# Anxiolytic and antidepressant-like effects of the hydroalcoholic extract from *Aloysia polystachya* in rats

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**Abstract**

Behavioral effects of a hydroalcoholic extract from leaves of *Aloysia polystachya* (Griseb.) Moldenke (Verbenaceae) were studied in female Sprague–Dawley rats. The extract was administered intraperitoneally and its effects on spontaneous motor activity (total motility, locomotion, rearing and grooming behavior) were monitored. Anxiolytic-like properties were studied in the elevated plus-maze (EPM) test and the possible antidepressant-like actions were evaluated in the forced swimming test (FST). The results revealed that high doses of the extract (25 and 50 mg/kg, i.p.) caused a significant decrease in total motility, locomotion, rearing and grooming behavior. All doses injected (from 1.56 to 50 mg/kg) increased the exploration of the EPM open arms in a similar way to that of diazepam (1 mg/kg, i.p.). In the FST, the extract (12.5, 25 and 50 mg/kg) was as effective as fluoxetine (10 mg/kg, i.p.) and imipramine (12.5 mg/kg, i.p.) in reducing immobility, along with a significant increase in swimming and climbing, respectively. These results suggest that some of the components of the hydroalcoholic extract of *A. polystachya*, such as thujone and carvone among others, may have sedative, anxiolytic and antidepressant-like properties which deserve further investigation.

*Keywords:* *Aloysia polystachya*; Sedative; Anxiolytic; Antidepressant; Forced swimming; Elevated plus-maze

## 1. Introduction

Anxiety and depressive disorders are the most frequent psychiatric conditions. More than 20% of the adult population suffer from these conditions at some time during their life. For many years, anxiety and depression were considered as two different mental diseases, with the benzodiazepines used as the drugs of choice for acute anxiety states and the amine uptake inhibitors and monoamine oxidase inhibitors to treat depression. Meanwhile the clinicians seem to have become less sure of the original mutually exclusive classification of the two diseases, with the margins between anxiety and depression becoming blurred (Elliott et al., 1992). In the treatment of anxiety disorders the benzodiazepines are now slowly replaced by antidepressants, which are not only efficacious in depression but also in the acute and long-term treatment of major anxiety disorders (Buller and Legrand, 2001). The clinical indications for many of the new compounds include both anxiety and depression. However, because of the limited efficacy of current drugs, the need for newer, better-tolerated and more efficacious treatments remains high. Herbal therapies could be considered as alternative/complementary medicines. The search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly in the past decade (Zhang, 2004). This is reflected in the large number of herbal medicines whose psychotherapeutic potential has been assessed in a variety of animal models. These studies have provided useful information for the development of new pharmacotherapies from medicinal plants for use in clinical psychiatry.

*Aloysia polystachya* (Griseb.), Moldenke, is an aromatic native plant of the Verbenaceae family which is widely distributed in subtropical regions of South America, mainly Paraguay and the north of Argentina. This species is popularly

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known as “burrito”, “poleo de castilla” or “poleo riojano”. The decoction of leaves and flowers from this plant is widely used in folk medicine to treat gastrointestinal disorders, such as pain, nausea, vomiting, dyspepsia and gastritis (Filipov, 1994; Martínez Crovetto, 1981). In addition, there are reports about the popular use of this plant as sedative or “tranquilliser” (González Torres, 1996). Nevertheless there is no scientific evidence about potential effects of *A. polystachya* in animal models of psychiatric diseases. The main components extracted from the essential oil of *A. polystachya* leaves are the monoterpenes carvone and α-thujone (Cabanillas et al., 2003; Fester et al., 1956; Gatto et al., 1981; Huergo and Retamar, 1973) which could take account of its digestive and central nervous system actions, respectively.

The present study was undertaken to investigate whether the administration of hydroalcoholic extract from the leaves of *Aloysia polystachya* produces behavioral modifications in rats. We evaluate spontaneous motor responses and the anxiolytic- and antidepressive-like effects were assessed in the elevated plus-maze test and in the forced swimming test, respectively.

