AMARISOLIDE A AND PEDALITIN AS BIOACTIVE COMPOUNDS IN THE ANTINOCICEPTIVE EFFECTS OF SALVIA CIRCINATA (LAMIACEAE)

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Abstract
Background: Salvia circinata is an endemic species of Mexico used in the folk medicine of Santiago Huauclilla, Oaxaca, mainly as remedy for gastrointestinal diseases.

Hypothesis: If the extracts of Salvia circinata have secondary metabolites with antinociceptive activity, then the behavior of nociception in the model of “whrithing” in mice will decrease.

Specie studied: Salvia circinata Cav. (Lamiaceae).

Study site and years of study: Salvia circinata was collected in Santiago Huauclilla, Oaxaca, in July 2014.

Methods: Firstly, the acute toxicity of S. circinata extracts was evaluated to calculate the LD50 with OECD method. Then, dose-response curves of the antinociceptive effect of S. circinata organic and aqueous extracts (1, 10, 30, 100, and 300 mg/kg) were obtained in the writhing test in mice. Furthermore, chromatographic techniques were applied to isolate the compounds and were identified by comparison of the values of 1H NMR, 13C NMR and ESIMS reported in the literature.

Results: Our data showed significant antinociceptive activity in all the tested extracts. Amarisolide A and pedalitin were isolated in the ethyl acetate and methanol extracts, respectively and assayed at doses of 1, 5 and 10 mg/kg, i.p. All the compounds decreased nociception in mice in at least 50 % from a minimal dosage of 1 mg/kg, i.p. and in a similar manner than the reference drug ketorolac (1 mg/kg, i.p.).

Conclusions: Our findings give evidence that Salvia circinata possesses antinociceptive activity depending on the presence of several known bioactive constituents, reinforcing its use in the Mexican traditional medicine to alleviate abdominal pain.

Key words: Abdominal pain, amarisolide A, Lamiaceae, pedalitin, Salvia circinata.

Resumen
Antecedentes: Salvia circinata es una especie endémica de México, utilizada en Santiago Huauclilla, Oaxaca como remedio para enfermedades gastrointestinales.

Hipótesis: Si los extractos de Salvia circinata tienen metabolitos con actividad antinociceptiva, entonces disminuirán la conducta nociceptiva en el modelo whrithing.

Especie estudiada: Salvia circinata Cav. (Lamiaceae).

Lugar de estudio y años de estudio: Salvia circinata se colectó en Santiago Huauclilla, Oaxaca, en julio de 2014.

Métodos: Se evaluó la toxicidad aguda (DL50) de los extractos de S. circinata mediante el método de la OECD. Se realizaron las curvas dosis-respuesta del efecto antinociceptivo de los extractos de S. circinata (1, 10, 30, 100, and 300 mg/kg) en el modelo de writhing en ratones. Además, se utilizaron técnicas cromatográficas para aislar los compuestos y se identificaron por comparación de los datos de 1H RMN, 13C RMN y ESIMS reportados en la literatura.

Resultados: Nuestros resultados muestran una actividad antinociceptiva significativa en todos los extractos evaluados. La amarisolida A y la pedalitina fueron aisladas de los extractos de acetato de etilo y metanol, respectivamente y evaluadas a dosis de 1, 5 y 10 mg/kg, i.p. Todos
Salvia cinnamata Cav. (syn. Salvia amarissima Ortega) is an endemic herbaceous plant widely distributed in Mexico (Martínez-Gordillo et al. 2013). In Santiago Huauclilla, Oaxaca, a Mexican region where traditional medicine using plants is extensively common, this plant is known as “bretónica”, and according to the citizens it is frequently used as an infusion for its analgesic and anti-inflammatory properties mainly to alleviate gastrointestinal illness that includes diarrhea and stomachache (Nambo 2015) and for the treatment of ulcers and diabetes (Castro et al. 2014, Flores-Bocanegra et al. 2017).

