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SPASMOLYTIC, ANTI-INFLAMMATORY, AND ANTIOXIDANT ACTIVITIES OF SALVIA GESNERIFLORA LINDLEY

Abraham Gómez-Rivera1,3, Manasés González-Cortazar2*, Ammy Joana Gallegos-García1, Armando Escobar-Ramos1, Gabriel Flores-Franco4 and Carlos Ernesto Lobato-García1*

1Universidad Juárez Autónoma de Tabasco, Carretera Cunduacán-Jalpa Km. 0.5, 86690 Cunduacán, Tabasco, México. 2Centro de Investigación Biomédica del Sur, Instituto Mexicano del Seguro Social, Argentina No.1, Col. Centro, 62790 Xochitepec, Morelos, México. 3Laboratorio de Química Productos Naturales y Farmacognosia, Facultad de Farmacia, Universidad Autónoma del Estado de Morelos, Av. Universidad No. 1001, Col. Chamilpa, 62209 Cuernavaca, Morelos, México. 4Centro de Investigación en Biodiversidad y Conservación (CIByC), Universidad Autónoma del Estado de Morelos, Av. Universidad No. 1001, Col. Chamilpa, 62209 Cuernavaca, Morelos, México

*Corresponding author’s E-mail: carloslobatogarcia@gmail.com; gmanases2000@gmail.com

Abstract

Background: Salvia gesneriflora Lindley is employed in traditional Mexican medicine for the treatment of several diseases. This work reports the spasmolytic, anti-inflammatory and antioxidant activities of salvia gesneriflora

Materials and Methods: The chromatographic profile of three extracts (SgH, SgD and SgM) of this plant allowed the identification of 11 components in SgH, the presence of rosmarinic (1), chlorogenic (2) and caffeic (3) acids and quercetin glucoside (4) in SgM and of ursolic acid (5) in SgD. The spasmolytic (electrically-induced contractions of guinea-pig ileum), anti-inflammatory (edema in mouse ear) and antioxidant potential (DPPH, ABTS and FRAP assays) of the extracts were evaluated.

Results: SgM showed the highest percentage of relaxation (80.67 ± 1.633%) with no significant difference (p<0.05) when compared to the reference drug employed (Papaverine, 76.16 ± 2.44%), the EC50 was 66.89 ± 1.6 μg/mL, respectively for SgH and 26.88 ± 1.9 μg/mL for Papaverine. Anti-inflammatory activity was 71.12 ± 4.9% for SgH, an effect which is similar to that of Indomethacin (reference drug) at the same dose (75.24 ± 2.4%). In the DPPH test, SsM reached the least IC50 (1.16±1.08 μg/mL). For ABTS, SgH reached the least IC50 (1.73 ± 0.5 µg/mL) and for the FRAP assay, SgD showed the highest reductive capacity (1,782.08 ± 2.1 equivalent mM of FeSO4).

Conclusion: S. gesneriflora extracts exhibited spasmolytic, anti-inflammatory and antioxidant activities; thus serving as co-adjuvants with regard to knowledge in the traditional medicine of this plant species and its application’s potential in other fields of pharmacy and foods.

Key words: Salvia gesneriflora Lindley, spasmolytic, spasmodic, anti-inflammatory, antioxidant activity.

Abbreviations: SgH, hexanic extract; SgD, dichloromethanic extract; SgM, methanolic extract; TLC, Thin-layer chromatography; NMR, Nuclear Magnetic Resonance; HPLC, High Performance Liquid Chromatography; GC-MS, Gas Chromatography-Mass Spectrometry; TPA, 12-O-tetradecanoylphorbol-13-acetate; DPPH, 2, 2-Diphenyl-1-picrylHydrazyl; ABTS, 2, 2’-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); FRAP, Ferric Reducing Antioxidant Power; EC50, Effective Concentration fifty; IC50, Inhibitory Concentration 50%.
Introduction

One promising alternative in the search for new pharmaceuticals is made up of medicinal plants. In this respect, the genus Salvia, comprising more than 900 species, belongs to the Lamiaceae family, which is composed of 220 genera and approximately 4,000 species (Frodin, 2004; Wu et al., 2012). In Mexico, the genus Salvia is found in mountainous zones, mainly in the Central-South zone of Mexico, and some of these plants are employed in Mexican traditional medicine. In this regard, it has been reported that they possess anti-inflammatory, antimicrobial, antihypertensive activities and against central nervous system (CNS) disorders, among others (Aguilar et al., 1994; Herrera-Ruiz et al., 2006; Esquivel-Gutiérrez et al., 2012; Bisio et al., 2015; Gómez-Rivera et al., 2018).