## 2. Material and methods

### 2.1. Plant material and preparation of the extract

With the support of professional collectors, aerial parts of *A. polystachya* were collected from the Botanical Garden for Medicinal Plants, Faculty of Chemical Sciences, San Lorenzo, Paraguay. A voucher sample was deposited in the Department of Botany under the code Ortiz 1498. Fresh samples were air-dried and ground, yielding 1474 g of powder. The powder was extracted with a mixture of ethanol : water (60 : 40) by a conventional reflux method for 1 h at 50 °C in a bathing apparatus. The extraction was repeated two times and the filtered hydro-ethanolic extracts were mixed and evaporated under reduced pressure. The concentrated extract was frozen and finally freeze-dried to yield 208.7 g of lyophilized powder extract (14.48%) and used in all biological studies.

### 2.2. Animals

Female Sprague–Dawley rats (200–250 g), from the breeding stock, were housed in groups of 6–8 per cage, for a minimum of 5 days prior to the pharmacological experiments, with free access to standard rodent pellet diet and tap water, and maintained on a 12/12 h light–dark cycle. Each experimental group consisted of at least 10 animals. The room temperature was 22 ± 1 °C. In order to avoid the influence of ovarian hormones fluctuations across the estrous cycle, only female rats during the stage of diestrus were used in the experiments. Vaginal smears were taken daily to determine the different stages of the estrous cycle. Only females exhibiting three or more consistent 4-day cycles were utilized. Behavioral observations took place in soundproof rooms at the same period of the day to reduce the confounding influence of diurnal variation in spontaneous behavior. Each animal was tested only once.

All experiments were conducted in accordance with international standards of animal welfare recommended by the Society for Neuroscience (Handbook for the Use of Animals in Neuroscience Research, 1997). The experimental protocols were approved by the local Animal Care and Use Committee. The minimum number of animals and duration of observation required to obtain consistent data were employed.

### 2.3. Drugs

Diazepam (F. Hoffmann-La Roche, Basel, Switzerland) was used as reference drug (positive control) for anxiolytic and sedative activities. Fluoxetine (Ely-Lilly Co., Indianapolis, USA) and imipramine (Novartis Chile S.A.) were used as standard drugs for antidepressant effect.

### 2.4. Treatment

The extract of *A. polystachya* was freshly dissolved in distilled water to be acutely administered intraperitoneally (i.p.) in rats. Doses of the extract and the time intervals were determined in preliminary tests. Diazepam (1 mg/kg) was dissolved in 40% propylene glycol. Both fluoxetine (10 mg/kg) and imipramine (12.5 mg/kg) were dissolved in distilled water. All administrations were performed in a dose volume of 1 ml/kg body.

### 2.5. Behavioral evaluation

#### 2.5.1. Spontaneous motor activity

Thirty minutes after the treatment with the extract (1.56, 6.25, 12.5, 25 and 50 mg/kg, i.p.) or the solvent, rats were individually placed in a Plexiglas cage (30 × 30 × 30 cm), located inside a soundproof chamber. The floor of the cage was an activity platform connected to an amplifier and an electromechanical counter to monitor total motility (Lafayette Instrument Co, USA). Locomotor activity was also recorded with an infrared photocell activity monitor (Columbus Instruments, USA), provided with one array of 15 infrared photocells spaced 1 in. (2.54 cm) apart. Total motility and locomotor activity were monitored every 5 min during a 30-min period and, simultaneously, the number of times each animal reared and the time (in seconds) spent in grooming behavior were recorded. Each animal was observed continuously via a video camera connected to a VHS tape-recorder.

