black-at the end | Dark green-black-at the end (++) | Dark green-black-at the end (++) | Dark green-black-at the end (++) |
| **Saponins** | Triterpenes/steroids | Foam on the liquid surface more than 2 mm in height and persistent for more than 2 minutes. (+) | Persistent for 3 minutes (++). | Persistent for 3 minutes (++). | Persistent for 3 minutes (++). |
| **Fehling** | Reducing sugars | Solution turns red or red precipitate appears. | Red color (+) | Red color (+) | Red color (+) |

+++ strong evidence, ++ evidence, + low evidence, - negative

**Table 3. Results of the different phytochemical screening assays of the plant extracts to determine the presence of secondary metabolites with potential anti-inflammatory activity.**

### Inflammation volume in carrageenan-induced rat paw edema model in *Rattus norvegicus*

Carrageenan-induced paw edema in rats served as the *in vivo* model for analyzing the anti-inflammatory effects of plant extracts from *Miconia pseudocentrophora, Brachyotum ledifolium,* and *Fuchsia loxensis*. The paw volume was measured after inducing inflammation and administering each plant extract to achieve this. The corresponding values are detailed in Table 4 and Figure 1. Both compounds, ethanolic and chloroform sub-extracts, show performance comparable to naproxen sodium. The most efficient appears to be chloroform sub-extract and Melastomataceae extracts after quickly reducing the volume of the inflamed paw. Besides, the peak of inflammation was seen at the third hour of the experiment and then started to decline.

| Time (h) | Carrageenan (Positive control) | Naproxen sodium (Negative control) | *Brachyotum ledifolium* | *Fuchsia loxensis* | *Miconia pseudocentrophora* |
|---|---|---|---|---|---|
| **Ethanolic extracts** | | | | | |
| **0** | 15.708 | 15.708 | 15.708 | 15.708 | 15.708 |
| **1** | 26.549 | 16.399 | 18.665 | 22.243 | 17.701 |
| **3** | 37.378 | 19.179 | 22.699 | 21.085 | 18.327 |
| **5** | 28.777 | 17.564 | 18.341 | 19.463 | 19.380 |
| **7** | 20.153 | 15.612 | 16.903 | 19.654 | 17.925 |
| **8** | 15.972 | 15.134 | 15.781 | 19.736 | 14.740 |
| **Chloroform sub-extracts** | | | | | |
| **0** | 15.708 | 15.708 | 15.708 | 15.708 | 15.708 |
| **1** | 26.549 | 16.399 | 17.257 | 17.701 | 17.522 |
| **3** | 37.378 | 19.179 | 17.620 | 17.788 | 17.167 |
| **5** | 28.777 | 17.564 | 16.944 | 16.635 | 15.832 |
| **7** | 20.153 | 15.612 | 15.903 | 16.394 | 16.476 |
| **8** | 15.972 | 15.134 | 15.337 | 15.134 | 14.822 |

