

## Article

# Antimicrobial, Antioxidant, Anti-Inflammatory, and Antinociceptive Activities of the Aqueous Fraction of a Methanolic Extract of *Salvia brandegeei* Root

Othoniel Hugo Aragon-Martinez <sup>1,†</sup>, Juan Ramón Zapata-Morales <sup>2,†</sup>, Angel Josabad Alonso-Castro <sup>2</sup> ,  
María Leonor González-Rivera <sup>2</sup> , Iván Agustín Amador-Beas <sup>3</sup> , Itzel Joselyn Mora-Falcón <sup>3</sup> ,  
Iván Córdova-Guerrero <sup>4</sup> , Laura Díaz-Rubio <sup>4</sup>, Nicolás Serafín-Higuera <sup>5</sup> , Eduardo Gómez-Sánchez <sup>6</sup>   
and Mario Alberto Isiordia-Espinoza <sup>3,\*</sup> 

- <sup>1</sup> Departamento de Farmacología, Facultad de Medicina, Universidad Autónoma de San Luis Potosí, San Luis Potosí 78210, San Luis Potosí, Mexico; hugo.aragon@uaslp.mx
  - <sup>2</sup> Departamento de Farmacia, División de Ciencias Naturales y Exactas, Universidad de Guanajuato, Guanajuato 36050, Guanajuato, Mexico
  - <sup>3</sup> Departamento de Clínicas, División de Ciencias Biomédicas, Centro Universitario de los Altos, Universidad de Guadalajara, Av. Rafael Casillas Aceves No. 1200, Tepatlán de Morelos 47620, Jalisco, Mexico; itzel.mora@alumnos.udg.mx (I.J.M.-F.)
  - <sup>4</sup> Facultad de Ciencias Químicas e Ingeniería, Universidad Autónoma de Baja California, Tijuana 22390, Baja California, Mexico; ldiaz3@uabc.edu.mx (L.D.-R.)
  - <sup>5</sup> Facultad de Odontología, Unidad de Ciencias de la Salud, Universidad Autónoma de Baja California, Mexicali 21100, Baja California, Mexico
  - <sup>6</sup> Departamento de Fisiología, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara 44340, Jalisco, Mexico
- \* Correspondence: mario.isiordia162@yahoo.com  
† These authors contributed equally to this work.

## Abstract

**Background/Objectives:** The objective of this study was to determine the secondary metabolites of the aqueous fraction of a methanolic extract of *Salvia brandegeei* root and their antimicrobial, antioxidant, anti-inflammatory and antinociceptive properties. **Methods:** Fifteen reference compounds were evaluated by HPLC-UV, of which nine were tentatively identified in the aqueous fraction of the methanolic extract of *Salvia brandegeei* root. The antimicrobial effect of *Salvia brandegeei* 100, 50, 25, and 12.5 mg/mL and the vehicle (saline solution) against four ATCC bacterial and three fungal ATCC strains was analyzed. The DPPH, ABTS, and FRAP methods were used to evaluate antioxidant action. To evaluate the anti-inflammatory action, the topical application of TPA to the ear of mice was used, while to evaluate the antinociceptive effect, formalin and acetic acid models were used. In these animal models, *Salvia brandegeei* 100, 50, 25, and 12.5 mg/kg and the vehicle (saline solution) were administered orally. In addition, the antinociceptive mechanisms of action of *Salvia brandegeei* were evaluated with L-NAME (5 mg/kg), naloxone (2 mg/kg), and glibenclamide (10 mg/kg). **Results:** A total of 9 secondary metabolites were tentatively identified in the aqueous fraction of a methanolic root extract of *Salvia brandegeei*. *Salvia brandegeei* inhibited the growth of all ATCC species against which it was tested and showed an antioxidant effect through all three methods used. *Salvia brandegeei* produced a dose-dependent antinociceptive effect in both animal models. **Conclusions:** This study presents the first evidence of preliminary biological activities, including antimicrobial, antioxidant, anti-inflammatory, and antinociceptive effects, of an aqueous fraction of a methanolic root extract of *Salvia brandegeei*.

**Keywords:** antimicrobial activity; antioxidant effect; anti-inflammatory action; antinociception



Academic Editor: Fabrizio Schifano

Received: 13 July 2026

Revised: 27 August 2026

Accepted: 29 August 2026

Published: 1 September 2026

**Copyright:** © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

## 1. Introduction

In the past few years, the World Health Organization has noted an increasing concern with multidrug-resistant microorganisms and even produced a priority list of pathogens that have some type of resistance to antimicrobial agents [1]. The organization explained that bacterial multidrug resistance is a major public health threat [2], and projected that in the next couple of decades, it could kill more people than cancer [3] and that approximately ten million people would die by 2050 from an infection caused by this type of bacteria [4]. In this context, the WHO issued a formal request to the world scientific community to increase the research on novel biologically active molecules suitable for further pharmaceutical development for human use [1].

There are even several drugs derived from the plant kingdom that are used to relieve some symptoms or cure diseases in humans, such as morphine [5], papaverine [6], noscapine [7], and paclitaxel [8]. Nature has the potential to provide molecules with bioactive potential [9]. Long before the development of modern pharmaceuticals, human populations utilized botanical resources as a primary means of addressing health disorders and maintaining well-being [9] and some of these herbs have been shown to have a high antimicrobial effect, such as garlic, ginger [10,11], and turmeric [10,12], among many others [13,14].

Considering the impact and severity of multidrug resistance in bacteria [1,15], harnessing plant-produced secondary metabolites with antimicrobial properties could be useful for the development of drugs for the treatment of human infections in the current clinical setting [16]. Several plants in the genus *Salvia* have shown significant antimicrobial activity against a wide variety of human pathogens, including *Salvia officinalis* [16], *Salvia multicaulis* Vahl [17], *Salvia apiana* [18] and other species of these plants. In addition, some plants of the *Salvia* genus have demonstrated anti-inflammatory [19–22] and antinociceptive [23–25] properties. Despite the reported biological activities of several *Salvia* species, the phytochemical composition and biological properties of *Salvia brandegeei* remain comparatively underexplored, particularly with respect to its root-derived preparations. Moreover, the biological activity of the aqueous fraction obtained from a methanolic root extract of *Salvia brandegeei*, including its antimicrobial, antioxidant, anti-inflammatory, and antinociceptive properties, has not been comprehensively characterized. Therefore, further experimental characterization of this preparation is warranted to determine its phytochemical profile and provide preliminary evidence of its biological activities. The purpose of this study was to evaluate the antimicrobial, antioxidant, anti-inflammatory, and antinociceptive activities of the aqueous fraction of a methanolic extract of *Salvia brandegeei* root.

## 2. Material and Methods

### 2.1. Collection and Identification of Plant Material

The roots of *Salvia brandegeei* were collected in Ejido Punta Colonet, Ensenada Municipality, Baja California, Mexico, at an elevation of 20 m above sea level (a.s.l.; 31°07'00" N, 116°16'09" W) in July 2015. Taxonomic identification and authentication were performed by botanist Dr. José Delgadillo Rodríguez (herbarium BCMEX).

### 2.2. Preparation of Extracts

Following air-drying under laboratory conditions, the plant material was ground, and 1.9 kg of the resulting dry powder were obtained. The powdered material was extracted by soaking in methanol for 72 h using a 1:10 (*w/v*) ratio. The liquid phase was separated by filtration and concentrated under vacuum, affording 258 g of crude methanolic extract.

The crude extract was then subjected to liquid–liquid partitioning based on differential solubility, producing four fractions: n-hexane (17 g), dichloromethane (45 g), n-butanol

(15 g), and aqueous (22 g). The aqueous fraction was selected for all subsequent phytochemical and biological analyses.

The crude methanolic extraction yielded 258 g, corresponding to approximately 13.6% (*w/w*) of the initial dried plant material. The aqueous fraction yielded 22 g, corresponding to approximately 8.5% of the crude methanolic extract and 1.2% relative to the initial dried plant material.

### 2.3. Chronology and Storage of the Plant Material and Tested Fraction

The roots were collected in July 2015 and processed the same year. After collection, the plant material was dried, ground, and extracted with methanol. The resulting crude extract was chromatographically fractionated shortly after extraction. After chromatographic separation, the fractions obtained, including the aqueous fraction evaluated in the present study, were lyophilized, sealed under a nitrogen atmosphere, and stored at  $-4^{\circ}\text{C}$  until subsequent chemical and biological evaluation. Thus, the material maintained during the prolonged storage period consisted of previously prepared and lyophilized chromatographic fractions rather than dried roots or liquid crude extracts.

Lyophilization was used to substantially reduce residual water, thereby limiting hydrolytic degradation, while storage at low temperature and sealing the samples under a nitrogen atmosphere were intended to minimize chemical and oxidative degradation during storage. Nevertheless, we acknowledge that prolonged storage may affect the abundance or stability of some phytochemical constituents; therefore, complete chemical stability throughout the storage period cannot be assumed. We explicitly acknowledge this limitation in our study.

The animal experiments were conducted in 2025, while the studies on the antioxidant effect and the HPLC-UV analysis were performed in 2026.

### 2.4. Identification and Quantification of Secondary Metabolites

The phytochemical profile of *Salvia brandegei* extracts was characterized through liquid chromatography (LC) following previously reported procedures with methodological adaptations [26,27]. Reference standards were prepared in ethanol and included apigenin (1.07–68  $\mu\text{g/mL}$ ), ascorbic acid (1.79–114  $\mu\text{g/mL}$ ), benzoic acid (1.77–113  $\mu\text{g/mL}$ ), caffeic acid (1.24–79  $\mu\text{g/mL}$ ), gallic acid (1.50–96  $\mu\text{g/mL}$ ), gentisic acid (1.92–122  $\mu\text{g/mL}$ ), kaempferol (1.09–70  $\mu\text{g/mL}$ ), *p*-coumaric acid (1.80–115  $\mu\text{g/mL}$ ), sinapic acid (1.22–78  $\mu\text{g/mL}$ ), *o*-coumaric acid (1.14–72  $\mu\text{g/mL}$ ), methyl gallate (1.94–124  $\mu\text{g/mL}$ ), myricetin (1.17–74  $\mu\text{g/mL}$ ), quercetin (1.09–70  $\mu\text{g/mL}$ ), rutin (1.54–98  $\mu\text{g/mL}$ ), and rosmarinic acid (19.33–232.00  $\mu\text{g/mL}$ ) [28]. Plant extracts were dissolved in deionized water before analysis.