In these zones, there are endemic species of this genus that are localized in coniferous forest, and among these is found the species Salvia gesneriflora Lindley, commonly known as myrtle. Its manner of employment is by cooking its aerial parts, and it is drunk for the treatment of stomach-ache and diarrhea (Monroy-Ortiz and Castillo-España, 2007). The only study found, to the best of our knowledge, for this species is one report about the antiamebic and antiigiardial activity of clerodane diterpenes from Mexican Salvia used for the treatment of diarrhea.

These two diterpenes, identified as salvifulgenolide and isosalvixalapadiene were isolated from Salvia gesneriflora and presented low antiparasitic activity when compared against metronidazole (Calzada et al., 2015). Thus, to the best of our knowledge, their employment has not been validated within traditional medicine for the treatment of stomachache. The purpose of this work was to evaluate the spasmyotic, anti-inflammatory, and antioxidant potential of the three extracts of increasing polarity of the species Salvia gesneriflora Lindley.

Materials and Methods

Plant material

Collection of the aerial parts (5 kg) of the plant was carried out in the town of Tres Marías, Hutizilac Municipality, state of Morelos, Mexico (19° 02’ 28.27” N; 99° 13’ 69.97” W, 2,735 msnm) in the month of November, 2015. A specimen was deposited at the HUMO-CIByC Herbarium of the Autonomous University of the State of Morelos (UAEM) for safe-keeping and taxonomic identification (Voucher no. 33908) headed by Gabriel Flores-Franco.

Preparation of plant extracts

The plant material was oven-dried at 40°C for 3 days and pulverized in a Pulvex MPP300 mill. One kg of the plant material was macerated with hexane (10 L, Merck) in triplicate, this was filtered and vacuum-concentrated employing a rotary evaporator (Heidolph G3, German) at 40°C followed by lyophilization (Heto Dpywinner DW3) until obtaining a powder denominated hexanic extract (SgH). The dried plant residue was macerated with dichloromethane (10 L, Merck) and later with methanol (10L, Merck) following the same procedure as previously described, until obtaining the dichloromethane (SgD) and methanol (SgM) extracts, respectively. A portion of each extract was used for conducting the biological-activity assays that are described later.

From the maceration of 1 kg of the dried and milled plant material, the following extracts in amounts and yields were obtained: SgH (8.3 g, 0.83%); SgD (12.3 g, 1.23%), and SgM (54.7 g, 5.47%).

HPLC analysis

Chromatographic analysis was performed in a Waters 2695 Separation Module System equipped with a Waters 996 Photodiode Array Detector and Empower Pro software (Waters Corporation, USA). Chemical separation was achieved using a Supelcosil LC-F column (4.6 mm x 250 mm i.d., 5-µm particle size) (Sigma-Aldrich, Bellefonte, PA, USA). Mobile phase consisted of a 0.5% trifluoroacetic acid aqueous solution (solvent A) and acetonitrile (solvent B). The gradient system was as follows: 0-1 min; 0% B; 2-3 min; 5% B; 4-20 min, 30% B; 21-23 min; 50% B; 24-25 min; 80% B; 26-27 100% B, and 28-30 min, 0% B. The flow rate was maintained at 0.9 mL/min and sample injection volume was 10 µL.

Chromatographic separation of the SgM and SgD extracts and identification of rosmarinic acid (1), caffeic acid (2), chlorogenic acid (3), quercetin glucoside (4) and ursolic acid (5).