#### 2.5.2. Elevated plus-maze test (EPM)

This test has been widely validated to measure anxiety in rodents (Pellow et al., 1985). Briefly, for rats, the apparatus consisted of two open arms (50 × 10 cm each), two enclosed arms (50 × 10 × 20 cm each) and a central platform (10 × 10 cm), arranged in such a way that the two arms of each type were opposite to each other. The maze was elevated 100 cm above the floor. The maze floor was constructed from plywood. Sixty minutes after the i.p. injection of the extract (1.56, 6.25, 12.5, 25 and 50 mg/kg) or diazepam (1 mg/kg) or the solvent,

namely immediately after spontaneous motor activity recording, each animal was placed at the center of the maze facing one of the enclosed arms. During the 5-min test period, the number of open and enclosed arms entries, plus the time spent in open and enclosed arms, was recorded (Pellow and File, 1986). Entry into an arm was defined as the point when the animal places all four paws onto the arm. Animal behavior was taped by using a video camera located above the maze. After the test, the maze was carefully cleaned with a wet tissue paper (10% ethanol solution).

### 2.5.3. Forced swimming test (FST)

The FST was originally described by Porsolt et al. (1977) and now is the most widely used pharmacological model for assessing antidepressant activity (Cryan et al., 2002a). The development of immobility when the rodents are placed in an inescapable cylinder of water reflects the cessation of persistent escape-directed behavior (Lucki, 1997). The apparatus consisted of a transparent Plexiglas cylinder (50 cm high × 20 cm wide) filled to a 30 cm depth with water at room temperature. In the pre-test, rats were placed in the cylinder for 15 min, 24 h prior to the 5-min swimming test. *A. polystachya* extract (6.25, 12.5, 25, 50 mg/kg), fluoxetine (10 mg/kg), imipramine (12.5 mg/kg) or distilled water was administered i.p. three times: immediately after the initial 15-min pre-test, 6 and 0.5 h prior to the swimming test. During the 5-min swimming test, the following behavioral responses were recorded by a trained observer: *climbing behavior* (or trashing), which is defined as upward-directed movements of the forepaws along the side of the swim chamber; *swimming behavior*, defined as movement throughout the swim chamber, which included crossing into another quadrant; and *immobility* was considered when the rat made no further attempts to escape except the movements necessary to keep its head above the water. Increases in active responses, such as climbing or swimming, and reduction in immobility, are considered as behavioral profiles consistent with an antidepressant-like action (Cryan et al., 2002a).

### 2.6. Statistical analysis

Data were analyzed by Prism Graph Pad software and presented as mean ± SEM values. The statistical tests used were one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison test. A probability level of 0.05 or less was accepted as significant.

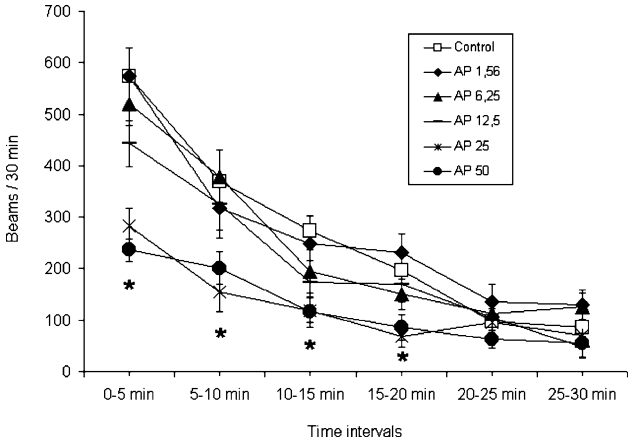


Fig. 1. The effects of *Aloysia polystachya* hydroalcoholic extract (AP-mg/kg i.p.) on locomotor activity in rats. Data represent mean ± SEM of locomotion during each 5-min interval. Animals were 26 in the solvent group and 11–12 in the treated groups. Comparisons were made by using a one-way ANOVA on each time interval followed by post hoc Dunnett's Multiple Comparison test: *$p < 0.05$ compared AP 25 and 50 mg/kg with control group.

