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# Antidepressant and anxiolytic effects of hydroalcoholic extract from *Salvia elegans*☆

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**Abstract**

*Salvia elegans* Vahl (Lamiaceae), popularly known as "mirto", is a shrub that has been widely used in Mexican traditional medicine for the treatment of different central nervous system (CNS) diseases, principally, anxiety. Nevertheless, the available scientific information about this species is scarce and there are no reports related to its possible effect on the CNS. In this work, the antidepressant and anxiolytic like effects of hydroalcoholic (60%) extract of *Salvia elegans* (leaves and flowers) were evaluated in mice. The extract, administered orally, was able to increase the percentage of time spent and the percentage of arm entries in the open arms of the elevated plus-maze, as well as to increase the time spent by mice in the illuminated side of the light–dark test, and to decrease the immobility time of mice subjected to the forced swimming test. The same extract was not able to modify the spontaneous locomotor activity measured in the open field test. These results provide support for the potential antidepressant and anxiolytic activity of *Salvia elegans*.


*Keywords:* Anxiety; Depression; *Salvia elegans*; Elevated plus-maze; Forced swimming test; Open field

## 1. Introduction

According to the World Health report (WHO, 2001), approximately 450 million people suffer from a mental or behavioral disorder, yet only a small minority of them receive even the most basic treatment. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020 (Reynolds, 2003). In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models (Zhang, 2004).

In Mexican traditional medicine, an infusion is prepared from the leaves and flowers of *Salvia elegans* Vahl (Lamiaceae), popularly known as "mirto", and administered orally for treating CNS diseases. This plant constitutes one of the most used species in Mexico for the treatment of anxiety, and insomnia (Aguilar et al., 1994). It is important to mention that the common name of this plant is shared with other members of the genus (Martínez, 1979; Bello, 1993). Despite the widely popular use of the plant, it was not possible to find pharmacological data confirming some activity of this plant on the mentioned diseases. The only evidence of probable CNS activity was found in a report showing that the methanolic extract of *Salvia elegans* was able to displace [$^3$H]-(*N*)-scopolamine from muscarinic receptors in homogenates of human cerebral cortical cell membranes (Wake et al., 2000). Despite the scarce pharmacological scientific information about this species, there are other members of the family which possess demonstrated CNS activities. *Salvia divinorium* Epling and

*Abbreviations:* CNS, central nervous system; DZP, diazepam; EPM, elevated plus maze; FST, forced swimming test; IMI, imipramine; LDT, light–dark test; OFT, open field test; PTX, pycrotoxine

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Játiva has hallucinogenic (Siebert, 1994; Chavkin et al., 2004) and antidepressant properties (Hanes, 2001), and *Salvia officinalis* L. possesses metabolites with benzodiazepine-like effects (Kavvadias et al., 2003), *Salvia miltiorrhiza* f. *alba* Wu and Li, used in Chinese traditional medicine, has shown neuroprotective effect, due to the NMDA receptor antagonistic activity (Sun et al., 2003), and *Salvia reuterana* Boiss recently showed anxiolytic effect in mice (Rabbani et al., 2005).

Twenty-eight volatile constituents were identified in essential oil from *Salvia elegans*, mono- and sesqui- terpenoids such as *trans*-ocimene, linalool, β-caryophyllene, germacrene D and spathulenol, aliphatic alcohols such as 2-propanol and 3-octanol, and trans-3-hexenal (Makino et al., 1996).

These data confirm that different members of the Lamiaceae family are able to modulate the physiology of the CNS. On this basis, the objective of the present study was to evaluate the anxiolytic and antidepressant effect produced by the hydroalcoholic extract from *Salvia elegans* in mice. Experiments were conducted with the ICR mice strain, which permits the analysis of the effects produced by *Salvia elegans* in different mice models.