Standard solutions and samples were filtered through 0.45  $\mu\text{m}$  membrane filters (Millipore Corporation, Bedford, MA, USA). Subsequently, 200  $\mu\text{L}$  of each filtrate was transferred to amber autosampler vials fitted with glass inserts. Chromatographic analyses were performed using an Agilent 1100 LC (Rev. B.02.01-SR1; Agilent Technologies, Palo Alto, CA, USA) platform equipped with a quaternary pump, vacuum degasser, autosampler, thermostatically controlled column compartment, and diode-array detector. Data acquisition and processing were carried out using Agilent ChemStation software for LC 3D systems (Rev. B.02.01-SR1; Agilent Technologies, Palo Alto, CA, USA).

Separation was achieved on a Synchronis C18 analytical column (250  $\times$  4.6 mm, 5  $\mu\text{m}$  particle size) maintained at 30  $^{\circ}\text{C}$  [29]. The mobile phase consisted of sodium acetate buffer (pH 3.0) and acetonitrile delivered under gradient conditions. The chromatographic program began with 95% buffer and 5% acetonitrile, reaching 80:20 at 28 min, 60:40 at 39 min, and 10:90 at 50 min. Initial conditions were restored at 55 min and maintained for an additional 10 min to ensure column re-equilibration.

Samples were injected at a volume of 20  $\mu\text{L}$  and eluted at a flow rate of 1 mL/min. Detection wavelengths were selected according to the absorption characteristics of each compound: 230 nm for benzoic acid; 270 nm for ascorbic acid, gallic acid, methyl galate, and *o*-coumaric acid; 310 nm for gentisic acid, caffeic acid, *p*-coumaric acid, sinapic acid, and rosmarinic acid; and 350 nm for rutin, myricetin, quercetin, apigenin, and kaempferol. The total running time was 65 min, while full UV spectra were recorded between 190 and 600 nm.

Compounds were tentatively identified by comparing their retention times and UV-visible spectral characteristics with those of authentic standards. Flavonoid assignment was further supported by the characteristic absorption bands associated with the cinnamoyl (Band I) and benzoyl (Band II) chromophore systems [30]. Quantification was performed using external calibration curves generated for each standard compound, and results were expressed as  $\mu\text{g}$  of compound per gram of extract.

### 2.5. Assessment of Antimicrobial Effect

The microorganisms were obtained from the company PROQUIFA in Guadalajara, Jalisco, Mexico. Using the agar well diffusion technique (well diameter = 5 mm) on nutrient agar [18], concentrations of 12.5 to 100 mg/mL of *Salvia brandegeei* and the vehicle (saline solution) in 20  $\mu\text{L}$  were evaluated against *Enterococcus faecalis* (ATCC 51575), *Streptococcus mitis* (ATCC 13770), *Streptococcus pyogenes* (ATCC 49399), *Staphylococcus aureus* (ATCC 11632), *Candida albicans* (ATCC 2091), *Candida glabrata* (ATCC 15126), and *Candida krusei* (ATCC 14243) ( $n = 3$ ). The samples were subjected to incubation at 37 °C for 24 h prior to assessment. Bacterial and yeast suspensions were adjusted to McFarland turbidity standards of 0.5 and 1.0, respectively, to ensure standardized cell density prior to exposure. No reference antimicrobial agent was included as a positive control for the assays.

### 2.6. Determination of Total Phenolics and Flavonoids

The concentration of total phenolic compounds was determined using the Folin–Ciocalteu colorimetric assay [31]. Briefly, 30  $\mu\text{L}$  of extract were combined with 150  $\mu\text{L}$  of 10% Folin–Ciocalteu reagent and 120  $\mu\text{L}$  of 7.5% sodium carbonate solution. Following a 30 min incubation period, absorbance was measured at 765 nm. Results were calculated from a gallic acid calibration curve and reported as  $\mu\text{g}$  gallic acid equivalent (GAE)/mg dry weight (DW).

Total flavonoid content was assessed by the aluminum chloride method [32]. Aliquots of extract (250  $\mu\text{L}$ ) were mixed with distilled water (1 mL) and 75  $\mu\text{L}$  of 5% sodium nitrite. After 3 min, 75  $\mu\text{L}$  of 10% aluminum chloride was added. Following an additional 3 min incubation, 500  $\mu\text{L}$  of 1 M sodium hydroxide were incorporated, and the reaction mixture was allowed to stand for 15 min. Absorbance was then recorded at 496 nm. Flavonoid content was calculated using a quercetin standard curve and expressed as  $\mu\text{g}$  quercetin equivalents (QE)/mg DW. Calibration models for both assays exhibited coefficients of determination ( $R^2$ ) of at least 0.99.

### 2.7. Antioxidant Capacity Assays

The antioxidant properties of the extracts were examined using DPPH and ABTS radical scavenging assays according to previously established procedures [28].

For the DPPH assay, 50  $\mu\text{L}$  of sample were mixed with 200  $\mu\text{L}$  of a 202  $\mu\text{mol/L}$  DPPH solution and incubated at 30 °C for 20 min. Thereafter, 150  $\mu\text{L}$  of the reaction mixture were transferred to a 96-well microplate and absorbance was measured at 517 nm using a Cytation™ 3 microplate reader operated with Gen5™ software (version 2.06, Biotek Instruments Inc., Winooski, VT, USA). Water served as the blank because it was also used as the extraction solvent. Radical scavenging activity was calculated as percentage inhibition

relative to the blank. Gallic acid (27.5 µg/mL) and citric acid (1586.3 µg/mL) were included as positive and negative controls, respectively, due to their known antioxidant and non-antioxidant properties [33].

For the ABTS assay, the radical cation was generated by incubating a 7.0 mmol/L ABTS solution with 2.4 mmol/L potassium persulfate for 16 h in darkness. The resulting solution was diluted with deionized water to obtain a final radical concentration of 95.7 µmol/L. Subsequently, 300 µL of the ABTS solution were mixed with 3 µL of sample and incubated at 30 °C for 4 min. An aliquot of 150 µL was then placed into a 96-well plate, and absorbance was recorded at 730 nm. The same blank and control solutions employed in the DPPH assay were used in this experiment, with gallic acid (440.0 µg/mL) and citric acid (50,760.0 µg/mL) serving as positive and negative controls, respectively. Antioxidant activity was expressed as percentage inhibition ( $n = 5$ ).

The reducing power of the extracts was further assessed using the ferric reducing antioxidant power (FRAP) assay [33]. Calibration standards were prepared from ferrous sulfate dissolved in deionized water. For each determination, 100 µL of sample or standard were combined with 250 µL of 300 mmol/L sodium acetate buffer (pH 3.6), 25 µL of 2,4,6-tris(2-pyridyl)-s-triazine, and 25 µL of ferric chloride solution. After incubation at 37 °C for 4 min, 200 µL of the reaction mixture were transferred to a 96-well plate, and absorbance was measured at 596 nm. FRAP values were calculated from the ferrous sulfate calibration curve and expressed as Fe(II) equivalents. As in the radical scavenging assays, gallic acid (27.7 µg/mL) and citric acid (1580.3 µg/mL) were employed as positive and negative controls, respectively ( $n = 5$ ).

## 2.8. Acute Inflammation with 12-O-Tetradecanoylphorbol 13-Acetate (TPA)

All experiments involving animals—including the formalin and abdominal writhing tests—utilized male BALB/c mice (28–30 g) sourced from the animal housing facility of the Division of Natural and Exact Sciences at the University of Guanajuato. Animals were maintained with ad libitum access to standard chow and water. The study was approved by the University of Guanajuato's ethical committee for research (protocol code CIBIUG-P48-2022 and 4 November 2022). In adherence to Mexican guidelines for lab animal care (NOM-062-ZOO-1999), animals were maintained under regulated thermal conditions with a 12 h photoperiod. Testing was scheduled exclusively between 08:00 and 14:00 to control for circadian fluctuation. Upon completion of all evaluations, euthanasia was carried out via cervical dislocation [34].

The TPA acute swelling assay was performed on the ears of rodents as previously described [35,36]. The studied groups ( $n = 6$  each group) included the following: vehicle in comparison to extract of *Salvia brandegeei* (2 mg/ear). An acetone-based preparation containing 2.5 µg of TPA in a final volume of 20 µL was topically applied to both sides of the right ear. Mice were euthanized 6 h post-treatment for evaluation of the inflammatory response. At the end of the experimental period, 6 mm punch biopsies were obtained from the central portion of both ears. Tissue weights were recorded, and the anti-inflammatory response was quantified by calculating the percentage inhibition relative to the untreated contralateral ear [35–37].

## 2.9. Formalin Test

The animal test of nociception was carried out as described previously [38]. Prior to each experiment, the animals were acclimated for 1 h in the experimentation room in individual observation chambers. Saline or the extract of *Salvia brandegeei* (100, 50, 25, or 12.5 mg/kg) were administered orally 30 min before the injection of 30 µL 3% formalin. The dose range was selected based on previous pharmacological studies of *Salvia* extracts evaluated

in murine nociception models, including a study reporting antinociceptive activity over a dose range of 3.2–100 mg/kg [25]. Based on this pharmacological precedent, doses of 12.5, 25, 50, and 100 mg/kg were selected to provide a graded exploratory dose range.