The SgM extract (50 g) was adsorbed on silica gel and applied to a silica gel column for gravity (150 g, 70-230 mesh, Merck, Darmstadt, Germany). A gradient of dichloromethane/methanol was utilized to elute the column, collecting 24 fractions that were grouped according to the TLC similarity of the compounds in nine (SgAg1-SgAg9) fractions of 500 mL each. The fractions were concentrated in a rotary evaporator under reduced pressure. Fractions SgAg5 and SgAg6 (3.6 g) were mixed and subjected to
chromatographic fractionation in a silica gel column and applied to a silica gel column (100 g, 70-230 mesh, Merck). A gradient of n-dichloromethane/methanol was used to elute the column with an increase in polarity of 5%, collecting 51 fractions of 50 mL each. The fractions were grouped according to their similarity in terms of TLC in nine fractions (SgR1-SgR9). Fractions SgR4 and SgR5, on TLC, exhibited a single spot, which corresponded to quercetin glucoside (4, 35 mg) and this was compared with a sample standard (Sigma, ≥90%) and Nuclear Magnetic Resonance (NMR) of 1H and 13C, while in fractions SgR8 and SgR9, it was identified the presence of rosmarinic (1), caffeic (2) and chlorogenic (3) acids by High-Performance Liquid Chromatography (HPLC) compared to sample standards (1, Sigma, ≥98%; 2, Sigma, ≥98%; 3, Sigma, 95%). The Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker Advance III HD-600 at 600 MHz for 1H and 13C at 150 MHz NMR in CD3OD. Chemical shifts reported ppm, while tetramethylsilane was utilized as internal reference.

The SgD extract was that with the lowest biological activity, thus, it was not fractionated and only one compound was identified by TLC and HPLC, which corresponded to ursolic acid (5), compared to a standard sample (Sigma, ≥90%), and the presence of other compounds more polar than 5 was observed, which were not identified.

**GC-MS analysis of hexane extract (SgH)**

The chemical composition of the SgH was analyzed on a Gas Chromatograph (GC) equipped with a quadruple mass detector in electron impact mode at 70 eV. Volatile compounds were separated on a HP 5MS capillary column (25 m long, 0.2 mm i.d., with 0.3-µm film thickness). Oven temperature was set at 40°C for 2 min, then programmed at 40–260°C for 10°C/min and maintained for 20 min at 260°C. Mass detector conditions were as follows: interphase temperature 200°C and mass acquisition range, 20-550.

Injector and detector temperatures were set at 250 and 280°C, respectively. Splitless injection mode was carried out with 1 µL of each fraction (3 mg/mL solution). The carrier gas was helium at a flow rate of 1 mL/min. Identification of volatiles was performed, comparing their mass spectra with those of the National Institute of Standards and Technology (NIST) 1.7 Library and comparing these with data in the literature (Adams, 2007).

**Spasmolytic and anti-inflammatory activities**

Experiments were performed according to the Official Mexican Regulation NOM-062-ZOO-1999 Guidelines (Technical Specifications for the Production, Care and Use of Laboratory Animals) and the international ethical guidelines for the care and use of experimental animals (Zimmermann, 1983).

**Model of guinea-pig isolated ileum**

To evaluate the spasmolytic activity, the experimental model of electrically-induced guinea-pig isolated ileum was performed. Guinea-pigs of either sex (250-500 g) were used. Animals were maintained at a temperature of 22°C ± 3°C, with 70% ± 5% humidity, with 12-h light/dark cycles, and with food/water *ad libitum* and subjected to cervical dislocation. Their abdomens were opened. Their ileum was removed and maintained in Petri dishes containing Tyrode solution, constantly aerated with carbogen gas (95%, O₂ +5% CO₂ gas mixture). Portions of about 1.5 cm length of the isolated tissue were mounted in a set of 3 mL chambers. One end of the tissue was attached to the bottom of the chamber to an electrode while the other end was attached with a silk thread to a force-displacement transducer, which was connected to an acquisition system (PanLab, BIOPAC Systems, USA). After 30 min adaptation period, the tissue was electrically stimulated (25 V, 5mS, 1 Hz, 5 S, every 2 min; with a Grass stimulator) by isolated tungsten electrodes connected to the end of the tissue. Induced contractions were recorded and after homogeneous responses were obtained, different concentrations of the drugs under study were added to the chamber and the ability to inhibit the electrically-induced contractions was evaluated (Cheng et al., 2013; Escobar-Ramos et al, 2017).