Phytochemical studies of S. cinnamata have reported the presence of diterpenoids such as amarissinins A-E (Bautista et al. 2016, Fragoso-Serrano et al. 2019) and teotihuacanin (Bautista et al. 2015, Fragoso-Serrano et al. 2019), and glucoside diterpenoids as amarisolides A-F (Maldonado et al. 1996, Flores-Bocanegra et al. 2017, Fragoso-Serrano et al. 2019). Flavonoids like pedalitin (Maldonado et al. 1996), apigenin-7-O-β-D-glucoside, the flavone 2-(3,4-dimethoxy-phenyl)-5,6-dihydroxy-7-methoxy-4H-chromen-4-one, and new biflavone (Flores-Bocanegra et al. 2017).

Pharmacological studies have reported the cytotoxic effect of teotihuacanin isolated from S. amarissima as potent compound with multidrug resistance (MDR) modulatory activity in the vinblastine-resistant MCF-7 cancer cell line (Bautista et al. 2015). Cytotoxicity of the amarissinins has been also reported against five human cancer cell lines, as well as MDR modulatory activity in a breast cancer cell line (MCF-7) resistant to vinblastine (Bautista et al. 2016). In addition, the in vivo antihyperglycemic activity and the α-glucosidase in vitro inhibitory effects have been reported for the extract of S. cinnamata aerial parts and its flavonoids and clerodane diterpene glucosides (Flores-Bocanegra et al. 2017). However, scientific studies supporting the efficacy and security of the use of this plant for abdominal pain are less studied. Other studies showing purity from 90 to 99 % were obtained by lyophilization (HETO FD3, Heto-Holten A/S, Denmark) to obtain a total yield of 10.6 g.

Plant material. Salvia cinnamata aerial parts were collected in Santiago Huauclilla, Oaxaca, in July 2014. This region is located at the parallels 17° 25’ and 17° 34’ latitude north and meridians 96° 56’ and 97° 08’ longitude west, and at altitude between 1,200 and 2,700 m (INEGI 2010). A voucher specimen (Number 16360) was identified by Dra. Martha J. Martínez Gordillo and deposited in the IMSS Herbarium of CDMX, Mexico.

Preparation of the extracts. Organic extracts were obtained by maceration of S. cinnamata, dry and ground aerial parts at room temperature, three successive extractions each 24 h were done using solvents (2.5 L) in increased polarity (hexane, ethyl acetate, and methanol analytical grade purchased in Tecsisquim, SA de CV, Mexico). Solvent excess was completely retired by evaporation in a rotovaporator RII (Büchi Labortechnik AG, Switzerland) to obtain a final yield of the crude extracts (Figure 1). To identify chemical compounds involved in the pharmacological activity of S. cinnamata, samples of the crude extracts (3 mg) were subjected to a high-performance liquid chromatographic (HPLC-DAD) analysis. Since the hexane extract was obtained in a less yield than the ethyl acetate and methanol extracts (Figure 1), only these two were fractionated to isolate individual pure compounds (Figure 1).

Aqueous extract of S. cinnamata dried aerial parts was obtained by pulverizing 50 g of plant material and boiled in 500 mL of distilled water for 10 min. Afterwards, the liquid was filtered at room temperature and then frozen in liquid nitrogen to be lyophilized (HETO FD3, Heto-Holten A/S, Denmark) to obtain a total yield of 10.6 g.

High performance liquid chromatography (HPLC-DAD). Bioactive constituents of S. cinnamata were determined and quantified using a HPLC apparatus Agilent Technologies, series 1100 equipped with a diode array detector. A sample of each extract (3 mg) was dissolved in methanol (1 mL, HPLC grade from J.T.Baker, USA) and filtered into Acrodisc® syringe filters Nylon membrane, diameter 25 mm, pore size 0.45 μm to inject 15 μL of each solution.

For identification and quantification of terpenes, a Zorbax Eclipse XDB-C8 column (125 × 4.0 mm diameter and a 5 μm particle size) was used with a mobile phase of acetonitrile (MeCN, HPLC grade from J.T. Baker, USA)/water, 80:20 with a flow rate of 1 mL/min and temperature at 40 °C. Equipment was calibrated at a wavelength of 215 and 220 nm in a running time of 21 min. Standard curves of calibration were built with five concentrations from 0.037 to 1.29 μg of standard terpenoids and amarisolide (99 % purity, determined by HPLC-DAD) obtained from S. cinnamata in this study. Other standards showing purity from 90 to 99 % were ursoic acid, oleancenic acid, α- and β-amyrin, and β-sitosterol purchased at Sigma-Aldrich (St. Louis Mo. USA). Interpolation was done with the ChemStation program, Agilent Tech version B.02.01.

