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Research Paper

Central nervous system effects of the essential oil of the leaves of *Alpinia zerumbet* in mice

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Abstract

Objectives *Alpinia zerumbet*, known in Brazil as colônia, is popularly used as a diuretic, antihypertensive, anti-ulcerogenic and sedative. Based on this, we have investigated the central effects of the essential oil isolated from *A. zerumbet* leaves.

Methods Mice were treated once with 50 or 100 mg/kg of the essential oil, intraperitoneally, 30 min before being submitted to behavioural models of: locomotor activity (open-field), catalepsy, anxiety (elevated plus maze), depression (forced swimming test and tail suspension tests) as well as apomorphine-induced stereotypy.

Key findings Results showed a dose-related decrease on locomotor activity and apomorphine-induced stereotypy. There was a decrease to the order of 55% of the grooming behaviour with both doses studied. The essential oil 100 mg/kg increased cataleptic activity (167%) and the immobility time in the forced swimming and tail suspension tests. Pretreatment with haloperidol (0.2 mg/kg, i.p.) alone also decreased locomotion, increased cataleptic activity and immobility time in the tail suspension test. No alterations in the elevated plus maze test were registered.

Conclusions The essential oil of *A. zerumbet* leaves had depressant and possible antipsychotic activity, since it could reverse the stereotypy induced by apomorphine, presenting effects comparable with those obtained with haloperidol treatment.

Keywords *Alpinia speciosa*; *Alpinia zerumbet*; apomorphine-induced stereotypy; central nervous system; depressant

Introduction

Alpinia zerumbet (Pers.) B. L. Burtt from the Zingiberaceae family is also cited in the literature as *Alpinia speciosa* K. Schum. This species is known in Brazil by the name colony (colônia) and is used in folk medicine and religious rituals. The major constituents present in its roots, leaves and stems are sesquiterpenoids and diterpenoids.[1]

In Brazil, *A. zerumbet* is used in the northeast and southeast as infusions and decoctions to achieve its diuretic, antihypertensive, anti-ulcerogenic and sedative properties.[2,3]

The pharmacological and toxicological evaluation of the alcoholic extract obtained from the plant leaves showed low toxicity after intraperitoneal administration in rats, with no alterations in parameters such as blood glucose, urea and creatinine after 30 days of administration, neither were there any histopathological alterations of liver, spleen, gut, lung or heart.[4]

Some effects of the essential oil of *A. zerumbet* leaves have been determined in animals, including arterial hypotension, dose-dependent blockage of the compound action potential, antinociceptive effect, probably involving the participation of opiate receptors, myorelaxant and antispasmodic effects.[5–8]

The most cited compounds isolated from this plant are flavonoids, a category of the secondary metabolites that exhibit vasodilative and vaso-strengthening bioactivity, which are effective in the prevention of strokes and hypertension.[9–11]

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Recently, medicinal plants including *Ginkgo biloba*, St John's wort, kava kava, valerian, *Bacopa monniera* and *Convolvulus pluricaulis* have been used widely for their reputed effectiveness in central nervous system (CNS) disorders.[12] The most frequent mental conditions treated using medicinal plants include mood disorders (mainly depression), anxiety disorders, age-related cognitive decline and sometimes psychotic disorders.[13]

Based on the fact that medicinal plants can be important tools for the treatment of some pathologies, including mental disorders, mainly as a result of their low toxicity and ease of access by the population, we decided to study the central effects of the essential oil of *A. zerumbet* leaves, since it is a popularly used sedative with few studies determining its effects on the CNS.

Materials and Methods

Animals

Swiss mice (25–30 g; $n = 6$–11 per group) were used for behavioural tests. The animals were housed in standard environmental conditions (22 ± 1°C, humidity 60 ± 5%, 12-h light : 12-h dark cycle) with free access to a standard commercial diet and with water freely available, following international recommendations.[14] All experiments were performed according to the Guide for the Care and Use of Laboratory Animals, from the US Department of Health and Human Services. The experimental protocol was approved by the Animal Ethics Committee of the Federal University of Ceara.