Once the painful stimulus into the sub-plantar space of the right hind paw was administered, the licking time that the animal performed on the injured area was quantified for 45 min (phase 1 from minute 0 to 15 min and phase 2 from 16 to 45 min) ( $n = 6$  mice per group/cage of animals) [34,35]. The decrease in the licking time was considered antinociception. Information regarding treatment allocation to the study arms was available exclusively to the first author and the corresponding author (approved protocol codeCIBIUG-P48-2022 and 4 November 2022).

Nociceptive responses were quantified in mice after intraplantar challenge with 3% formalin by measuring the duration of pain-related behavioral activity. The behavioral response was analyzed according to the two established periods of the formalin assay, and the measurements obtained for each treatment group were subsequently compared with those recorded in the saline control group [38]. During the formalin assay, behavioral scoring was conducted with the evaluator blinded to the treatment received by each animal, minimizing potential observer-related bias in the assessment of nociceptive responses.

#### 2.10. Writhing Test

The model of writhing mice was made as previously reported [39,40]. Prior to each experiment, the animals were acclimated for 1 h in the experimentation room in individual observation chambers. Saline or the extract of *Salvia brandegeei* (100, 50, 25, or 12.5 mg/kg) were administered orally 30 min before the intraperitoneal injection of a 1% acetic acid solution. An intraperitoneal injection of 10 mL/kg of a 1% acetic acid solution was used like a painful stimulus. Then, the number of abdominal contractions was counted during the next 30 min (in 5 min-periods) ( $n = 6$  mice per group/cage of animals). The decrease in the number of abdominal contractions was considered antinociception. Information regarding allocation to the study arms was available exclusively to the second author and the corresponding author. For the abdominal writhing assay, behavioral responses were recorded and quantified by an evaluator who was unaware of the treatment assignment, thereby reducing the potential influence of observer bias (approved protocol codeCIBIUG-P48-2022 and 4 November 2022).

The antinociceptive mechanisms of action of *Salvia brandegeei* were evaluated with L-NAME (5 mg/kg), naloxone (2 mg/kg), and glibenclamide (10 mg/kg). The evaluation of each route was conducted using four experimental groups: vehicle, antagonist plus vehicle, extract, and antagonist plus extract. These antagonists were administered via intraperitoneal at −45 min, *Salvia brandegeei* extract 100 mg/kg was administered by oral way at −30 min, and acetic acid was given intraperitoneally at time 0 min, at which point the evaluation of the animals' nociceptive behavior began, as explained in the previous paragraph ( $n = 6$  mice per group) [39,40] (approved protocol codeCIBIUG-P48-2022 and 4 November 2022).

#### 2.11. Data Analysis

Sample size estimation was performed prior to the study using G\*Power 3.1.9.7 software. Setting  $\alpha = 0.05$ , a power of 0.80, and a large effect size ( $f = 0.50$ ), the analysis indicated a minimum cohort of  $n = 6$  per group across 2 conditions for the TPA model, 6 for the formalin assay, and 6 for the abdominal writhing test. A large effect magnitude was anticipated due to the marked differences in behavioral and inflammatory outcomes. This sample size reconciles statistical validity with the reduction principle of the 3Rs

framework, although we acknowledge that larger cohorts are occasionally employed in similar literature [41,42].

GraphPad Prism software (version 5.01, San Diego, CA, USA) was employed for all statistical procedures. Experimental outcomes are expressed as mean values together with their corresponding standard errors. Because the data did not meet the assumption of normality, nonparametric statistical methods were used. Comparisons among multiple independent groups were performed using the Kruskal–Wallis test followed by Dunn’s multiple-comparison test with Holm adjustment. Data from the formalin and writhing time-course experiments are presented descriptively, whereas inferential analyses were performed on the predefined summary measures. Differences were interpreted as significant when the associated probability value was lower than 0.05.

### 3. Results

#### 3.1. Determination of Secondary Metabolites

Analysis of the extract composition revealed nine detectable metabolites. Quantitative assessment showed that rosmarinic acid was the dominant constituent, occurring at higher levels than any other identified compound. Figure 1 shows a chromatogram monitored at 310 nm, where rosmarinic acid is the greatest peak in comparison with the rest of peaks. Between the compounds tentatively identified by HPLC-UV, myricetin and sinapic acid were the second and third most abundant compounds in the aqueous fraction of a methanolic extract of *Salvia brandegeei* (Table 1). Information regarding method validation is presented in Table S1. These findings indicate that the aqueous fraction contains several phenolic and flavonoid-related compounds, with rosmarinic acid being the most abundant compound detected.

#### 3.2. Antimicrobial Effect

Overall, the antimicrobial effect results showed that bacteria were more sensitive to the aqueous fraction of a methanolic extract of *Salvia brandegeei* root than to *Candida* species. The extract’s greatest antimicrobial effect was observed against *Enterococcus faecalis*, while antifungal activity was observed against *Candida albicans* (Figure 2).

**Table 1.** Compounds tentatively identified and quantified in the aqueous fraction of a methanolic extract of *Salvia brandegeei*.

| Compound                | Concentration (µg/g) |
|-------------------------|----------------------|
| Rosmarinic acid         | 7282.8 ± 152.6       |
| Myricetin               | 1722.5 ± 141.5       |
| Sinapic acid            | 1615.3 ± 21.2        |
| Rutin                   | 951.5 ± 121.2        |
| Gentisic acid           | 734.3 ± 51.2         |
| Caffeic acid            | 468.6 ± 259.7        |
| Gallic acid             | 332.0 ± 4.7          |
| <i>p</i> -Coumaric acid | 293.6 ± 43.5         |
| <i>o</i> -Coumaric acid | 60.1 ± 15.4          |
| Kaempferol              | Not detected         |
| Quercetin               | Not detected         |
| Apigenin                | Not detected         |
| Methyl gallate          | Not detected         |
| Benzoic acid            | Not detected         |
| Ascorbic acid           | Not detected         |

Measurements were conducted in triplicate, and the results are reported as the corresponding mean accompanied by the standard deviation.

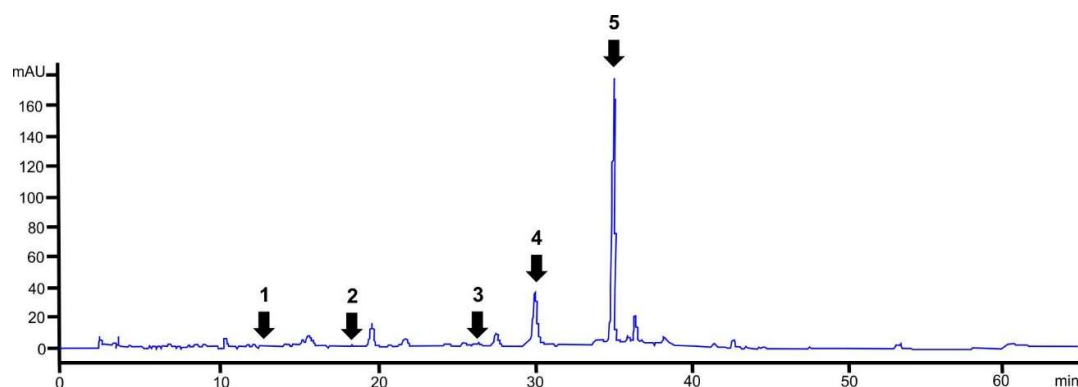


**Figure 1.** Representative chromatogram obtained at 310 nm of the aqueous fraction of a methanolic extract of *Salvia brandegeei* ( $n = 3$ ). Compound 1, gentisic acid; compound 2, caffeic acid; compound 3, *p*-coumaric acid; compound 4, sinapic acid; and compound 5, rosmarinic acid.

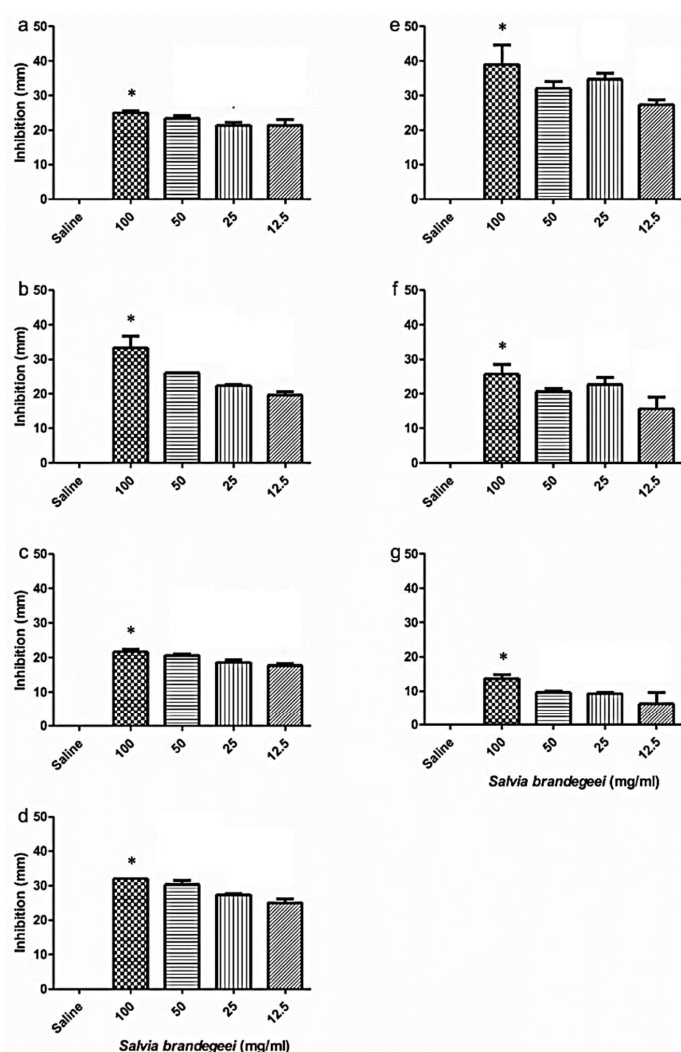


**Figure 2.** Antimicrobial activity of *Salvia brandegeei* against (a) *Staphylococcus aureus* (ATCC 11632), (b) *Streptococcus pyogenes* (ATCC 49399), (c) *Streptococcus mitis* (ATCC 13770), (d) *Enterococcus faecalis* (ATCC 51575), (e) *Candida albicans* (ATCC 2091), (f) *Candida glabrata* (ATCC 15126), and (g) *Candida krusei* (ATCC 14243). \*  $p \leq 0.05$  *Salvia brandegeei* versus saline ( $n = 3$ ).