The Tyrode solution was prepared with the following in mM: NaCl (137); C₃H₅O₇ (5); NaHCO₃ (11.9); CaCl₂;2H₂O (2.7); KCl (5.4); MgCl₂ 6H₂O (0.5); NaH₂PO₄ H₂O (0.45) and was diluted to 2 L volume with distilled water. The pH was adjusted to 7.4. The extracts were diluted in Tyrode solution to obtain final concentrations in the bath of 50 to 200 µg/mL, which were compared with Papaverine as positive control. For SgH, a dose-response curve (12.5-200 µg/mL) was obtained to determine the EC₅₀.

**Anti-inflammatory activity**

Male ICR mice weighing 25-30 g each, were utilized (*n* = 5 for each treatment). Mice were maintained at a temperature of 22°C ± 3°C, with 70% ± 5% humidity, with 12-h light/dark cycles, with food/water *ad libitum*. 
Animal inflammation was induced following the method previously described by Payá et al. 1993. The dose evaluated for the extracts was 1.0 mg/ear. A control group received acetone as vehicle and Indomethacin Indo (Sigma) 1.0 mg/ear was utilized as anti-inflammatory positive control.

All extracts/drugs were dissolved in acetone and applied topically to both ears immediately after the administration of TPA. Six hours after administration of the inflammatory agent, the animals were sacrificed by cervical dislocation. Circular sections (6 mm in diameter) were taken from both the treated (t) and non-treated (nt) ears, and weighed to determine the magnitude of inflammation. Percentage of inhibition was obtained by using the following expression:

\[
\text{Inhibition} \% = \left[ \frac{(\text{DW control} - \text{DW treatment})}{\text{Dw control}} \right] \times 100
\]

Where: Dw = wt - wnt

wt is the weight of the section of the treated ear
wnt is the weight of the section of the non-treated ear.

Evaluation of antioxidant capacity
Antioxidant capacity for the 2, 2-Diphenyl-1-pycrylHydrazyl (DPPH) assay

For the DPPH assay, the method described by MacDonald-Wicks et al. (2006), was used with minor modifications. The activities of the extracts and reference compounds measured by means of the targeting of a methanolic solution of 2,2-Diphenyl-1-pycryl-hydrazyl, purple-colored DPPH (Sigma). 25 µL of the different concentrations of the organic extracts (156.25, 312.5, 625, 1,250, 2,500, and 5,000 µg/mL) were evaluated against 175 µL of a solution of DPPH (0.025 mg/mL). The target contained all of the reagents, with the exception of the positive control extracts, which included the following: the standardized extract of *Camellia sinensis*, which contains epigallocatechin-3-galate 94% (Teavigo®, 2014). Absorbencies were measured in an Ultraviolet (UV)-Vis spectrophotometer at 515 nm, in an Enzyme-Linked Immuno-Sorbent Assay (ELISA) plate reader (Perkin-Elmer Lambda 40 UV-Vis). The determinations were carried out in triplicate. Percentage of inhibition was calculated according to the following expression:

\[
\text{Inhibition} \% = \left[ \frac{A_0 - A_1}{A_0} \right] \times 100
\]

Where: A₀ is the target absorbency and A₁ is the absorbency of the extracts-to-evaluate.

The elaborated linear equation for each of the extracts was determined by linear regression analysis, and the inhibitory concentration 50% (IC₅₀), defined as the concentration necessary to inhibit the formation of 50% of the DPPH radicals.