Plant material

The essential oil was extracted from the leaves of *A. zerumbet*, collected from the Medicinal Plants Garden of the Laboratory of Natural Products of the Federal University of Ceara, Ceara State, Brazil, during December 2008. A voucher specimen of *A. zerumbet* has been deposited at Herbarium Prisco Bezerra (# 10858), as identified by Drs Edson Paula Nunes and Peres Martins. The isolation of the essential oil was carried out at the Department of Organic and Inorganic Chemistry of the Federal University of Ceara, according to the method described by Craveiro *et al*.[15] Briefly, freshly chopped plant leaves were placed in a glass flask, connected at one end to a glass vessel with water and at the other end to a water-cooled condenser. The water was heated to boiling, and the steam percolated through the chopped plant leaves and collected in the condenser. After condensation, the watery phase with its solutes, here called 'aqueous extract' was separated from an oily phase, the essential oil. The composition of the essential oil was determined by gas chromatography and mass spectrometry (Shimadzu QP5050 GCMS gas chromatograph, Shimadzu Corporation, Kyoto, Japan). The identification of the constituents was performed by a computer library search, retention indices and visual interpretation of the mass spectra.[16,17] The presence of the main constituents was confirmed by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy.

Drugs

The essential oil was emulsified with 2% Tween 80 (Sigma-Aldrich Corporation, St Louis, MO, USA) in distilled water. Diazepam (1 mg/kg, União Química, São Paulo, Brazil) and imipramine (10 mg/kg, Geigy Pharmaceuticals, Ardsley, NY, USA) were used as positive standards. Haloperidol (0.2 mg/kg, Sigma Pharma, Hortolândia, São Paulo, Brazil) was used as a standard antipsychotic drug.

Pharmacological evaluation

Experimental protocol

Animals were treated once with the essential oil of *A. zerumbet* 50 or 100 mg/kg (i.p.) 30 min before the experiments. Control animals received vehicle (10 ml/kg 2% solution Tween 80). Mice were tested during the light period and were observed in a closed room at constant temperature (23 ± 1°C), poorly illuminated with a 15-V red light. In the forced swimming test illumination was with a normal light. Thirty minutes after the treatment, the behavioural tests were performed on different days using different groups of animals. The animals treated with haloperidol (0.2 mg/kg) only, used as a standard antipsychotic, were submitted to open-field, catalepsy and tail suspension tests.

Open-field test

The open-field area was made of acrylic (transparent walls and black floor, 30 × 30 × 15 cm) divided into nine squares of equal area. The open field was used to evaluate the exploratory activity of the mouse.[18] Each mouse was placed in the centre of the arena and the number of squares crossed with the four paws (locomotor activity) was recorded for 5 min. The observed parameters were number of squares crossed (locomotor activity), number of groomings (the number of body cleaning with paws, picking of the body and pubis with mouth, and face-washing actions), and rearings (number of times the animal stood on its hind legs or with its forearm against the wall of the observation cage or in the free air). Before introducing each animal, the arena was cleaned with 5% alcohol to eliminate the possible bias due to the odour that could be left by the previous animal.

Catalepsy

The inability of an animal to correct an externally imposed posture (catalepsy time) was measured by placing the animals on a flat horizontal surface with both limbs on a 3 cm high wooden bar.[19] The length of time that the animals stayed on the bar without any voluntary movement was recorded, with a cut-off time of 120 s. In this test haloperidol (0.2 mg/kg) was used as a cataleptic drug.

Evaluation of apomorphine-induced stereotypy

Mice were given an injection of vehicle or the essential oil followed by vehicle (10 ml/kg, i.p.) or apomorphine (20.0 mg/kg, i.p.) 30 min later. Immediately after drug injections, mice were placed three per cage and evaluated for stereotyped behaviour by a blind experimenter as described previously.[20,21] Animals were observed for 1 min at 10-min intervals during 1 h. Stereotyped behavioural response is well-defined with the mouse remaining stationary and exhibiting rapid, repetitive head and forelimb

movements.[22] Behaviours scored were sniffing and climbing. Climbing (i.e. all four paws on the cage, above the floor) and sniffing (uninterrupted sniffing for at least 3 s during these 10 s sampling periods). The score for climbing or sniffing could vary from 0 to 10 for the entire observation period. Mice were injected and tested on a single occasion.