### 3.3. Antioxidant Activity

Gallic acid and *Salvia brandegeei* root extract showed a significant antioxidant effect in all analytical tests when compared to citric acid (Figure 3).

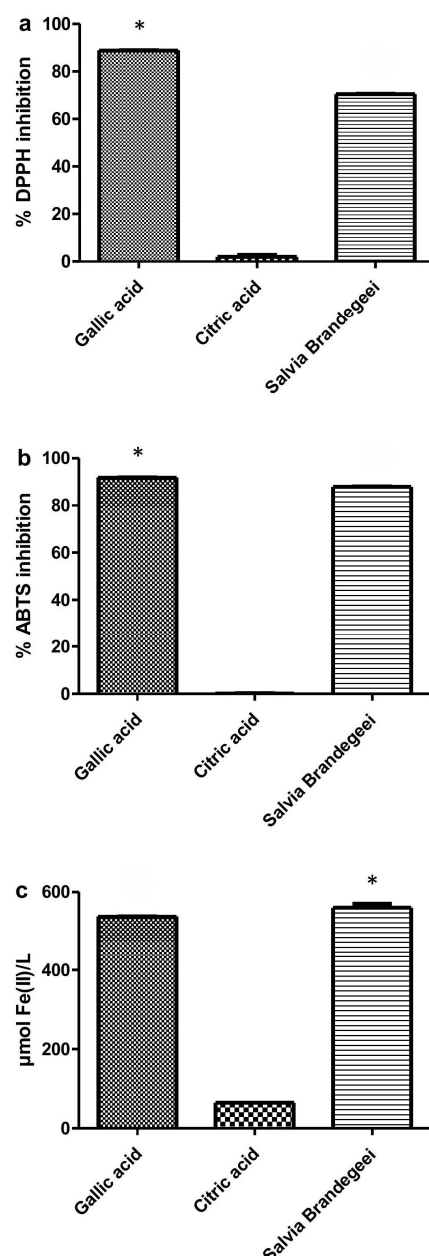


**Figure 3.** Antioxidant effect of *Salvia brandegeei* in (a) DPPH, (b) ABTS, and (c) FRAP methods ( $n = 5$ ). \*  $p \leq 0.05$  *Salvia brandegeei* versus citric acid ( $n = 5$ ).

### 3.4. Anti-Inflammatory Activity

The aqueous fraction of the methanolic extract of *Salvia brandegeei* showed a mean anti-inflammatory effect of  $0.013 \pm 0.002$ , whereas the mean for the control group was  $0.017 \pm 0.001$  in the TPA-induced edema model ( $p > 0.05$ ).

### 3.5. Formalin Test

Time curves show the painful behavior of the animals over time after intraplantar administration of 3% formalin as well as the antinociceptive effect over time of different concentrations of the aqueous fraction of a methanolic extract of *Salvia brandegeei* root (Figure 4a–d).

It is important to note that *Salvia brandegeei* at doses of 100 and 50 mg/kg showed a statistically difference when compared to saline in the first phase (Figure 5a), while only the 100 dose of *Salvia brandegeei* showed a statistically significant difference when compared to saline in the second phase of the formalin trial (Figure 5b).

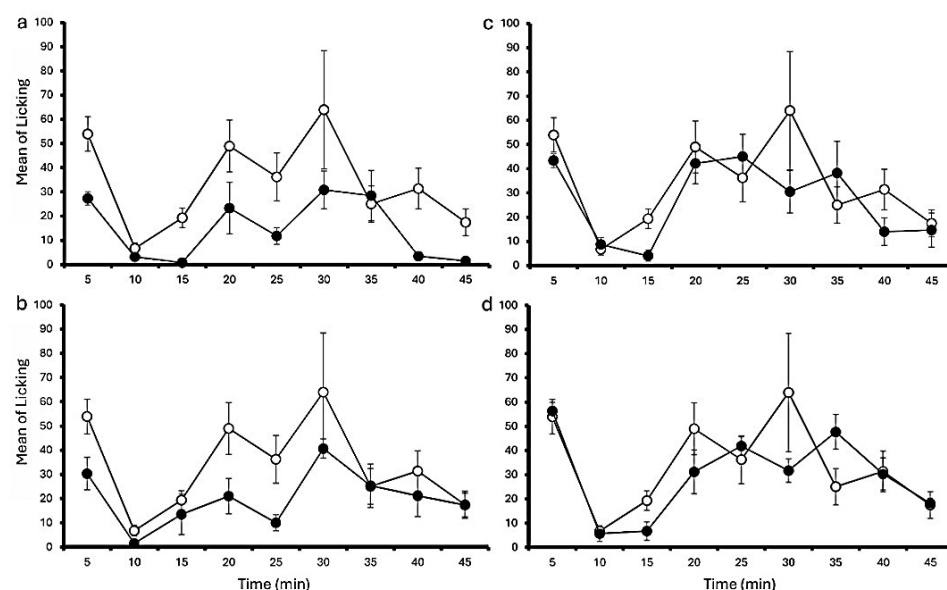


**Figure 4.** Descriptive time-course profiles of the antinociceptive action of the aqueous fraction of a methanolic extract of *Salvia brandegeei*: (a) 100, (b) 50, (c) 25, and (d) 12.5 mg/kg in the formalin test. The white circles correspond to the curve of the saline solution plus formalin (○), while the black circles (●) show the effect of the different concentrations of the aqueous fraction of a methanolic extract of *Salvia brandegeei* ( $n = 6$  per group).

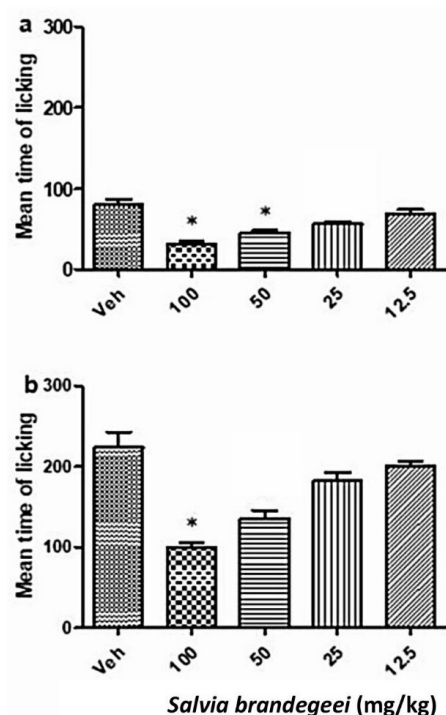


**Figure 5.** Antinociceptive effect of *Salvia brandegeei* in the (a) first and (b) second phases of the formalin test. In both phases of this nociception model, a dose-dependent effect can be observed. \*  $p \leq 0.05$  *Salvia brandegeei* versus saline ( $n = 6$  per group).

### 3.6. Writhing Test

The time curves show that intraperitoneal administration of 1% acetic acid induced characteristic behavior in mice, while the curves of the animals administered with the aqueous fraction of the methanolic extract of *Salvia brandegeei* induced a decrease in nociceptive behavior (Figure 6a–d).

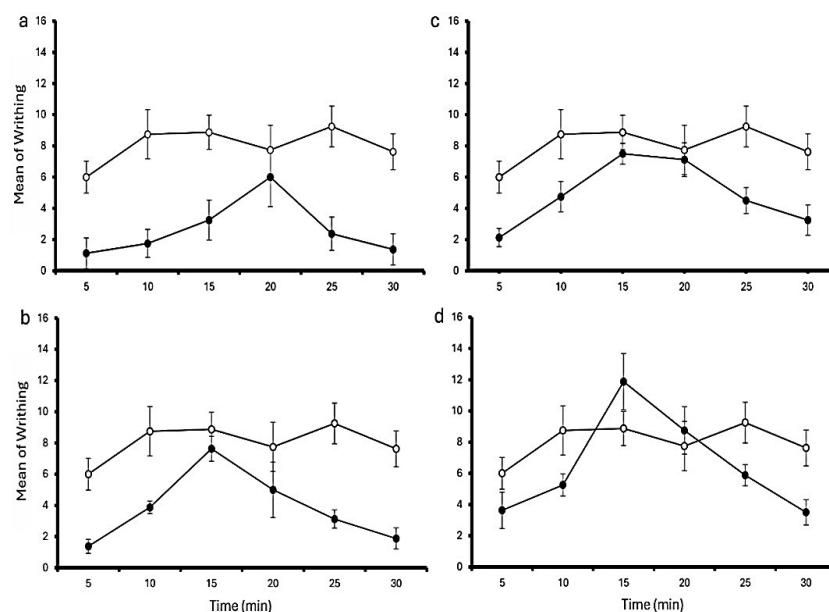


**Figure 6.** Descriptive time-course profiles of the antinociceptive activity of the aqueous fraction of a methanolic extract of *Salvia brandegeei*: (a) 100, (b) 50, (c) 25, and (d) 12.5 mg/kg in the writhing test. The white circles correspond to the curve of the saline solution plus acetic acid (○), while the black circles (●) show the curves of the antinociceptive effect of the different concentrations of the aqueous fraction of the methanolic extract of *Salvia brandegeei* root ( $n = 6$  per group).