For identification and quantification of phenolic acids, a Nucleosil 100 A column (125 × 4.0 mm of diameter and a 5 μm particle size). Mobile phase has a flow rate of 1 mL/min with a gradient of water at pH 2.5 using trifluoroacetic acid/MeCN: 0-10 min, 85 % water: 15 % MeCN; 10-20 min, 65 %...
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**Figure 1.** Diagram showing chromatographical fractionation of the *S. cirinata* extracts to isolate pure compounds.

**Fractionation of the organic extracts.** The ethyl acetate (17.2 g) and methanol (31.7 g) extracts were partitioned using a chromatographic column packed with silica gel (Macherey-Nagel). The elution program started with hexane, to be continued with ethyl acetate, and finally methanol. The ethyl acetate fraction was separated on silica gel column chromatography in a proportion of 1:15, extract-eluent. The elution started with hexane followed by using a gradient of increasing polarity of hexane-ethyl acetate (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9), ethyl acetate (100%), ethyl acetate–methanol mixture (9:1, 8:2, 7:3), and finally methanol. A total of 58 subfractions (100 mL each) were collected and then grouped by similarity according to their profiles acquired by thin layer chromatography (TLC) (Figure 1). From subfractions 51-56 eluted with ethyl acetate-methanol (8:2 and 7:3) was obtained compound 1, which was crystallized from methanol-acetone and analyzed through ESIMS, 1H and 13C NMR. Identification of 1 was determined by comparison of its spectroscopic data with those described for amarisolide A, which were the same. Allowed purifying by crystallization a pure compound (m.p. 206 °C, Fisher Johns equipment) that was analyzed by ESIMS in positive mode with a Cap LC coupled Micromass Q-ToF Ultima ESI system (Waters Corp., Milford, MA), as well as 1H NMR and 13C NMR analysis (Bruker, Avance DPX400). The NMR signals matched with those previously reported for this compound preliminary isolated from *S. amarissima* (Maldonado et al. 1996, Flores-Bocanegra et al. 2017).

Amarisolide A (1, yield 108 mg): white powder, mp 206 °C; 1H NMR (400 MHz, CD3OD) δ = 7.40 (t, J = 1.4 Hz, 1H, H-15), 7.36 (s, 1H, H-16), 7.01 (d, J = 6.5 Hz, 1H, H-3), 6.25 (dd, J = 1.4, 0.8 Hz, 1H, H-14), 4.62 (m, 1H, H-2), 4.56 (d, J = 7.8 Hz, 1H, H-1′), 4.41 (d, J = 8.0 Hz, 1H, H-19a), 4.07 (dd, J = 8.0, 2.0 Hz, 1H, H-19b), 3.95 (dd, J = 11.0, 4.2 Hz, 1H, H-6a), 3.78 (dd, J = 11.3, 3.2 Hz, 1H, H-6b), 3.50 (t, J = 8.5 Hz, 1H, H-3′), 3.43 (t, J = 9.0 Hz, 1H, H-4′), 3.35

**Table 1.** Composition and yield of the organic extracts. 7.70 (dd, J = 11.3, 3.2 Hz, 1H, H-6b), 3.50 (t, J = 8.5 Hz, 1H, H-3′), 3.43 (t, J = 9.0 Hz, 1H, H-4′), 3.35

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Acute toxicity. Mice receiving the acute administration of the methanol and aqueous extracts at a maximal dosage of 2,000 mg/kg i.p. allowed by OECD (2001) were observed for 14 days to register toxicological manifestations such as: loss of the consciousness, ataxia or respiratory depression, as well as possible death. At the end of the observation period of 14 days, surviving mice were euthanized to analyze macroscopically possible tissue alteration.