**Table 4. Inflammation volume ($cm^3$) after treatment with the studied plant extracts.**

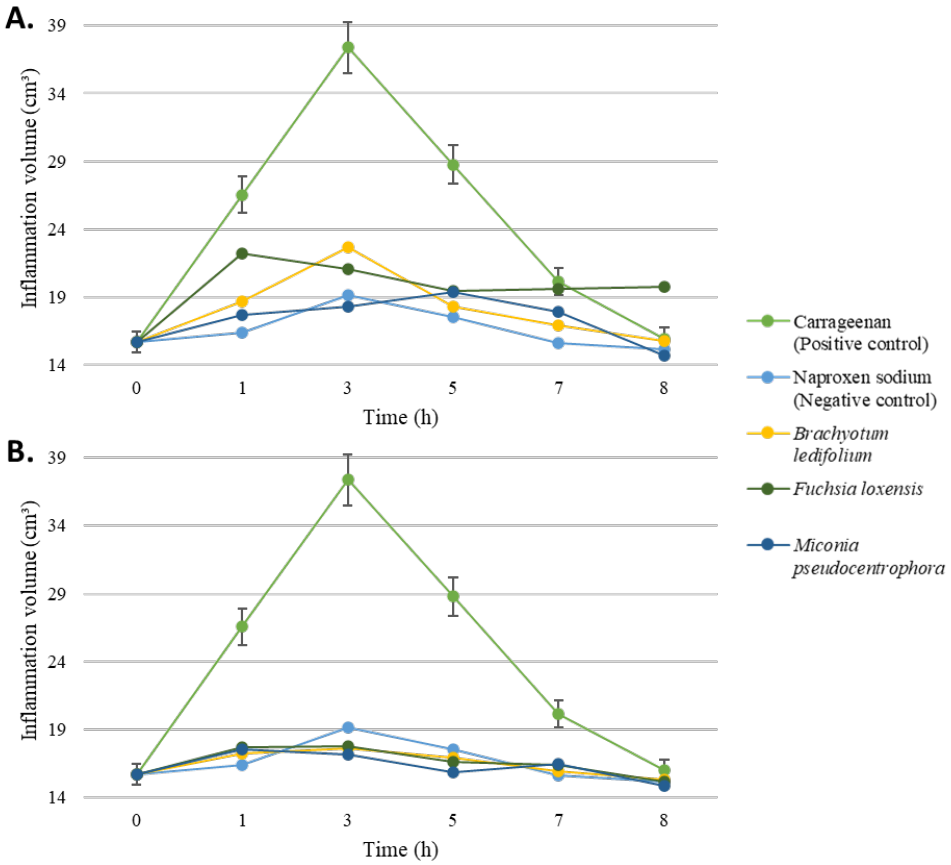


**Figure 1. Effect through time of ethanolic (A) and chloroform-based (B) plant extracts over inflammation on rats' paw. Control positive is carrageenan alone (green), while negative control is given by the administration of Naproxen sodium (light blue). Error percentage bars are included.**

### Inhibition percentage produced by the ethanolic extracts and chloroform sub-extracts

To conduct a comprehensive statistical analysis of the anti-inflammatory activity of the different obtained extracts, assays were performed in batches of three rats, using a negative control group and a positive control group as reference. These groups were analyzed at six different time points (0, 1, 3, 5, 7, and 8 hours) during the inflammation process, as shown in Table 5. After one hour of conducting the study, it was observed that all groups exhibited a similar percentage of inflammation inhibition, around 33%. The only extract showing a relatively lower performance is the alcoholic extract of *Fuchsia loxensis* with an inhibition percentage of 16%. Later on, the trend is that at 3 hours, the percentage of inflammation inhibition increases, reaching a maximum of 54%, and from that point onward, it begins to decline. At 7 hours, the average inflammation is 15%, and by 8 hours, it drops to 1%. Notably, the chloroform sub-extract of *Miconia pseudocentrophora* performs the best, while the ethanolic extract of *Fuchsia loxensis* shows the lowest performance.

| **Time (h)** | **Naproxen sodium** | ***Brachyotum ledifolium*** | | ***Fuchsia loxensis*** | | ***Miconia pseudocentrophora*** | |
|---|---|---|---|---|---|---|---|
| | | Ethanolic extract (100%) | chloroform sub-extract (100%) | Ethanolic extract (100%) | chloroform sub-extract (100%) | Ethanolic extract (100%) | chloroform sub-extract (100%) |
| **0** | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **1** | 38.231 | 29.696 | 34.999 | 16.218 | 33.327 | 33.327 | 34.002 |
| **3** | 48.690 | 39.273 | 52.860 | 43.591 | 52.412 | 50.970 | 54.073 |
| **5** | 38.967 | 36.266 | 41.119 | 32.367 | 42.193 | 32.654 | 44.984 |
| **7** | 22.535 | 16.128 | 21.089 | 2.479 | 18.654 | 11.059 | 18.248 |
| **8** | 5.245 | 1.192 | 3.973 | -23.566 | 5.245 | 7.711 | 7.198 |

**Table 5. The anti-inflammatory activity of ethanolic extracts and chloroform sub-extracts is represented as the average percentage of inflammation inhibition (%).**