Antioxidant capacity for 2, 2’-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

The antioxidant capacity of the ABTS was estimated according to the method described by MacDonald-Wicks et al. (2006). In general, the ABTS complex was produced in situ by the reaction of a solution of the 2, 2’-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (Sigma) with potassium persulfate (Sigma). 230 µL were taken and mixed this with 20 µL of the extracts established for the DPPH assay. The target contained all the reagents except the extracts and was also evaluated. The positive controls of the standardized extract of *Camellia sinensis*. Absorbencies were read at 734 nm, while percentage of inhibition and inhibitory concentration 50% (IC₅₀) were calculated according to the method described previously for the DPPH assay. The determinations were carried out in triplicate.

Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was followed according to the method reported by Firuzi et al. (2005), with minor modifications. For the latter, the FRAP reagent was prepared by mixing the following: in a tampon of acetates 300 mM (3.1 g de CH₂COONa in 16 mL of glacial acetic acid); 10-mM of 2, 4, 6-Tris-(2-pyridyl)-s-triazine (TPTZ) (Sigma) in 40 mM of hydrochloric acid, and a 20-mM solution of FeCl₂•6H₂O, the latter at a 10:1:1 proportion (v/v). The extracts analyzed were at the concentrations previously indicated in the DPPH assay. 175 µL portions were taken from recent preparations of CARF reagent, incubated at 37°C, and together with a target of reagents, this was read at 595 nm. Later, 25 µL of the extracts at different concentrations and 50 µL of methanol were added. A calibration curve was prepared with a standard solution of FeSO₄. Results were expressed as equivalent (mM) of FeSO₄ per gram of extract. All experiments were carried out in triplicate.

Statistical analysis

Results are expressed as means ± standard error mean (SEM) of the means. Statistical analyses were performed utilizing the SPSS™ ver. 23.0 statistical package and statistical differences were determined by means of analysis of variance (ANOVA) and the Tukey test. The EC₅₀ was determined by
linear regression employing GraphPad Prism™ ver. 6.0 statistical software. Differences were considered significant at $p < 0.05$.

**Results**

**Chromatographic profile by HPLC of the SgM and SgD extracts**

Analysis by high-performance liquid chromatography (HPLC) of the SgM extract (Figure 1) permitted us to identify a major compound, which displayed a retention time of 11.13 min ($\lambda_{\text{nm}} = 221.6, 330.3$), which corresponds to rosmarinic acid (1). The compounds with retention times at 8.83 min were identified as chlorogenic acid (2, $\lambda_{\text{nm}} = 242.7, 327.9$ nm), at 9.24 min as caffeic acid (3, $\lambda_{\text{nm}} = 242.7, 325.5$ nm), and at 9.54 min as quercetin glucoside (4, $\lambda_{\text{nm}} = 211, 255.7, 355.3$ nm).

Identification of compounds 1 to 3 was performed by means of comparison with commercial standards and compound 4 was characterized by Nuclear Magnetic Resonance (NMR) of $^1$H and $^{13}$C and by comparison of the data of the chemical shifts with data described in the literature.

For the SgD extract (Figure 2), ursolic acid (5) was identified with a 27.92-min retention time and three compounds of higher polarity than were not identified, with retention times of 25.52, 25.88 and 26.35 min were observed.

![Figure 1: HPLC chromatogram of SgH of Salvia gesneriflora.](image)

![Figure 2: HPLC chromatogram of SgD of Salvia gesneriflora.](image)

**Profile by GC-MS analysis of the SgH**

Analysis of SgH by GC–MS identified 11 components, in the Table 1. These components are listed in order of elution, highlighting the presence of esterified aliphatic and aliphatic compounds such as hentriacontane (29.9%) and hecadaecanoic acid, methyl ester (2.64%); sesquiterpenes such as spathulenol (3.35%) and triterpenes such as $\alpha$, $\beta$-sitosterol (21.51 and 2.87%), $\beta$-amyrin (2.87%) and lupeol (6.46%).