## 2. Methods

### 2.1. Plant material and extract preparation

Leaves and flowers of *Salvia elegans* were collected from the state of Puebla, south west of Mexico. Plant material was identified by Abigail Aguilar-Contreras, M.Sc., the IMSSM Herbarium Director (located in National Medical Center, Mexico City). Voucher specimens were stored at this site for future reference (IMSSM-14588). From the collected plant, the leaves and flowers were selected and dried under dark conditions at room temperature for 2 weeks. Dry material was milled in an electrical grinder (Pulvex) obtaining particles less than 4 mm. Milled material was extracted in 60% ethanol solution at 50 °C for 2 h. Afterwards, the extract was filtered through a Wattman #1 paper and extracted once again (under the same conditions) with a new solvent. The obtained extracts were reunited and the solvent was evaporated to dryness with a rotary evaporator under reduced pressure. The yield of the extract was quantified (16.48%) and the obtained material was protected from direct light and stored under 4 °C until its use.

### 2.2. Drugs

The hydroalcoholic extract from *Salvia elegans* was used as experimental extract (25–2000 mg/kg; dissolved in saline solution), and as positive controls were used: diazepam (DZP, 1.0 mg/kg, Sigma) as an anxiolytic drug; picrotoxin (PTX, 2.0 mg/kg, Sigma St. Louis, USA) as an anxiogenic drug; and imipramine hydrochloride (IMI, 15 mg/kg, Sigma St. Louis, USA) as an antidepressant drug.

### 2.3. Animals and treatments

All experiments were conducted in accordance with international standards of animal welfare recommended by the Society for Neuroscience (USA). The experimental protocol was approved by the Institutional Research Committee. The minimum number of animals and duration of observation required to obtain consistent data were employed.

Male ICR mice (32–38 g) purchased at Harlan Mexico were used. All animals were maintained under controlled conditions of temperature (22 ± 2 °C), and illumination (12 h light–dark cycle), with free access to food (Harlan rodent lab diet) and water. Groups of eight animals were organized and, in order to reduce the influence of day variation, all assays were conducted from 8 to 13 h, in a special noise-free room with controlled illumination.

Mice were treated orally with different doses of the hydroalcoholic extract of *Salvia elegans* (125, 250, 500, 1000 and 2000 mg/kg). A negative control group was included which received physiological saline solution (p.o.). Positive control groups were administered with 15 mg/kg of IMI i.p. (for forced swimming test) or with 1.0 mg/kg of DZP i.p. (for the other tests) and an anxiogenic group was treated with 2.0 mg/kg of PTX i.p. (for elevated plus-maze, light–dark test and open field test). All treatments were administered 1 h before the test, with the exception of forced swimming test, in which IMI and the plant extract were administered 24, 18 and 1 h before the test.

### 2.4. Elevated plus-maze (EPM)

This test has been widely validated to measure anxiety in rodents (Pellow et al., 1985; Lister, 1987). This apparatus was made of Plexiglas and consisted of two open arms (30 cm × 5 cm) and two closed arms (30 cm × 5 cm) with 25 cm walls. The arms extended from a central platform (5 cm × 5 cm). The maze was elevated 38.5 cm from the room floor.

Each animal was placed at the center of the maze, facing one of the enclosed arms. Number of entries and the time spent in enclosed and open arms was recorded for 5 min test. Entry into an arm was defined as the animal placing all four paws onto the arm. All tests were taped by using a video camera. After each test, the maze was carefully cleaned up with a wet tissue paper (10% ethanol solution).

### 2.5. Forced swimming test (FST)

The FST is the most widely used pharmacological in vivo model for assessing antidepressant activity (Porsolt et al., 1977). The development of immobility when the mice are placed in an inescapable cylinder filled with water, reflects the cessation of persistent escape-directed behavior (Lucki, 1997). The apparatus consisted of a clear plexiglas cylinder (20 cm high × 12 cm diameter) filled to a 15 cm depth with water (24 ± 1 °C). In the pre-test session, every animal was placed individually into the cylinder for 15 min, 24 h prior to the 5 min swimming test. *Salvia elegans* extract, imipramine and distilled water were administered three times: immediately after the initial 15-min pre-test, 18 and 1 h prior to the swimming test. During the test session a trained observer registered the immobility time, considered to be