It can be observed that *Salvia brandegeei* induced a dose-dependent antinociceptive effect with a statistically significant difference when compared to saline (Figure 7a). However, no antagonist affected the antinociceptive activity of *Salvia brandegeei* (Figure 7b–d).

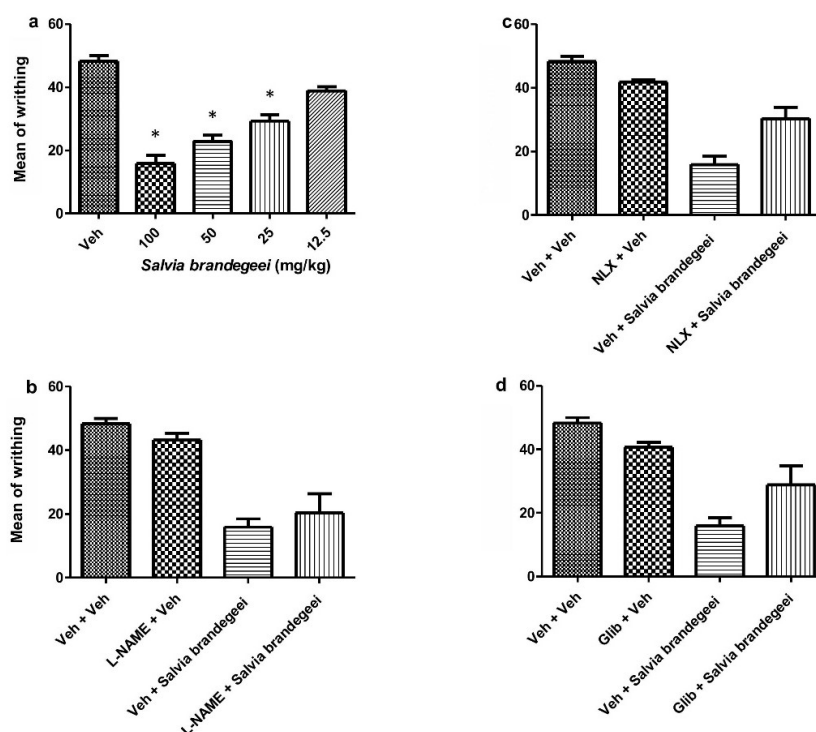


**Figure 7.** (a) Dose-dependent antinociceptive action of the aqueous fraction of a methanolic extract of *Salvia brandegeei*, (b) lack of action of L-NAME on the antinociceptive effect of *Salvia brandegeei*, (c) lack of activity of naloxone (NLX) on the antinociceptive effect of *Salvia brandegeei*, and (d) lack of effect of glibenclamide on the antinociceptive action of *Salvia brandegeei*. \*  $p \leq 0.05$  *Salvia brandegeei* versus saline ( $n = 6$  per group).

#### 4. Discussion

This is the first scientific study using the aqueous fraction of a methanolic extract of *Salvia brandegeei* root demonstrating the different properties of its secondary metabolites. The results of our study show that the aqueous fraction of the *Salvia brandegeei* extract has antimicrobial, antioxidant, anti-inflammatory, and antinociceptive effects, which may be associated with the phenolic and flavonoid constituents detected in the fraction; however, their individual contributions were not established [43–54] (Table 1).

Among the secondary metabolites detected in the aqueous fraction, rosmarinic acid was the most abundant [43], followed by others including gentisic acid [49], gallic acid [50,51], and *p*- and *o*-coumaric acid [46,53,54] (Table 1). These compounds may contribute to the observed antimicrobial activity; however, their individual contributions were not established. The antimicrobial effect of *Salvia* plants is widely known, with *Salvia officinalis* being the most studied. This plant has demonstrated an antimicrobial effect against *S. mutans*, *Lactobacillus* spp. [55], *Listeria monocytogenes* [56], *Porphyromonas gingivalis* [57], *Aggregatibacter actinomycetemcomitans* [57], *Prevotella intermedia* [57], *Prevotella nigrescens* [57], *Fusobacterium nucleatum* [57], and *Prevotella melaninogenica* [57]. Other plants of the genus *Salvia* that have demonstrated antimicrobial effects are *Salvia multicaulis* Vahl [17], *Salvia apiana* [18], *Salvia munzii* [58], *Salvia rosmarinus* Spenn [59], *Salvia bracteata* [60] and *Salvia rubifolia* [60], *Salvia blepharophylla* [61], *Salvia greggii* [61], *Salvia aegyptiaca* [62], and *Salvia lanigera* [62], and possibly many other plants.

The antimicrobial effect of this *Salvia brandegeei* extract could be related to other metabolites that produce antioxidant or redox activity [63–66]. Some flavonoid compounds can interact with membranes, proteins, and nucleic acids, which could increase oxidative stress and affect the viability of microorganisms [63–66]. In this same context, these compounds can interfere with catalase or superoxide dismutase, increasing the susceptibility of microorganisms to oxidative damage [67–69]. This highlights the potential of antioxidant-rich plant extracts as promising antimicrobial agents. Some plants of this genus that have demonstrated an antioxidant effect are *Salvia officinalis* [70], *Salvia aratocensis* [71], *Salvia miltiorrhiza* [72], *S. nemorosa* [73], and *Salvia hispanica* [74], among other plants [75].

In this study, *Salvia brandegeei* extract showed anti-inflammatory activity in one murine model and antinociceptive activity in two murine models. Both the anti-inflammatory and antinociceptive effects may be associated with the secondary metabolites present in our *Salvia brandegeei* extract; however, their individual contributions were not established [43–54]. The presence of rosmarinic acid provides a plausible hypothesis for part of the observed antinociceptive activity, based on previous pharmacological evidence; however, causality cannot be established from the present whole-extract study. Boonyarikpunchai et al., 2014 demonstrated that rosmarinic acid produces antinociception in formalin- and acetic-acid-induced mouse models [76]. Furthermore, this study showed that the antinociceptive effect of rosmarinic acid was reversed by naloxone, indicating the involvement of opioid receptors [76]. A study by Hernández-León et al., 2016 [77] demonstrated that the secondary metabolite rutin exhibits antinociceptive activity and that this action involves the participation of opioid receptors and the descending modulation of the ventrolateral periaqueductal gray matter. Rodrigues et al., 2012 demonstrated that *Salvia officinalis* induces an antinociceptive effect in the formalin nociception model in mice and that this antinociceptive effect was partially reversed by carnosol, indicating the involvement of the TRPA1 receptor pathway [78]. Quiñonez-Bastidas et al., 2025 reported that an extract of *Salvia divinorum* produced antinociception in the murine model of formalin-induced trigeminal pain and that this effect is mediated through the CB1R and TRPV1 receptors [25].

Several limitations should be considered when interpreting the findings of the present study. First, the biological activities were evaluated using the aqueous fraction obtained from a methanolic extract of *Salvia brandegeei* root, and therefore the observed effects

cannot be attributed to a single compound or specific constituent. Although the chromatographic method was evaluated for linearity, accuracy, precision, stability, and lower limits of quantification (Table S1), complementary structural confirmation by LC-MS or NMR and additional validation parameters, including recovery and matrix effects, were not assessed. In addition, the extract was not subjected to formal batch-to-batch standardization, and further studies should establish appropriate chemical markers and evaluate the reproducibility of independently prepared batches. Second, the antimicrobial evaluation was limited to ATCC reference strains and inhibition-zone assays. MIC, MBC, and MFC values, as well as activity against clinical or multidrug-resistant isolates, were not determined; therefore, the antimicrobial findings should be interpreted as preliminary activity under the experimental conditions employed and should not be extrapolated to clinical efficacy. Third, although the TPA-induced ear edema model demonstrated an anti-inflammatory effect, no reference anti-inflammatory drug was included, limiting the pharmacological comparison and preventing an assessment of relative potency. Fourth, the antinociceptive experiments provide evidence of activity in the formalin- and acetic-acid-induced writhing models; however, in the present study, pretreatment with naloxone, glibenclamide, or L-NAME did not significantly modify the antinociceptive response produced by the aqueous fraction in the acetic-acid-induced writhing test. Therefore, these experiments do not provide sufficient pharmacological evidence to support the involvement of opioid, ATP-sensitive potassium, or nitric oxide pathways. Likewise, the present study did not evaluate additional pathways, such as cannabinoid, TRPV1, or TRPA1 signaling, nor were the antagonist experiments replicated across all nociceptive models. Fifth, the animal experiments were conducted with relatively small experimental groups, and additional studies with adequately powered designs and independent replication would strengthen the robustness of the findings. Sixth, cytotoxicity assays, acute systemic toxicity studies, and pharmacokinetic or bioavailability evaluations were not performed. Consequently, the present data do not establish the safety profile, therapeutic index, pharmacokinetic behavior, or a therapeutically appropriate dose of the extract. The human-equivalent doses calculated from the experimental mouse doses are useful only for interspecies pharmacological comparison and should not be interpreted as proposed human doses (equation  $HED = \text{animal dose} \times 3/37$ ). Using this conversion factor, the HED values corresponding to 12.5, 25, 50, and 100 mg/kg in mice are approximately 1.01, 2.03, 4.05, and 8.11 mg/kg, respectively. Finally, the biological effects observed in the present study should be considered preliminary preclinical evidence and should not be interpreted as demonstrating therapeutic efficacy in humans. Further investigations integrating chemical standardization, mechanistic pharmacology, toxicological evaluation, pharmacokinetic characterization, dose–response optimization, and validation in additional experimental models will be necessary to determine the translational potential of the aqueous fraction of *Salvia brandegeei*.

In conclusion, this study presents the first evidence of preliminary biological activities, including antimicrobial, antioxidant, anti-inflammatory, and antinociceptive effects of an aqueous fraction of a methanolic root extract of *Salvia brandegeei*.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/futurepharmacol6030049/s1>, Table S1. Analytical performance and validation parameters of the HPLC-UV method used for the determination of the reference compounds [27].