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Molecular weight (a.m.u.)</th>
<th>Compound</th>
<th>% In the sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.34</td>
<td>220</td>
<td>Spathulenol</td>
<td>3.35</td>
</tr>
<tr>
<td>15.42</td>
<td>220</td>
<td>Caryophylene oxide</td>
<td>6.22</td>
</tr>
<tr>
<td>18.93</td>
<td>270</td>
<td>Hexadecanoic acid, methyl ester</td>
<td>2.64</td>
</tr>
<tr>
<td>19.59</td>
<td>284</td>
<td>Hecadecanoic acid, ethyl ester</td>
<td>0.45</td>
</tr>
<tr>
<td>30.44</td>
<td>408</td>
<td>Nonacosane</td>
<td>9.33</td>
</tr>
<tr>
<td>32.97</td>
<td>436</td>
<td>Hentriacontane</td>
<td>29.9</td>
</tr>
<tr>
<td>36.44</td>
<td>464</td>
<td>Trihariacontane</td>
<td>17.26</td>
</tr>
<tr>
<td>36.95</td>
<td>414</td>
<td>$\alpha$-Sitosterol</td>
<td>21.51</td>
</tr>
<tr>
<td>36.95</td>
<td>414</td>
<td>$\beta$-Sitosterol</td>
<td>2.87</td>
</tr>
<tr>
<td>37.62</td>
<td>426</td>
<td>$\beta$-Amyrin</td>
<td>2.87</td>
</tr>
<tr>
<td>38.64</td>
<td>426</td>
<td>Lupeol</td>
<td>6.46</td>
</tr>
</tbody>
</table>

Characterization by NMR of the Isolated Compound (4)
Chromatographic fractionation of SgF4 allowed us to obtain compound (4) as a yellow powder that was compared with a standard sample (quercetin glucoside). In the UV light spectrum, the compound showed $\lambda_{\text{max}}$ at 211, 255.7, and 355.3 nm, which are distinctive of the flavonol structure.

According to these spectroscopic data analyses (Table 1) and by comparison with the data described in the literature, this compound (Figure 3) was identified as quercetin glucoside (Quercetin-3-O-glucoside) (Abdelhady et al., 2015).

![Figure 3: Structure of the quercetin glucoside isolated from Salvia gesneriflora (4).](image)

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta^{13}$C-NMR</th>
<th>$\delta^1$H-NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>2</td>
<td>158.58</td>
<td>----</td>
</tr>
<tr>
<td>3</td>
<td>135.92</td>
<td>----</td>
</tr>
<tr>
<td>4</td>
<td>179.68</td>
<td>----</td>
</tr>
<tr>
<td>5</td>
<td>162.25</td>
<td>----</td>
</tr>
<tr>
<td>6</td>
<td>98.64</td>
<td>6.20 (1H, dd, 2.1)</td>
</tr>
<tr>
<td>7</td>
<td>164.76</td>
<td>----</td>
</tr>
<tr>
<td>8</td>
<td>94.87</td>
<td>6.40 (1H, d, 2.1)</td>
</tr>
<tr>
<td>9</td>
<td>158.61</td>
<td>----</td>
</tr>
<tr>
<td>10</td>
<td>105.55</td>
<td>----</td>
</tr>
<tr>
<td>1'</td>
<td>122.9</td>
<td>----</td>
</tr>
<tr>
<td>2'</td>
<td>116.23</td>
<td>7.84 (1H, d, 2.1)</td>
</tr>
<tr>
<td>3'</td>
<td>145.95</td>
<td>6.86 (1H, d, 8.3)</td>
</tr>
<tr>
<td>4'</td>
<td>148.43</td>
<td>----</td>
</tr>
<tr>
<td>5'</td>
<td>117.93</td>
<td>----</td>
</tr>
<tr>
<td>6'</td>
<td>123.08</td>
<td>7.58 (1H, dd, 2.1, 8.3)</td>
</tr>
<tr>
<td>1''</td>
<td>100.05</td>
<td>5.16 (1H, d, 7.7)</td>
</tr>
<tr>
<td>2''</td>
<td>73.32</td>
<td>3.81-3.83, m</td>
</tr>
<tr>
<td>3''</td>
<td>75.23</td>
<td>3.54-3.58, m</td>
</tr>
<tr>
<td>4''</td>
<td>70.17</td>
<td>3.63-3.66, m</td>
</tr>
<tr>
<td>5''</td>
<td>77.32</td>
<td>3.45-3.49, m</td>
</tr>
<tr>
<td>6' a, b</td>
<td>62.09</td>
<td>3.83-3.87, m; 3.83-3.87, m</td>
</tr>
</tbody>
</table>