Nociceptive test (writhing). To build dose-response curves, all the extracts and pure bioactive compounds (1 and 11) were tested in independent groups at doses of 1, 10, 30, 100 and 300 mg/kg. The antinociceptive activity was evaluated 30 min after their administration. The test consisted in the induction of an exaggerated extension of the abdomen combined with the outstretching of the hind limbs as previously reported (Collier et al. 1968). This nociceptive behavior was induced after i.p. administration of 10 mL/kg of diluted acetic acid solution at 1%. The number of writhes was immediately counted each 5 min for a total period of 30 min after the injection of the nociceptive agent (Viana et al. 2003).

Statistical analysis. The area under the curve (AUC) values were calculated from the respective temporal course curves obtained in the nociceptive behavior assays using the trapezoidal rule and they were considered as an expression of the nociceptive behavior in the writhing test. Data are expressed as the mean ± standard error of the mean (SEM) of 6 animals. The statistical analysis was performed using one-way ANOVA followed by Dunnnett’s post hoc test. Graphpad Prism® version 6.0 for Windows (Graphpad Software, San Diego, CA, USA). A $P < 0.05$ was considered statistically significant.

Results

Phytochemical analysis. According to the phytochemical analysis using several chromatographic techniques, the presence of possible bioactive metabolites was obtained as follows:

Terpenoids. *S. circinata* showed five terpenoids in the hexane and ethyl acetate extracts: amarisolide A (1) (Figure 2), ursolic acid (2), oleanolic acid (3), α-amyrin (4), and β-sitosterol (5) (Table 1); as well as in the methanol extract with exception of 4. The most abundant terpenoids in the hexane extract from major to lower were 2, 3, and 1; and

![Figure 2](image-url)  
**Figure 2.** Structure of amarisolide A (1) and pedaltalin (11).
Antinociceptive effects of *Salvia circinata* and bioactive compounds

Table 1. Analysis of terpenoids, phenolic acids and flavonoids obtained from *Salvia circinata* aerial parts

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Compound</th>
<th>Terpenoids retention time (min)</th>
<th>Ethyl acetate (µg/mg)</th>
<th>Methanol (µg/mg)</th>
<th>Aqueous (µg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amarisolide A</td>
<td>1.69</td>
<td>55.33</td>
<td>255.33</td>
<td>182.60</td>
</tr>
<tr>
<td>2</td>
<td>Ursolic acid</td>
<td>2.73</td>
<td>96.33</td>
<td>12.76</td>
<td>7.35</td>
</tr>
<tr>
<td>3</td>
<td>Oleoletic acid</td>
<td>4.65</td>
<td>67.00</td>
<td>197.83</td>
<td>n.d.</td>
</tr>
<tr>
<td>4</td>
<td>α-amyrin</td>
<td>5.76</td>
<td>22.66</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>5</td>
<td>β-sitosterol</td>
<td>18.79</td>
<td>3.86</td>
<td>40.30</td>
<td>1.02</td>
</tr>
<tr>
<td>6</td>
<td>Chlorogenic acid</td>
<td>4.97</td>
<td>n.d.</td>
<td>40.39</td>
<td>44.93</td>
</tr>
<tr>
<td>7</td>
<td>Caffeic acid</td>
<td>7.32</td>
<td>n.d.</td>
<td>5.79</td>
<td>5.96</td>
</tr>
<tr>
<td>8</td>
<td>Ferulic acid</td>
<td>10.20</td>
<td>n.d.</td>
<td>12.26</td>
<td>12.32</td>
</tr>
<tr>
<td>9</td>
<td>Rutin</td>
<td>4.94</td>
<td>n.d.</td>
<td>18.33</td>
<td>21.43</td>
</tr>
<tr>
<td>10</td>
<td>Phlorizin</td>
<td>6.98</td>
<td>n.d.</td>
<td>27.28</td>
<td>10.39</td>
</tr>
<tr>
<td>11</td>
<td>Pedalitin</td>
<td>10.20</td>
<td>n.d.</td>
<td>134.06</td>
<td>5.16</td>
</tr>
<tr>
<td>12</td>
<td>Quercetin</td>
<td>10.82</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

n.d. = not detected.

in the ethyl acetate and methanol extracts was principal the terpenoid 1 (Table 1). In the case of the aqueous extract, the compounds identified were 1, 2 and 5, with the compound 1 as the most abundant (Table 1); all they were corroborated in the HPLC-DAD chromatograms (Figure 3).