## 3. Results

### 3.1. Spontaneous motor activity

The overall effects of the i.p. administration of the extract of *A. polystachya* on rat spontaneous motor activity, during the 30-min period, are summarized in Table 1. One-way ANOVA revealed significant effects of the treatment on total activity, $F(5,78) = 18.40$, $p < 0.0001$; locomotor activity, $F(5,78) = 8.54$, $p < 0.0001$; rearing behavior, $F(5,78) = 22.96$, $p < 0.0001$; and grooming behavior, $F(5,78) = 4.54$, $p < 0.005$. Subsequent Dunnett's Multiple Comparison test demonstrated that the extract induced a significant and dose-dependent decrease in total motor activity, locomotor activity and rearing behavior. Grooming behavior was also decreased, but a dose–response relationship was not observed. In order to verify, data from locomotor activity are also presented in 5-min intervals in Fig. 1. The depressant effects of 25 and 50 mg/kg of the extract were significant from the first until the fourth interval, but they were not different to the controls in the last two intervals.

Table 1
Effect of *Aloysia polystachya* hydroalcoholic extract on spontaneous motor activity in rats[a]

| Dose (mg/kg i.p.) | *n* | Total motility (counts) | Locomotor activity (counts) | Rearing (number) | Grooming (seconds) |
|---|---|---|---|---|---|
| Control | 26 | 1232.0 ± 85.9 | 1597.8 ± 100.5 | 57.7 ± 3.0 | 309.5 ± 25.9 |
| 1.56 | 11 | 1478.6 ± 124.4 | 1638.5 ± 167.7 | 61.7 ± 5.4 | 369.8 ± 54.3 |
| 6.25 | 12 | 1120.5 ± 140.4 | 1483.8 ± 137.4 | 51.2 ± 6.1 | 284.9 ± 55.9 |
| 12.5 | 12 | 534.5 ± 70.2* | 1353.9 ± 197.4 | 33.1 ± 3.6* | 227.0 ± 36.8 |
| 25.0 | 12 | 358.8 ± 53.4* | 793.3 ± 79.4* | 18.4 ± 3.7* | 150.8 ± 18.7* |
| 50.0 | 11 | 468.2 ± 102.3* | 761.0 ± 71.0* | 15.4 ± 2.5* | 183.8 ± 28.2* |

[a]Data are means ± SEM; *n* = number of rat per group.
* $p < 0.05$ compared with control animals (ANOVA followed by Dunnett's Multiple Comparison test).

### 3.2. Elevated plus-maze

The ANOVA revealed significant effects of the *A. polystachya* extract treatment on the percentage of entries into the open arms, $F(6,89)=3.93$, $p<0.01$]; and on the percentage of time spent in the open arms $F(6,89)=4.20$, $p<0.001$, of the elevated plus-maze (Fig. 2). Dunnett's post hoc analysis showed a significant increase of the percentage of entries after the doses of 1.56, 6.25, 12.5 and 50 mg/kg. This effect was accompanied by a significant increase in the percentage of time spent in the open arms of the maze after 1.56, 12.5 and 50 mg/kg. The effects on the open arms exploration elicited by the extract were not significantly different from those observed after diazepam administration (1 mg/kg; i.p.).

### 3.3. Forced swimming test

The effects of the *A. polystachya* extract, fluoxetine and imipramine on active behaviors in the FST of rats are shown in Fig. 3. The ANOVA revealed significant effects of treatment on immobility, $F(6,88)=7.47$, $p<0.0001$; swimming behavior, $F(6,88)=2.83$, $p<0.05$; and climbing behavior, $F(6,88)=2.91$, $p<0.05$. Post hoc analysis demonstrated that *A. polystachya* 12.5, 25 and 50 mg/kg significantly shortened the immobility time in comparison to control values. This effect was accompanied by significant increases in climbing behavior and swimming behavior, after 25 and 50 mg/kg. Both fluoxetine and imipramine significantly decreased the immobility time during the 5-min test session while inducing corresponding increases in swimming and climbing behaviors, respectively. There was no significant difference between the effects of *A. polystachya* 12.5 mg/kg and those observed after fluoxetine and imipramine on the immobility time. Moreover, higher doses of the extract (25 and 50 mg/kg) resulted more effective than fluoxetine and imipramine in reducing this behavior.