**Table 2: NMR spectroscopic data for compound 4 (600 MHz, CD$_3$OD, $\delta$ ppm, $J$ in Hz)**

**Spasmodolytic Activity**

The results presented in Figure 4 demonstrate that all of the extracts of *S. gesneriflora* induced the concentration-dependent inhibition of the electrical stimulation-induced contractions in guinea-pig isolated ileum, using papaverine as positive control. At 200 $\mu$g/mL, the percentage relaxation achieved was 80.67 ± 1.63% for SgH, 52.41 ± 0.82% for SgD, and 54.99 ± 2.03% for SgM; in the case of papaverine at 100 $\mu$g/mL, this was 76.16 ± 2.44%. Statistical comparison among the extracts and against the papaverine revealed that SgH did not present a significant difference in percentage of relaxation ($p <0.05$). From the result of the statistical analysis, we carried out a dose-response curves of SgH and of papaverine (Figure 5), from which EC$_{50}$ values of 66.89 ± 1.6 $\mu$g/mL for SgH and 26.88 ± 1.9 $\mu$g/mL for papaverine respectively, were obtained.
Figure 4: Dose-response curves of SgH, SgD and SgM extracts from Salvia gesneriflora and papaverine (100 µg/mL)-induced contractions in guinea-pig ileum isolated. Values are reported as means ± standard error of the means (SEM). n = 5. ANOVA, Tukey *p<0.05 Papav100.

Figure 5: Dose-response curves of SgH and papaverine-induced contractions in guinea-pig ileum isolated.

Anti-inflammatory Activity

The results corresponding to the anti-inflammatory effect of the extracts are presented in Figure 6 where, at a same dose of 1.0 mg/ear, all of the extracts showed anti-inflammatory activities; the percentage of inhibition decreased according to the polarity of the solvent employed, that is, 71.12 ± 4.9% for SgH, 43.86 ± 4.1% for SgD, and 29.12 ± 0.3% for SgM. Statistical comparison among the extracts and against the reference drugs revealed that SgH did not present a significant difference in percentage inhibition of inflammation (p <0.05).
Figure 6: Percentage inhibition of inflammation (%) of SgH, SgD and SgM extracts from Salvia gesneriflora and Indo (Indomethacin) in edema induced by TPA in mouse ear at 1.0 mg/ear. Values are presented as means ± standard error of the means (SEM). n = 5. ANOVA, Tukey * p <0.05 Indo.

Antioxidant Capacity of the Extracts of Salvia gesneriflora

The results obtained in the three assays in which the antioxidant capacity of the extracts from S. gesneriflora were evaluated are shown in Table 2. In the DPPH assay, SgH extract presented very low antioxidant capacity in terms of the concentrations assayed; thus, it was not possible to determine their IC_{50}; the highest IC_{50} reached (0.04 ± 0.09 µg/mL) was of Cs followed by SgM (1.16 ± 1.08 µg/mL).

Of the three extracts of S. gesneriflora evaluated in ABTS, SgH produced the highest IC_{50} (1.73 ± 0.5 µg/mL), only surpassed by Cs (1.54 ± 0.6 µg/mL), while in the FRAP assay, SgD showed the greatest reductive capacity (1782.08 ± 2.1 Equivalent mM FeSO_{4}).