Phenolic acids.- Phenolic acids were identified as follows: ferulic acid (8) was majority in the ethyl acetate extract that showed also caffeic acid (7) and chlorogenic acid (6) (Table 1 and Figure 3). These three compounds were identified again in the aqueous and methanol extracts, where 6 was the most abundant in both (Table 1 and Figure 3).

Flavonoids.- Quercetin (12) and phloretin (13) were identified in the ethyl acetate extract; whereas rutin (9), phlorizin (10), and pedalitin (11) (Figure 2) were obtained in the methanol and aqueous extracts. The compound 11 was the most abundant flavonoid in the methanol extract and compound 9 in the aqueous extract as corroborated by HPLC-DAD analysis (Figure 3).

Pharmacological analysis. Regarding to the pharmacological evaluation, all the treatments including organic extracts (Figure 4A-4F), aqueous (Figure 5A, B) and individual pure compounds (1 and 11, Figure 5C-5F), significantly decreased (*P < 0.05*) the number of writhes from a dosage of 1 mg/kg, except for the aqueous extract that produce its significant antinociceptive response after a dosage of 10 mg/kg (Figure 5A, B) in comparison to the group receiving vehicle. Antinociceptive response produced by extracts and the pure compounds resembled the effect of the reference drug ketorolac (1 mg/kg), the pharmacological response was dose-dependent in the evaluation with the medium polar (ethyl acetate) and polar extracts (methanol and aqueous) (Figures 4 and 5).

Acute toxicity of the organic and aqueous extracts was calculated to be > 2,000 mg/kg. Mice did not show weight loss during the 14-days observation period and it was not observed macroscopic tissue injury in those surviving suggesting that low toxicity might be expected in the use of this species to alleviate abdominal pain.

Discussion

The present study demonstrates for the first time that organic and aqueous extracts, as well as some isolated and purified compounds from *Salvia circinata*, reduce nociception in mice. The three organic extracts (hexane, ethyl acetate and methanol) of *S. circinata* showed a similar pharmacological profile in the antinociceptive responses in mice treated with a range of doses in a logarithmic increase from 1 to 300 mg/kg showing a dose-dependent effect in case of the ethyl acetate and methanol extracts, but not with the hexane extract and the pure compounds amarisolide A and pedalitin.

Compounds 1, 2, 3 and 5 were identified in all the organic extracts, as well as in the aqueous extract, with an exception of oleanolic acid. While, α-amyrin was determined in the hexane and ethyl acetate extracts. In the case of amarisolide A, its presence was abundant mainly in the ethyl acetate and methanol extracts. Pharmacological antinociceptive activity for this terpenoids is lacking in literature; consequently, investigation about it is important to explore and describe. Recently, this terpenoid was isolated from aerial parts of *S. circinata*, and its antihyperglycemic activity was evaluated (Flores-Bocanegra et al. 2017). In the present study, the antinociceptive activity was tested using a model of abdominal pain in which it produced at least 50% inhibition from a minimal dose of 1 mg/kg. The effect did not show increase by increasing doses and it was like that produced by different organic extracts even in those in which it was detected in greater abundance. It is possible that amarisolide A is one of the main bioactive metabolites responsible for the antinociceptive activity of this plant species. Nevertheless, there was detected other compounds that likely contribute to the
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final effect of the crude extracts. The mechanism of action of amarisolide A was not explored in this study; however, it was isolated from *Salvia rubescens* and reported as anti-inflammatory by inhibition of the elastase and myeloperoxidase enzymes (22 ± 4 % and 38 ± 10 %, respectively) at 100 μM in a model of murine inflammation (Rodriguez et al. 2005).