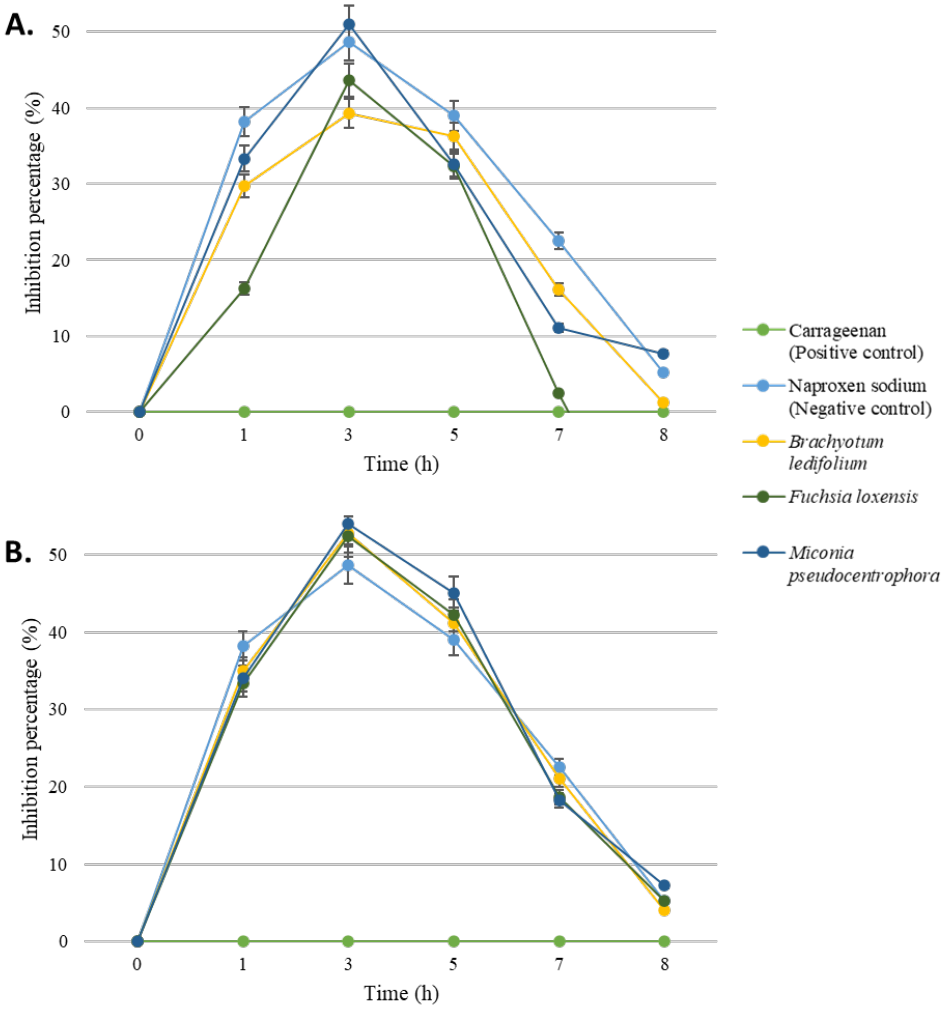


**Figure 2. Inflammation inhibition percentage through time of ethanolic (A) and chloroform-based (B) plant extracts over carrageenan-induced model. Error percentage bars are included.**

The ANOVA statistical test was employed to conduct a comprehensive analysis and ascertain the efficacy of each tested extract, followed by post hoc multiple comparisons using the Tukey HSD test at a 95% confidence level. The null hypothesis ($H_0$) postulates that there is no significant difference among the study groups concerning their anti-inflammatory activity, while the alternative hypothesis asserts that at least one of the study groups displays a statistically significant difference in anti-inflammatory activity. The decision to accept or reject the null hypothesis hinges on the p-value; if it falls below 0.05, the null hypothesis is rejected, and the alternative hypothesis is accepted. In terms of the p-values, after 1 hour of experimentation is $0.0002\times10^{-5}$, at 3 hours it is $0.0008\times10^{-2}$, at 5 hours it is $0.0006\times10^{-5}$, at 7 hours it is 0.0006, and at 8 hours it is $0.0003\times10^{-1}$. Consequently, at every point in time, we reject $H_0$ and accept the alternative hypothesis, affirming the existence of statistical differences between groups.

## CONCLUSIONS

This study is presented as the first-time evaluation of the ethnopharmacological properties of the extracts from *Miconia pseudocentrophora, Brachyotum ledifolium,* and *Fuchsia loxensis* collected in situ. The extracts, characterized with attention to quality through humidity and ash content assays, exhibited ethanolic extract yields of *Miconia pseudocentrophora*: 97.5%, *Brachyotum ledifolium*: 78.6%, and *Fuchsia loxensis*: 89.2%, along with chloroform sub-extract yields of *Miconia pseudocentrophora*: 1%, *Brachyotum ledifolium*: 3.5%, and *Fuchsia loxensis*: 1.8%.

The comprehensive characterization of these extracts provided insights into their purity based on various physicochemical properties. A phytochemical screening employing multiple qualitative assays unveiled the presence of diverse secondary metabolites, notably flavonoids, alkaloids, quinones, triterpenes, and reducing sugars. Given flavonoids' well-known anti-inflammatory properties, our observed inflammation reduction in animal models is noteworthy. Although attributing the effect to a specific secondary metabolite remains challenging, further studies are imperative to explore this aspect further. The remarkable reduction in paw inflammation volume in our rat models, comparable to naproxen sodium, supports the potential anti-inflammatory activity of *Miconia pseudocentrophora*, *Brachyotum ledifolium*, and *Fuchsia loxensis* across leaves, stems, and fruits. These findings validate the traditional medicinal properties of these plants, warranting additional research into toxicology, determination of minimum effective doses, and stability of the phytopharmaceuticals, with potential implications for the pharmaceutical industry.

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