Table 3: Antioxidant capacity of the extracts (µg/mL) from Salvia gesneriflora at Inhibitory Concentration 50% (IC_{50})

<table>
<thead>
<tr>
<th>Extract</th>
<th>DPPH IC_{50} (µg/mL)</th>
<th>ABTS IC_{50} (µg/mL)</th>
<th>FRAP* Equivalent mM FeSO_{4}</th>
</tr>
</thead>
<tbody>
<tr>
<td>SgH</td>
<td>ND</td>
<td>1.73 ± 0.5</td>
<td>947.64 ± 2.0</td>
</tr>
<tr>
<td>SgD</td>
<td>2.72 ± 0.07</td>
<td>1.93 ± 0.7</td>
<td>1782.08 ± 2.1</td>
</tr>
<tr>
<td>SgM</td>
<td>1.16 ± 1.08</td>
<td>2.37 ± 0.6</td>
<td>1135.14 ± 2.2</td>
</tr>
<tr>
<td>Cs</td>
<td>0.04 ± 0.09</td>
<td>1.54 ± 0.6</td>
<td>1612.64 ± 0.7</td>
</tr>
</tbody>
</table>

Extracts SgH, SgD, SgM, and Cs (Camellia sinensis). Values are presented as means ± standard error of the means (SEM), n = 3. ND (Not Determined). *Determined for extracts at a concentration of 5,000 µg/mL, and for the compounds, at a concentration of 2.5 mM.

Discussion

The present study revealed that extracts SgH, SgD and SgM showed dose-dependent spasmolytic activity and diminished according to the polarity of the solvents employed to obtain the extracts, that is, SgH demonstrated the greatest inhibition of the electrically-induced ileal contractions. The EC_{50} of the extract was 2.49 times greater than that calculated for papaverine, whose effect could be associated with the presence of fatty acids such as hexadecenoic acid and hydrocarbons such as hentriacontane (major compound) as presented in the chromatographic profile. This finding is in agreement with what has been reported for the genus Salvia. In several of this species in which the antispasmodic activity has been evaluated. For example, S. officinalis. Antispasmodic activity is associated with the presence of these groups of compounds. Therefore, in our study, fatty acids could be responsible for such activity (Kan et al., 2011, Raal et. al., 2007). Similarly, for anti-inflammatory activity, SgH produced the highest activity,
similar to that of Indo at the same dose. This corroborated by hentriacontane which exhibited suppressive
potential on NO, PGE2 and LTB4 on LPS- induced translocation of NF-kB in RAW 264.7 (Khajuria et al., 2017). However, this was mainly due to the presence of compounds such as spathulenol which was
tested in a pleurisy model that induces inhibition of inflammatory parameters such as leukocyte migration
and protein extravasation (Do Nascimento, et al., 2018), and to the triterpenes, α, β-sitosterol, β-amyrin
and lupeol that in various assay models, demonstrated high anti-inflammatory activity (Melo et al., 2011;
Valerio et al., 2011; Sánchez-Burgos et al., 2015).

In relation to antioxidant capacity by DPPH, SsM extract achieved the highest IC50, which is
statistically similar to that achieved by Cs, which was used as reference extract. The elevated activity of
SsM in this assay is probably related to the presence of rosmarinic acid, as well as that of caffeic acid,
chlorogenic acid and quercetin glucoside, which have been reported in various species of this genus and
which possess high antioxidant capacity (Rungsimakan, et al., 2014; Bahadori et al., 2017; Zengin, et al.,
2018). Likewise, it is noteworthy that the SgH extract showed the highest antioxidant capacity in the
ABTS assay, demonstrating that it is the extract that has the least IC50 in comparison with the other two
extracts. Thus, it is likely that the fatty acids and triterpenes previously mentioned in this assay again
exhibited good activity. Finally, the greatest reductive capacity in the FRAP assay was for SgD, which
reached highest equivalent mM of FeSO4, even surpassing those of Cs. The latter could be related to the
presence of terpenic derivatives, which were identified in this species and which have been identified in
Salvia species with antioxidant activity (Wang et al., 1998; Tepe et al., 2006; Bahadori et al., 2015;
Alipić, 2017).

Conclusion

Hexane, dichloromethane and methanol extracts of the aerial parts of Salvia gesneriflora
produced spasmylytic, anti-inflammatory and antioxidant activities, in which compounds of the following
types were identify: fatty acids; sterols; phenols and flavonoids. Therefore, these compounds may act as
coadjuvants in terms of the knowledge of traditional medicine of this species and suggests possible
potential applications of the species in other fields of pharmacy and foods.

Conflict of Interest: The authors declare no conflict of interest.

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