**Author Contributions:** Conceptualization, M.A.I.-E., O.H.A.-M., and J.R.Z.-M.; methodology, M.A.I.-E., O.H.A.-M., and J.R.Z.-M.; software, A.J.A.-C.; validation, M.L.G.-R., O.H.A.-M., N.S.-H., and J.R.Z.-M.; formal analysis, A.J.A.-C., I.C.-G., N.S.-H., and L.D.-R.; investigation, M.A.I.-E., O.H.A.-M., J.R.Z.-M., M.L.G.-R., I.A.A.-B., I.C.-G., L.D.-R., and I.J.M.-F.; resources, I.A.A.-B. and I.J.M.-F.; data curation, I.A.A.-B., I.J.M.-F., and E.G.-S.; writing—original draft preparation, M.A.I.-E., O.H.A.-

M., and J.R.Z.-M.; writing—review and editing, N.S.-H., M.A.I.-E., I.C.-G., L.D.-R., and M.L.G.-R.; visualization, E.G.-S.; supervision, A.J.A.-C., O.H.A.-M., and J.R.Z.-M.; project administration, E.G.-S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no grant from any funding agency.

**Institutional Review Board Statement:** The study was approved by the University of Guanajuato's ethical committee for research (protocol code CIBIUG-P48-2022 and 4 November 2022).

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data are available from the corresponding author upon reasonable request.

**Acknowledgments:** During the preparation of this manuscript, the authors used ChatGPT-5 (OpenAI, San Francisco, CA, USA) as a language-support tool to improve grammar, spelling, clarity, readability, and overall manuscript organization. The AI system was not used to generate, analyze, interpret, or validate scientific data, nor to formulate scientific conclusions. All scientific content, data interpretation, critical analysis, and final decisions regarding the manuscript were performed exclusively by the authors. The authors carefully reviewed and edited all AI-assisted outputs and assumed full responsibility for the accuracy, integrity, and originality of the work.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

1. Available online: <https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed> (accessed on 15 January 2025).
2. Isiordia-Espinoza, M.A.; Terán-Rosales, F.; Serafín-Higuera, N.A.; Alonso-Castro, Á.J.; Aragon-Martínez, O.H. Isobolographic analysis of the ciprofloxacin-gentamicin combination against beta-lactamase-producing *Staphylococcus aureus*. *Fundam. Clin. Pharmacol.* **2023**, *37*, 1198–1204. [[CrossRef](#)] [[PubMed](#)]
3. Superbugs to Kill ‘More than Cancer’ by 2050—BBC News. Available online: <https://www.bbc.com/news/health-30416844> (accessed on 15 January 2025).
4. Levy, M.; Chowdhury, P.P.; Nagpal, P. Quantum dot therapeutics: A new class of radical therapies. *J. Biol. Eng.* **2019**, *13*, 48. [[CrossRef](#)] [[PubMed](#)]
5. Marton, J.; Cumming, P.; Rice, K.C.; Linders, J.T.M. Morphinan Alkaloids and Their Transformations: A Historical Perspective of a Century of Opioid Research in Hungary. *Int. J. Mol. Sci.* **2025**, *26*, 2736. [[CrossRef](#)] [[PubMed](#)]
6. Ashrafi, S.; Alam, S.; Sultana, A.; Raj, A.; Emon, N.U.; Richi, F.T.; Sharmin, T.; Moon, M.; Park, M.N.; Kim, B. Papaverine: A Miraculous Alkaloid from Opium and Its Multimedicinal Application. *Molecules* **2023**, *28*, 3149. [[CrossRef](#)] [[PubMed](#)]
7. Nemati, F.; Ata Bahmani Asl, A.; Salehi, P. Synthesis and modification of noscapine derivatives as promising future anticancer agents. *Bioorg. Chem.* **2024**, *153*, 107831. [[CrossRef](#)] [[PubMed](#)]
8. Gallego-Jara, J.; Lozano-Terol, G.; Sola-Martínez, R.A.; Cánovas-Díaz, M.; de Diego Puente, T. A Compressive Review about Taxol®: History and Future Challenges. *Molecules* **2020**, *25*, 5986. [[CrossRef](#)] [[PubMed](#)]
9. Subramani, R.; Narayanasamy, M.; Feussner, K.D. Plant-derived antimicrobials to fight against multi-drug-resistant human pathogens. *3 Biotech* **2017**, *7*, 172. [[CrossRef](#)] [[PubMed](#)]
10. Khatri, P.; Rani, A.; Hameed, S.; Chandra, S.; Chang, C.M.; Pandey, R.P. Current Understanding of the Molecular Basis of Spices for the Development of Potential Antimicrobial Medicine. *Antibiotics* **2023**, *12*, 270. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
11. Akullo, J.O.; Kiage, B.; Nakimbugwe, D.; Kinyuru, J. Effect of aqueous and organic solvent extraction on in-vitro antimicrobial activity of two varieties of fresh ginger (*Zingiber officinale*) and garlic (*Allium sativum*). *Heliyon* **2022**, *8*, e10457. [[CrossRef](#)] [[PubMed](#)]
12. Ranjbar, R.; Bagheri, H.; Ghasemi, F.; Guest, P.C.; Sahebkar, A. Effects of Curcumin and Its Analogues on Infectious Diseases. *Adv. Exp. Med. Biol.* **2021**, *1291*, 75–101. [[CrossRef](#)] [[PubMed](#)]
13. Liu, Q.; Meng, X.; Li, Y.; Zhao, C.N.; Tang, G.Y.; Li, H.B. Antibacterial and Antifungal Activities of Spices. *Int. J. Mol. Sci.* **2017**, *18*, 1283. [[CrossRef](#)] [[PubMed](#)]
14. Frankova, A.; Vistejnova, L.; Merinas-Amo, T.; Leheckova, Z.; Daskocil, I.; Wong Soon, J.; Kudera, T.; Laupua, F.; Alonso-Moraga, A.; Kokoska, L. In vitro antibacterial activity of extracts from Samoan medicinal plants and their effect on proliferation and migration of human fibroblasts. *J. Ethnopharmacol.* **2021**, *264*, 113220. [[CrossRef](#)] [[PubMed](#)]
15. Available online: <https://www.who.int/es/news-room/fact-sheets/detail/antimicrobial-resistance> (accessed on 15 January 2025).