Other terpenoids identified in *S. circinata* were the ursolic and oleanolic acids; these compounds have been already reported in *S. officinalis* due to their antinociceptive activity, which was observed at the dose of 30 mg/kg, producing inhibition in the inflammatory phase of the formalin test, and antinociception in the mechanical allodynia induced by cinnamaldehyde possibly through TRPA1-receptors (Rodrigues et al. 2012). On the other hand, terpenoids both have been isolated as responsible bioactive metabolites of pharmacological antinociceptive effects of *Rosmarinus officinalis* (Martínez et al. 2012) and *Agastache mexicana* (Verano et al. 2013), showing dose-dependent effects with an ED_{50} = 1.6 mg/kg and 2.1 mg/kg, respectively. A participation of cGMP pathway and serotonergic neurotransmission through 5-HT_{1A} receptors, as well as TRPV1 receptors were also considered in the antinociceptive responses of ursolic acid in the writhing and capsaicin tests in mice (Verano et al. 2013). In the case of oleanolic acid, its antinociceptive effects were mediated by an opioidergic and serotonergic, but not by adrenergic receptors, in glutamate-induced pain (Park et al. 2013). Regarding β-sitosterol, its antinociceptive properties were responsible of the activity of *Buddleja thrysoides* (Fialho et al. 2017) and *Moringa oleifera* at doses of 18, 25 and 35 mg/kg significantly attenuated hyperalgesia and tactile allodynia in a neurophatic pain model (Raa-fat & Hdaib 2017). Finally, α-amyrin obtained in the hexane and ethyl acetate extracts is other possible responsible bioactive metabolite of *S. circinata* since it has been reported that alone or combined with β-amyrin produces inhibition of pain like orofacial induced by formalin and capsaicin (Holanda-Pinto et al. 2008). Its antinociceptive effects have been associated with inhibition of COX-2 enzyme and diminution in the pro-inflammatory cytokines (Medeiros et al. 2007). The effects of the extracts were observed in a dose-dependent manner when fenolic acids and flavonoids were present together with terpenoids. These results suggest likely synergistic interac-

**Figure 3.** HPLC-DAD chromatograms of the *S. circinata* showing terpenoids, phenolic acids, and/or flavonoids as identified compounds in the hexane (A), ethyl acetate (B, E, and H), methanol (C, F, and I), and aqueous (D, G, and J) extracts. Amarisolide A (1), ursolic acid (2), oleanolic acid (3), α-amyrin (4), β-sitosterol (5), chlorogenic acid (6), caffeic acid (7), ferulic acid (8), rutin (9), phlorizin (10), pedalitin (11), quercetin (12), phloretin (13).
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**Figure 4.** Temporal course curves (TCC) of the nociceptive activity induced by 1% acetic acid in the presence of the vehicle, ketorolac (reference drug) and the crude organic extracts hexane (A), ethyl acetate (C), and methanol (E). The corresponding antinociceptive activity expressed as percentage from the TCC is indicated in the panels (B, D, and F, respectively). Each point and bars represent the mean ± standard error of 6 mice. One-way ANOVA followed by Dunnett’s test, *P < 0.05* was considered significant.
Figure 5. Temporal course curves (TCC) of the nociceptive activity induced by 1% acetic acid in the presence of the vehicle, ketorolac (reference drug) and the aqueous (A) and individual pure compounds amarisolide A (C) and pedalitin (E). The corresponding antinociceptive activity in percentage obtained from the TCC is indicated in the panels (B, D, and F, respectively). Each point and bars represent the mean ± standard error of 6 mice. One-way ANOVA followed by Dunnett’s test, *P < 0.05 was considered significant.
tions in this plant that will be interesting to study in a future investigation.

This is the first time that antinociceptive activity is evidenced for *S. circinata* in agreement and reinforcing this activity already reported for other species of *Salvia* genus. For example, the *S. wiedemannii* chloroform extract from its aerial parts produced antinociceptive effects in the tail-flick and acetic acid-induced writhing tests in mice (Ustun & Sezik 2011); the *S. officinalis* hexane and chloroform extracts inhibited in a dose-dependent fashion the croton oil-induced ear oedema in mice (Baricevic et al. 2001), as well as in the aqueous and butanol leaf extracts in the hot plate and formalin tests in rats (Qnais et al. 2010). *S. hypoleuca* and *S. limbata* reported antinociceptive activity in the methanol and aqueous extracts of the aerial parts using the hot plate model in mice (Karami et al. 2013). Nevertheless, *S. circinata* demonstrated better antinociceptive potency in comparison to these species from the same genus, since we observed significant and maximal response from 1 to 10 mg/kg, i.p. in comparison to the significant response observed at 500 mg/kg, i.p. in the case of *S. wiedemannii* in the same nociceptive test (Ustun & Sezik 2011). Other species has demonstrated antinociceptive effect in other tests using higher dosage; for example: *Salvia hypoleuca* and *S. limbata* using a minimal dose of 100 mg/kg and a maximal of 1,500 mg/kg, i.p. (Karami et al. 2013). In contrast, there are species from this genus without antinociceptive efficacy as reported for *S. halophila* and *S. virgata* in the writhing test (Küpeli et al. 2008). This difference is probably associated with the chemical composition. According to the phytochemical background analyzed in *S. circinata* in this investigation, mainly the identified terpenoid content, might play an important role in the antinociceptive activity of this genus species suggesting its potential for the pain therapy and reinforcing the medical traditional use of this plant.