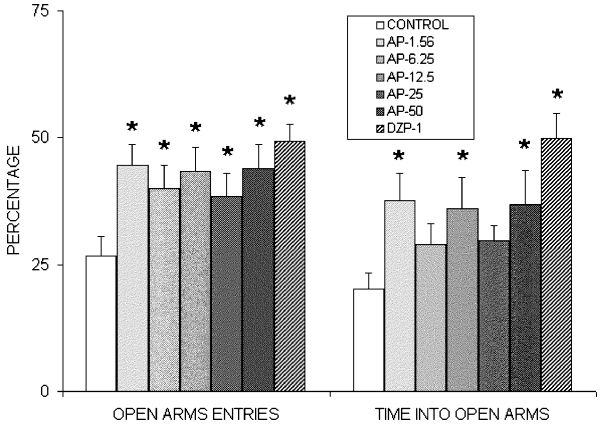


Fig. 2. The effects of *Aloysia polystachya* hydroalcoholic extract (AP-mg/kg i.p.) and diazepam (DZP 1 mg/kg i.p.) on the percentage of entries and the time spent in open arms of the elevated plus-maze during the 5-min test session. Data represent means ± SEM. Animals were 26 in the solvent group and 11–12 in the treated groups. Comparisons were made by using a one-way ANOVA followed by post hoc Dunnett's Multiple Comparison test: *$p<0.05$ compared with control group.

## 4. Discussion

The present study investigated the behavioral effects of the hydroalcoholic extract from the leaves of *A. polystachya*, a plant used in Latin America folk medicine mainly in gastrointestinal disorders. The results demonstrated that the extract was also able to promote a motor depressant effect in rats after the i.p. injection. Thus, given acutely at single doses of 25 and 50 mg/kg, the extract of *A. polystachya* produced significant decreases in total motility, locomotor activity and rearing behavior. In general, these findings indicate a remarkable sedative effect of this plant. In addition, the *A. polystachya* extract demonstrated to provoke antidepressant and anxiolytic-like activities.

The evaluation of the putative anxiolytic activity of *A. polystachya* was performed with the elevated plus-maze (EPM). The primary measures in the EPM are the proportion of entries into the open arms and of the time spent on the open arms. According to Barrett (1991), an anxiolytic effect is suggested when the drug increases open arms entries without altering the total number of arm entries. Treated rats showed a significant increase of both the percentage of entries and the percentage of time spent in the open arms of the maze, similar to the effects observed after the reference anxiolytic drug diazepam. These results could indicate an anxiolytic-like activity to the extract from *A. polystachya* leaves. It is interesting that this activity appears even after low doses, such as 1.56 mg/kg, that failed to reduce spontaneous motility.

The forced swimming test demonstrated that the *A. polystachya* extract acted like an antidepressant drug in the rat. Doses of 12.5 mg/kg and higher were able to reduce immobility time and, simultaneously, to enhance active behaviors, like climbing and swimming. Reduction of immobility was comparable to that observed after the i.p. administration of the reference antidepressant drugs fluoxetine and imipramine. In agreement with previous reports (Page et al., 1999), the decrease in immobility induced by fluoxetine was accompanied by an increase in swimming, whereas climbing duration was not affected by this drug. On the other hand, imipramine increased climbing duration without modifying swimming. It has been demonstrated that swimming is sensitive to serotoninergic compounds, such as the selective serotonin reuptake inhibitor fluoxetine, and that climbing is sensitive to tricyclic antidepressants and drugs with selective effects on noradrenergic transmission (Detke et al., 1995; Cryan and Lucki, 2000; Cryan et al., 2002b). Although other kind of studies is obviously necessary to elucidate the mechanism of action of *A. polystachya* in the CNS, the pattern of effects observed in the FST suggests the involvement of both serotoninergic and catecholaminergic neurotransmitter systems on its antidepressant-like effect.