16. Purgato, G.A.; Píccolo, M.S.; Moreira, M.A.S.; Pizziolo, V.R.; Diaz-Muñoz, G.; Rossi, C.C.; Diaz, M.A.N. Isolation and identification of antimicrobial multicyclic terpenoids from the medicinal plant *Salvia officinalis* and development of a formulation against clinical *Staphylococcus aureus* strains. *Lett. Appl. Microbiol.* **2024**, *77*, ovae077. [CrossRef] [PubMed]
17. Fahed, L.; Stien, D.; Ouaini, N.; Eparvier, V.; El Beyrouthy, M. Chemical Diversity and Antimicrobial Activity of *Salvia multicaulis* Vahl Essential Oils. *Chem. Biodivers.* **2016**, *13*, 591–595. [CrossRef] [PubMed]
18. Córdova-Guerrero, I.; Aragon-Martinez, O.H.; Díaz-Rubio, L.; Franco-Cabrera, S.; Serafín-Higuera, N.A.; Pozos-Guillén, A.; Soto-Castro, T.A.; Martinez-Morales, F.; Isiordia-Espinoza, M. Antibacterial and antifungal activity of *Salvia apiana* against clinically important microorganisms. *Rev. Argent. Microbiol.* **2016**, *48*, 217–221. [CrossRef] [PubMed]
19. d'Agostino, M.; Giori, A.M.; Vassallo, V.; Schiraldi, C.; D'Agostino, A. Protective and Anti-Inflammatory Effect of Novel Formulation Based on High and Low Molecular Weight Hyaluronic Acid and *Salvia haenkei*. *Int. J. Mol. Sci.* **2025**, *26*, 1310. [CrossRef] [PubMed]
20. Kostić, M.; Kitić, D.; Petrović, M.B.; Jevtović-Stoimenov, T.; Jović, M.; Petrović, A.; Živanović, S. Anti-inflammatory effect of the *Salvia sclarea* L. ethanolic extract on lipopolysaccharide-induced periodontitis in rats. *J. Ethnopharmacol.* **2017**, *199*, 52–59. [CrossRef] [PubMed]
21. Tubon, I.; Zannoni, A.; Bernardini, C.; Salaroli, R.; Bertocchi, M.; Mandrioli, R.; Vinuesa, D.; Antognoni, F.; Forni, M. In Vitro Anti-Inflammatory Effect of *Salvia sagittata* Ethanolic Extract on Primary Cultures of Porcine Aortic Endothelial Cells. *Oxidative Med. Cell. Longev.* **2019**, *2019*, 6829173. [CrossRef] [PubMed]
22. Zhao, Y.; Liu, H.; Lin, K.; Chen, C.; Chi, Q.; Zhang, M.; Li, J.; Pan, W.; Lou, H. Chemical components of *Salvia cavaleriei* and their anti-inflammatory effect via NF- $\kappa$ B signaling pathway. *Fitoterapia* **2025**, *182*, 106406. [CrossRef] [PubMed]
23. Ghorbani, A.; Esmaeilzadeh, M. Pharmacological properties of *Salvia officinalis* and its components. *J. Tradit. Complement. Med.* **2017**, *7*, 433–440. [CrossRef] [PubMed]
24. Ortiz-Mendoza, N.; Monribot-Villanueva, J.L.; Guerrero-Analco, J.A.; Martínez-Gordillo, M.J.; Basurto-Peña, F.A.; Aguirre-Hernandez, E.; Soto-Hernández, M. Comparative Metabolomic Analysis and Antinociceptive Effect of Methanolic Extracts from *Salvia cinnabarina*, *Salvia lavanduloides* and *Salvia longispicata*. *Molecules* **2024**, *29*, 5465. [CrossRef] [PubMed]
25. Quiñonez-Bastidas, G.N.; Tixta-Ramírez, E.D.; Balderas-López, J.L.; Vargas-Durán, P.A.; Pérez-Vásquez, A.; Navarrete, A. Antinociceptive effect of salvinorin A from the extract of *Salvia divinorum* in formalin-evoked trigeminal pain behavior in mice: Underlying mechanisms. *J. Ethnopharmacol.* **2025**, *353*, 120311. [CrossRef] [PubMed]
26. Seal, T. Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, *Sonchus arvensis* and *Oenanthe linearis* of North-Eastern region in India. *J. Appl. Pharm. Sci.* **2016**, *6*, 157–166. [CrossRef]
27. US Department of Health and Human Services; Food and Drug Administration; Center for Drug Evaluation and Research; Center for Veterinary Medicine. Guidance for Industry: Bioanalytical Method Validation. 2018. Available online: <https://www.fda.gov/media/70858/download> (accessed on 15 January 2025).
28. Aragon-Martinez, O.H.; González-Chávez, M.M.; Galicia-Cruz, O.G.; Isiordia-Espinoza, M.A.; Arias-Alvarez, B.; López-Rodríguez, J.F.; Martinez-Morales, F. Selection of a natural extract rich in antioxidants to reduce lipid and protein oxidation in rabbit meatballs during refrigerated storage. *J. Food Process. Preserv.* **2026**, *2026*, 6257671. [CrossRef]
29. Aragon-Martinez, O.H.; González-Chávez, M.M.; Galicia-Cruz, O.G.; Méndez-Gallegos, S.J.; Isiordia-Espinoza, M.A.; Martinez-Morales, F. Evaluation of *Dactylopius opuntiae* Extract for Xanthine Oxidase Inhibition and Serum Uric Acid Reduction in a Hyperuricemic Mouse Model. *Pharmaceuticals* **2024**, *17*, 1575. [CrossRef] [PubMed]
30. Mabry, T.J.; Markham, K.R.; Thomas, M.B. The ultraviolet spectra of flavones and flavonols. In *The Systematic Identification of Flavonoids*; Mabry, T.J., Markham, K.R., Thomas, M.B., Eds.; Springer: Berlin, Germany, 1970; pp. 41–164. [CrossRef]
31. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178. [CrossRef]
32. Zhishen, J.; Mengcheng, T.; Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* **1999**, *64*, 555–559. [CrossRef]
33. González-Rivera, M.L.; Martinez-Morales, F.; Alonso-Castro, A.J.; Lopez-Rodriguez, J.F.; Zapata-Morales, J.R.; Aranda Romo, S.; Aragon-Martinez, O.H. Validated and rapid measurement of the ferric reducing antioxidant power in plasma samples. *Chem. Pap.* **2018**, *72*, 2561–2574. [CrossRef]
34. NORMA Oficial Mexicana NOM-062-ZOO-1999, Especificaciones Técnicas para la Producción, Cuidado y uso de los Animales de Laboratorio. Official Journal of the Federation (Diario Oficial de la Federación), 1999. Available online: <https://www.fmvz.unam.mx/fmvz/principal/archivos/062ZOO.PDF> (accessed on 20 January 2025).
35. Barragan-Galvez, J.C.; Gonzalez-Rivera, M.L.; Jiménez-Cruz, J.C.; Hernandez-Flores, A.; de la Rosa, G.; Lopez-Moreno, M.L.; Yañez-Barrientos, E.; Romero-Hernández, M.; Deveze-Alvarez, M.A.; Navarro-Santos, P.; et al. A Patent-Pending Ointment Containing Extracts of Five Different Plants Showed Antinociceptive and Anti-Inflammatory Mechanisms in Preclinical Studies. *Pharmaceutics* **2024**, *16*, 1215. [CrossRef] [PubMed]

36. De Young, L.M.; Kheifets, J.B.; Ballaron, S.J.; Young, J.M. Edema and cell infiltration in the phorbol ester-treated mouse ear are temporally separate and can be differentially modulated by pharmacologic agents. *Agents Actions* **1989**, *26*, 335–341. [[CrossRef](#)] [[PubMed](#)]
37. Salinas-Sánchez, D.O.; Herrera-Ruiz, M.; Pérez, S.; Jiménez-Ferrer, E.; Zamilpa, A. Anti-inflammatory activity of hautriwaic acid isolated from *Dodonaea viscosa* leaves. *Molecules* **2012**, *17*, 4292–4299. [[CrossRef](#)] [[PubMed](#)]
38. Hunskar, S.; Hole, K. The formalin test in mice: Dissociation between inflammatory and non-inflammatory pain. *Pain* **1987**, *30*, 103–114. [[CrossRef](#)] [[PubMed](#)]
39. Zapata-Morales, J.R.; Alonso-Castro, Á.J.; Granados-Soto, V.; Sánchez-Enriquez, S.; Isiordia-Espinoza, M.A. Assessment of the antinociceptive and ulcerogenic activity of the tapentadol-diclofenac combination in rodents. *Drug Dev. Res.* **2018**, *79*, 38–44. [[CrossRef](#)] [[PubMed](#)]
40. Franco de la-Torre, L.; Alonso-Castro, Á.J.; Zapata-Morales, J.R.; Rivas-Carrillo, J.D.; Vidaurrazaga-Lugo, J.; Partida-Castellanos, E.M.; Granados-Soto, V.; Isiordia-Espinoza, M.A. Antinociception and less gastric injury with the dexketoprofen-tapentadol combination in mice. *Fundam. Clin. Pharmacol.* **2021**, *35*, 371–378. [[CrossRef](#)] [[PubMed](#)]
41. Faul, F.; Erdfelder, E.; Lang, A.G.; Buchner, A. G\*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* **2007**, *39*, 175–191. [[CrossRef](#)] [[PubMed](#)]
42. Charan, J.; Kantharia, N.D. How to calculate sample size in animal studies? *J. Pharmacol. Pharmacother.* **2013**, *4*, 303–306. [[CrossRef](#)] [[PubMed](#)]
43. Hitl, M.; Kladar, N.; Gavarić, N.; Božin, B. Rosmarinic Acid-Human Pharmacokinetics and Health Benefits. *Planta Med.* **2021**, *87*, 273–282. [[CrossRef](#)] [[PubMed](#)]
44. Taheri, Y.; Suleria, H.A.R.; Martins, N.; Sytar, O.; Beyatli, A.; Yeskaliyeva, B.; Seitimova, G.; Salehi, B.; Semwal, P.; Painuli, S.; et al. Myricetin bioactive effects: Moving from preclinical evidence to potential clinical applications. *BMC Complement. Med. Ther.* **2020**, *20*, 241. [[CrossRef](#)] [[PubMed](#)]
45. Chen, C. Sinapic Acid and Its Derivatives as Medicine in Oxidative Stress-Induced Diseases and Aging. *Oxidative Med. Cell. Longev.* **2016**, *2016*, 3571614. [[CrossRef](#)] [[PubMed](#)]
46. Chen, F.; Zhang, X.; Wang, J.; Wang, F.; Mao, J. P-coumaric Acid: Advances in Pharmacological Research Based on Oxidative Stress. *Curr. Top. Med. Chem.* **2024**, *24*, 416–436. [[CrossRef](#)] [[PubMed](#)]
47. Yong, D.O.C.; Saker, S.R.; Chellappan, D.K.; Madheswaran, T.; Panneerselvam, J.; Choudhury, H.; Pandey, M.; Chan, Y.L.; Collet, T.; Gupta, G.; et al. Molecular and Immunological Mechanisms Underlying the Various Pharmacological Properties of the Potent Bioflavonoid, Rutin. *Endocr. Metab. Immune Disord. Drug Targets* **2020**, *20*, 1590–1596. [[CrossRef](#)] [[PubMed](#)]
48. Forouzanfar, F.; Sahranavard, T.; Tsatsakis, A.; Iranshahi, M.; Rezaee, R. Rutin: A pain-relieving flavonoid. *Inflammopharmacology* **2025**, *33*, 1289–1301. [[CrossRef](#)] [[PubMed](#)]
49. Abedi, F.; Razavi, B.M.; Hosseinzadeh, H. A review on gentisic acid as a plant derived phenolic acid and metabolite of aspirin: Comprehensive pharmacology, toxicology, and some pharmaceutical aspects. *Phytother. Res.* **2020**, *34*, 729–741. [[CrossRef](#)] [[PubMed](#)]
50. Ahmed, H.A.; Abdel-Fattah, A.; Salama, Z.A.; Abd El-Wahab, A.E. Evaluation of antimicrobial potential of free gallic acid and its polyvinyl-based nano-formulation. *Sci. Rep.* **2025**, *15*, 34281. [[CrossRef](#)] [[PubMed](#)]
51. Kahkeshani, N.; Farzaei, F.; Fotouhi, M.; Alavi, S.S.; Bahramsoltani, R.; Naseri, R.; Momtaz, S.; Abbasabadi, Z.; Rahimi, R.; Farzaei, M.H.; et al. Pharmacological effects of gallic acid in health and diseases: A mechanistic review. *Iran. J. Basic Med. Sci.* **2019**, *22*, 225–237. [[CrossRef](#)] [[PubMed](#)]
52. Lim, E.Y.; Lee, C.; Kim, Y.T. The Antinociceptive Potential of *Camellia japonica* Leaf Extract, (-)-Epicatechin, and Rutin against Chronic Constriction Injury-Induced Neuropathic Pain in Rats. *Antioxidants* **2022**, *11*, 410. [[CrossRef](#)] [[PubMed](#)]
53. Pei, K.; Ou, J.; Huang, J.; Ou, S. p-Coumaric acid and its conjugates: Dietary sources, pharmacokinetic properties and biological activities. *J. Sci. Food Agric.* **2016**, *96*, 2952–2962. [[CrossRef](#)] [[PubMed](#)]
54. Roychoudhury, S.; Sinha, B.; Choudhury, B.P.; Jha, N.K.; Palit, P.; Kundu, S.; Mandal, S.C.; Kolesarova, A.; Yousef, M.I.; Ruokolainen, J.; et al. Scavenging Properties of Plant-Derived Natural Biomolecule Para-Coumaric Acid in the Prevention of Oxidative Stress-Induced Diseases. *Antioxidants* **2021**, *10*, 1205. [[CrossRef](#)] [[PubMed](#)]
55. Tardugno, R.; Pellati, F.; Iseppi, R.; Bondi, M.; Bruzzesi, G.; Benvenuti, S. Phytochemical composition and in vitro screening of the antimicrobial activity of essential oils on oral pathogenic bacteria. *Nat. Prod. Res.* **2018**, *32*, 544–551. [[CrossRef](#)] [[PubMed](#)]
56. Gál, R.; Čmiková, N.; Prokopová, A.; Kačániová, M. Antilisterial and Antimicrobial Effect of *Salvia officinalis* Essential Oil in Beef Sous-Vide Meat during Storage. *Foods* **2023**, *12*, 2201. [[CrossRef](#)] [[PubMed](#)]
57. Mendes, F.S.F.; Garcia, L.M.; Moraes, T.D.S.; Casemiro, L.A.; Alcântara, C.B.; Ambrósio, S.R.; Veneziani, R.C.S.; Miranda, M.L.D.; Martins, C.H.G. Antibacterial activity of *Salvia officinalis* L. against periodontopathogens: An in vitro study. *Anaerobe* **2020**, *63*, 102194. [[CrossRef](#)] [[PubMed](#)]