The chromatographic fractionation of the methanol extract allowed identify and purify also some flavonoids like pedalin, which was the most abundant compound showing significant antinociceptive effects from a dose of 1 mg/kg. This pharmacological activity might be associated to the inhibition property on the mediators like NO, TNF-α and IL-12 production (Rao et al. 2009). Other biological activities of pedalin are the antioxidant effects by inhibition of myeloperoxidase and as a scavenger of free radicals (Fernandes et al. 2008), as well as antihyperglycemic (Flores-Bocanegra et al. 2017).

Regarding to *S. circinata* aqueous extract, this was less active than the organics extracts since its significant response was observed at 10 mg/kg in comparison to 1 mg/kg, respectively. The most abundant chemical metabolites were amarisolide A, chlorogenic acid, and rutin. Chlorogenic acid has been involved in the antinociceptive effects of other species with this property (Küpeli et al. 2012, Martínez-González et al. 2016). In case of rutin, this flavonoid glycoside possesses antinociceptive properties mediated by central opioidergic neurotransmission (Selvaraj et al. 2014, Hernandez-Leon et al. 2016). This flavonoid has been even combined with a clinical analgesic to improve the efficacy against pain (Alonso-Castro et al. 2017).

The acute toxicity evaluation in vivo allowed to calculate a LD₅₀ > 2,000 mg/kg, i.p., for all the aqueous and organic extracts of *S. circinata*, at least at a maximal dosage recommended in the normativity of the OECD (2001), placing these extracts in category 5 of the globally harmonized system of classification and labeling of non-toxic chemical products. These results are consistent with previous data conducted in the aerial parts of the same species using 5 g/kg, p.o. (Flores-Bocanegra et al. 2017) and in other *Salvia* species like in *S. leriifolia* seeds aqueous extract determined as LD₅₀ = 19.5 g/kg, i.p. in mice (Hosseinizadeh et al. 2003) and in *S. officinalis* leaves hydroalcoholic extract with a LD₅₀ = 44.75 g/kg, p.o. (Rodrigues et al. 2012).

In conclusion, the present investigation gives pharmacological evidence of the potential use of *S. circinata* in the pain therapy due to the presence of diversity of bioactive compounds like terpenoids, phenolic acids, and flavonoids to validate the use of this species in the Mexican Traditional Medicine reported by the inhabitants of Santiago Huauclilla, Oaxaca, Mexico.

Acknowledgements

This work was supported by PAPIIT-IN218418, CONACYT 256448/256454. The authors thanks to Enrique Pinzón Estrada, Ismael Torres Saldaña, Alberto Hernandez Leon, Lizeth M. Zavala-Ocampo, and Verónica Muñoz Occtero for their technical assistance, as well as Marissa González for proof-reading the manuscript.

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Antinociceptive effects of *Salvia cirkinata* and bioactive compounds

DOI: https://doi.org/10.1016/j.jep.2011.11.042

DOI: https://doi.org/10.1055/s-0031-1282811


**Associated editor**: Elihu Bautista

**Author Contributions**: GFMP participated in the performance of experimental work and preparation of the manuscript. MEGT participated in the performance of nociceptive test, preparation and revision of the manuscript. MJMG and FABP participated in the collected and identified the plant material and revision of the manuscript. RSMC participated in the HPLC analysis and revision of the manuscript. ADG participated in the structure elucidation and revision of the manuscript. EAH participated in the isolation and purification of compounds, preparation and revision of the manuscript.