The results obtained after i.p. administration of *A. polystachya* extract in rats demonstrate the high potency of this plant on the CNS. Dosages of the drug seem to be crucial to the type of effect obtained. Although high doses (25 and 50 mg/kg) induced a significant reduction in locomotor activity, they increased both exploration of the EPM and active behaviors in

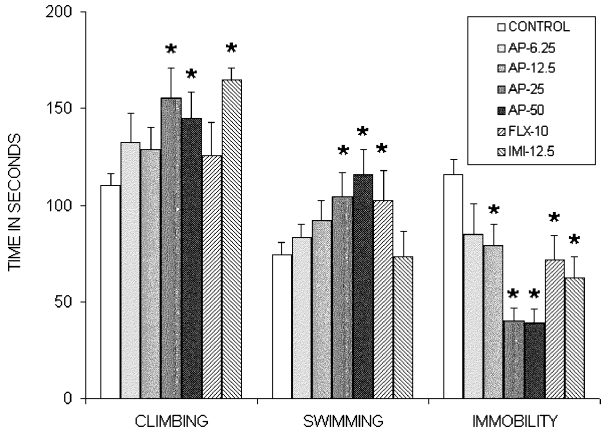


Fig. 3. The effects of *Aloysia polystachya* hydroalcoholic extract (AP-mg/kg i.p.), fluoxetine (FLX 10 mg/kg i.p.) and imipramine (12.5 mg/kg i.p.) on the forced swimming test in rats. Data represent means ± SEM of the duration of climbing, swimming and immobility during the 5-min test session. Animals were 25 in the solvent group and 10–12 in the treated groups Comparisons were made by using a one-way ANOVA followed by post hoc Dunnett's Multiple Comparison test: $*p<0.05$ compared with control group.

the FST. These results seem to be contradictory but they could be explained by the different environmental conditions of the tests applied which, in turn, determine different responses in the animals. Control animals placed into the activity monitor show a high explorative behavior which declines with the time due to habituation. On the other hand, the depressant effects of high doses of the extract on locomotor activity were evident only in the first 20 min but not in the last 10 min of observation. EPM and FST are extremely aversive for the animal, leading it to develop defensive behaviors which can be enhanced by the treatment. In addition, even low doses, such as 1.56 and 6.25 mg/kg, which did not modify total motility and locomotion, showed anxiolytic activity in the EPM but no antidepressant-like effect in the FST.

The active compounds present in *A. polystachya* are unknown until now and we can not discard the possibility that more than one compound are the responsive for its behavioral effects. Chemical studies have reported the presence of several monoterpenoid compounds in the essential oil of *A. polystachya*, primarily α- and β-thujone and carvone, along with limonene, sabinene, β-pinene, carvacrol and eucarvone (Cabanillas et al., 2003; Fester et al., 1956; Gatto et al, 1981; Huergo and Retamar, 1973). α-thujone is reported to have antinociceptive activity in mice (Rice and Wilson, 1976), to be neurotoxic in rats (Millet et al., 1981) and to produce mood elevation and antidepressant effects (Olsen, 2000). Recently, the effects of common monoterpenoid alcohol and ketones were investigated on recombinant human $GABA_A$ receptors (Hall et al., 2004). GABA currents were enhanced by coapplications of carvone enantiomers. By contrast, thujone diastereomers inhibited $GABA_A$ currents. This study reveals neuroactive roles for monoterpenoids as modulators of inhibitory ligand-gated channels. According with this evidence, we could speculate that carvone and thujone could take account, at least in part, for the anxiolytic and antidepressant-like effects of *A. polystachya* described in this study. The effects observed after each dose of the extract may be the consequence of a balance between thujone and carvone effects on CNS, not to mention other herbal ingredients of essential oil of *A. polystachya*.

In spite of the presence of the neurotoxic thujone and the widely non-controlled use of decoctions of *A. polystachya* leaves in popular medicine, we have no evidence about human intoxications. Besides, preliminary studies of acute toxicity in mice indicate that high doses of the extract (up to 3.0 g/kg p.o. and 1.0 g/kg i.p.) do not have lethal effects and are well tolerated.

In conclusion, our results make evident that the hydroalcoholic extract of the leaves of *A. polystachya* possesses sedative–anxiolytic and antidepressant properties in rats. However further studies are necessary to confirm and extend these results. Somehow, the findings presented here are relevant because they validate the folk uses of *A. polystachya*, an important medicinal plant used in South America.

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