58. González-Padilla, J.; Aceves-Franco, J.G.; Díaz-Rubio, L.; Hernández-Gómez, A.; Isiordia-Espinoza, M.A. Efecto antimicrobiano de un extracto de la raíz de *Salvia munzii* contra microorganismos de importancia odontológica (Antimicrobial effect of an extract from the root of *Salvia munzii* against microorganisms of dental importance). *Contexto Odontológico (Enero-Junio)* **2023**, *13*, 16–22.
59. Köse, Y.B.; İşcan, G.; Göger, F.; Demirci, B. Phytochemical Composition and Evaluation of Antimicrobial Activities of Five *Salvia* Species. *Processes* **2025**, *13*, 2011. [[CrossRef](#)] [[PubMed](#)]
60. Cardile, V.; Russo, A.; Formisano, C.; Rigano, D.; Senatore, F.; Arnold, N.A.; Piozzi, F. Essential oils of *Salvia bracteata* and *Salvia rubifolia* from Lebanon: Chemical composition, antimicrobial activity and inhibitory effect on human melanoma cells. *J. Ethnopharmacol.* **2009**, *126*, 265–272. [[CrossRef](#)] [[PubMed](#)]
61. Geremew, A.; Gonzalles, J., 3rd; Peace, E.; Woldesenbet, S.; Reeves, S.; Brooks, N., Jr.; Carson, L. Green Synthesis of Novel Silver Nanoparticles Using *Salvia blepharophylla* and *Salvia greggii*: Antioxidant and Antidiabetic Potential and Effect on Foodborne Bacterial Pathogens. *Int. J. Mol. Sci.* **2024**, *25*, 904. [[CrossRef](#)] [[PubMed](#)]
62. Nasr, A.; Yosuf, I.; Turki, Z.; Abozeid, A. LC-MS metabolomics profiling of *Salvia aegyptiaca* L. and *S. lanigera* Poir. with the antimicrobial properties of their extracts. *BMC Plant Biol.* **2023**, *23*, 340. [[CrossRef](#)] [[PubMed](#)]
63. Donadio, G.; Mensitieri, F.; Santoro, V.; Parisi, V.; Bellone, M.L.; De Tommasi, N.; Izzo, V.; Dal Piaz, F. Interactions with Microbial Proteins Driving the Antibacterial Activity of Flavonoids. *Pharmaceutics* **2021**, *13*, 660. [[CrossRef](#)] [[PubMed](#)]
64. Mahmud, A.R.; Ema, T.I.; Siddiquee, M.F.; Shahriar, A.; Ahmed, H.; Mosfeq-Ul-Hasan, M.; Rahman, N.; Islam, R.; Uddin, M.R.; Mizan, M.F.R. Natural flavonols: Actions, mechanisms, and potential therapeutic utility for various diseases. *Beni Suef Univ. J. Basic. Appl. Sci.* **2023**, *12*, 47. [[CrossRef](#)] [[PubMed](#)]
65. Cushnie, T.P.; Lamb, A.J. Recent advances in understanding the antibacterial properties of flavonoids. *Int. J. Antimicrob. Agents* **2011**, *38*, 99–107. [[CrossRef](#)] [[PubMed](#)]
66. Xie, Y.; Yang, W.; Tang, F.; Chen, X.; Ren, L. Antibacterial activities of flavonoids: Structure-activity relationship and mechanism. *Curr. Med. Chem.* **2015**, *22*, 132–149. [[CrossRef](#)] [[PubMed](#)]
67. Majumder, D.; Das, A.; Saha, C. Catalase inhibition an anti-cancer property of flavonoids: A kinetic and structural evaluation. *Int. J. Biol. Macromol.* **2017**, *104*, 929–935. [[CrossRef](#)] [[PubMed](#)]
68. Krych, J.; Gebicki, J.L.; Gebicka, L. Flavonoid-induced conversion of catalase to its inactive form—Compound II. *Free Radic. Res.* **2014**, *48*, 1334–1341. [[CrossRef](#)] [[PubMed](#)]
69. Jomova, K.; Raptova, R.; Alomar, S.Y.; Alwasel, S.H.; Nepovimova, E.; Kuca, K.; Valko, M. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. *Arch. Toxicol.* **2023**, *97*, 2499–2574. [[CrossRef](#)] [[PubMed](#)]
70. Al-Mijalli, S.H.; Assaggaf, H.; Qasem, A.; El-Shemi, A.G.; Abdallah, E.M.; Mrabti, H.N.; Bouyahya, A. Antioxidant, Antidiabetic, and Antibacterial Potentials and Chemical Composition of *Salvia officinalis* and *Mentha suaveolens* Grown Wild in Morocco. *Adv. Pharmacol. Pharm. Sci.* **2022**, *2022*, 2844880. [[CrossRef](#)] [[PubMed](#)]
71. Henríquez, J.C.; Duarte, L.V.; Sierra, L.J.; Fernández-Alonso, J.L.; Martínez, J.R.; Stashenko, E.E. Chemical Composition and In Vitro Antioxidant Activity of *Salvia aratocensis* (Lamiaceae) Essential Oils and Extracts. *Molecules* **2023**, *28*, 4062. [[CrossRef](#)] [[PubMed](#)]
72. Zhang, K.; Wang, Z.; Zhang, L.; Wu, H.; Liu, J.; Zhang, M.; Deng, Z.; Liu, R. Pharmacologically inherited carbon dots from *Salvia miltiorrhiza* with potent antioxidant activity and multi-pathway modulation for myocardial ischemia-reperfusion injury therapy. *Theranostics* **2026**, *16*, 1855–1876. [[CrossRef](#)] [[PubMed](#)]
73. Moshari-Nasirkandi, R.; Alirezalu, A.; Chamanabad, H.R.M.; Amato, J.; Alipour, H.; Asghari, A.; Moshari-Nasirkandi, A. Screening of native wild *Salvia nemorosa* populations for chemical compositions, antioxidant activity and UHPLC-HRMS profiling. *Sci. Rep.* **2024**, *14*, 32064. [[CrossRef](#)] [[PubMed](#)]
74. Motyka, S.; Kusznerewicz, B.; Ekiert, H.; Korona-Głowniak, I.; Szopa, A. Comparative Analysis of Metabolic Variations, Antioxidant Profiles and Antimicrobial Activity of *Salvia hispanica* (Chia) Seed, Sprout, Leaf, Flower, Root and Herb Extracts. *Molecules* **2023**, *28*, 2728. [[CrossRef](#)] [[PubMed](#)]
75. Moshari-Nasirkandi, A.; Iaccarino, N.; Romano, F.; Graziani, G.; Alirezalu, A.; Alipour, H.; Amato, J. Chemometrics-based analysis of the phytochemical profile and antioxidant activity of *Salvia* species from Iran. *Sci. Rep.* **2024**, *14*, 17317. [[CrossRef](#)] [[PubMed](#)]
76. Boonyarikpunchai, W.; Sukrong, S.; Towiwat, P. Antinociceptive and anti-inflammatory effects of rosmarinic acid isolated from *Thunbergia laurifolia* Lindl. *Pharmacol. Biochem. Behav.* **2014**, *124*, 67–73. [[CrossRef](#)] [[PubMed](#)]
77. Hernandez-Leon, A.; Fernández-Guasti, A.; González-Trujano, M.E. Rutin antinociception involves opiodergic mechanism and descending modulation of ventrolateral periaqueductal grey matter in rats. *Eur. J. Pain.* **2016**, *20*, 274–283. [[CrossRef](#)] [[PubMed](#)]
78. Rodrigues, M.R.; Kanazawa, L.K.; das Neves, T.L.; da Silva, C.F.; Horst, H.; Pizzolatti, M.G.; Santos, A.R.; Baggio, C.H.; Werner, M.F. Antinociceptive and anti-inflammatory potential of extract and isolated compounds from the leaves of *Salvia officinalis* in mice. *J. Ethnopharmacol.* **2012**, *139*, 519–526. [[CrossRef](#)] [[PubMed](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.