Elevated plus maze test

The elevated plus maze for mice consisted of two perpendicular open arms (30 × 5 cm) and two closed arms (30 × 5 × 25 cm) also in a perpendicular position.[23] The open and closed arms were connected by a central platform (5 × 5 cm). The platform and the lateral walls of the closed arms were made of transparent acrylic. The floor was made of black acrylic. The maze was 45 cm above the floor. Thirty minutes or 1 h after intraperitoneal and oral treatments, respectively, the animal was placed at the centre of the plus maze with its nose in the direction of one of the closed arms, and observed for 5 min, according to the following parameters: number of entries in the open and closed arms, and time of permanence in each of them. The time of permanence measured the time spent by the animal in the open and closed arms. Anxiolytic compounds reduce the animal's natural aversion to the open arms and promote the exploration thereof. On the other hand, the forced or voluntary passages of the animal into the closed arms of the elevated plus maze are associated with hormonal and behavioural changes indicative of increased anxiety. Diazepam (1 mg/kg) was used as a standard anxiolytic drug.

Forced swimming test

The forced swimming test has been used as a model predictive of antidepressant effect.[24,25] Mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm water (depth) at 25 ± 1ºC; the total duration of immobility was recorded for 5 min. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant-like effect.[26] After the initial 2–3 min of vigorous activity the animals showed a period of immobility by floating with minimum movements. Imipramine (10 mg/kg) was used in this test as a standard antidepressant drug.

Tail suspension test

The total duration of immobility induced by tail suspension was measured according to the method described by Steru *et al.*[27] in animals treated with the essential oil or haloperidol (0.2 mg/kg; i.p.). Briefly, mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was chronometered by an observer for 6-min. Each animal was used only once.

Statistical analysis

All results are expressed as mean ± SEM. In the apomorphine-induced stereotypy test, results were expressed as a mean of the six times that the animals were observed during 1 h. Treated groups were compared with the controls and differences were estimated by means of analysis of variance followed by Student–Newman–Keuls *post hoc* test for multiple comparisons. In all comparisons $P < 0.05$ was considered to indicate statistical significance.

Results

Chemical composition of the essential oil of *A. zerumbet* leaves

The constituents of the essential oil were (%): terpinen-4-ol, 25.7; 1,8-cineole, 24.61; γ-terpinene, 14.28; sabinene, 10.99; ρ-cimene, 5.88; α-thujene, 4.49; α-terpinene, 2.89; β-pinene, 2.82; α-pinene, 1.72; terpinolene, 1.5; cariophilene, 1.73; α-terpineol, 1.21; linalool, 1.05, cariophilene oxide, 1.14. Their chemical structures, determined by mass spectrometry, are shown in Figure 1.

Effect of the essential oil of *A. zerumbet* leaves on the locomotor and exploratory activity in the open-field test and cataleptic activity

Acute administration of the essential oil decreased the number of squares crossed in the open field test in a dose-dependent manner, the mice also exhibiting a significant decrease of grooming behaviour of approximately 55% with both doses studied. No alteration was seen in rearing activity. Haloperidol (0.2 mg/kg) also decreased locomotor activity and grooming, but increased rearing as compared with control group. The essential oil only at the high dose (100 mg/kg) increased the cataleptic activity by approximately 167%, as compared with control, an effect comparable with animals treated acutely with haloperidol 0.2 mg/kg (103.7% increase) (Table 1).

Effect of the essential oil of *A. zerumbet* leaves on apomorphine-induced stereotypy

The results obtained showed a significant, dose-related decrease in the apomorphine-induced stereotypy after pretreatment with the essential oil (Figure 2).

Determination of the anxiolytic and antidepressant effects of the essential oil of *A. zerumbet* leaves

The essential oil did not present anxiolytic activity on the elevated plus maze model as can be seen in Table 2. The evaluation of a possible antidepressant effect of the oil at the high dose (100 mg/kg), using the models of the forced swimming test (Table 3) and the tail suspension test (Table 4), showed an increase in the immobility time as compared with control (64% and 40% in relation to forced swimming and tail suspension tests, respectively), which determined a depressant profile of this oil. An increase in the immobility time in the tail suspension test was observed after the administration of haloperidol (0.2 mg/kg) as compared with control animals (Table 4).

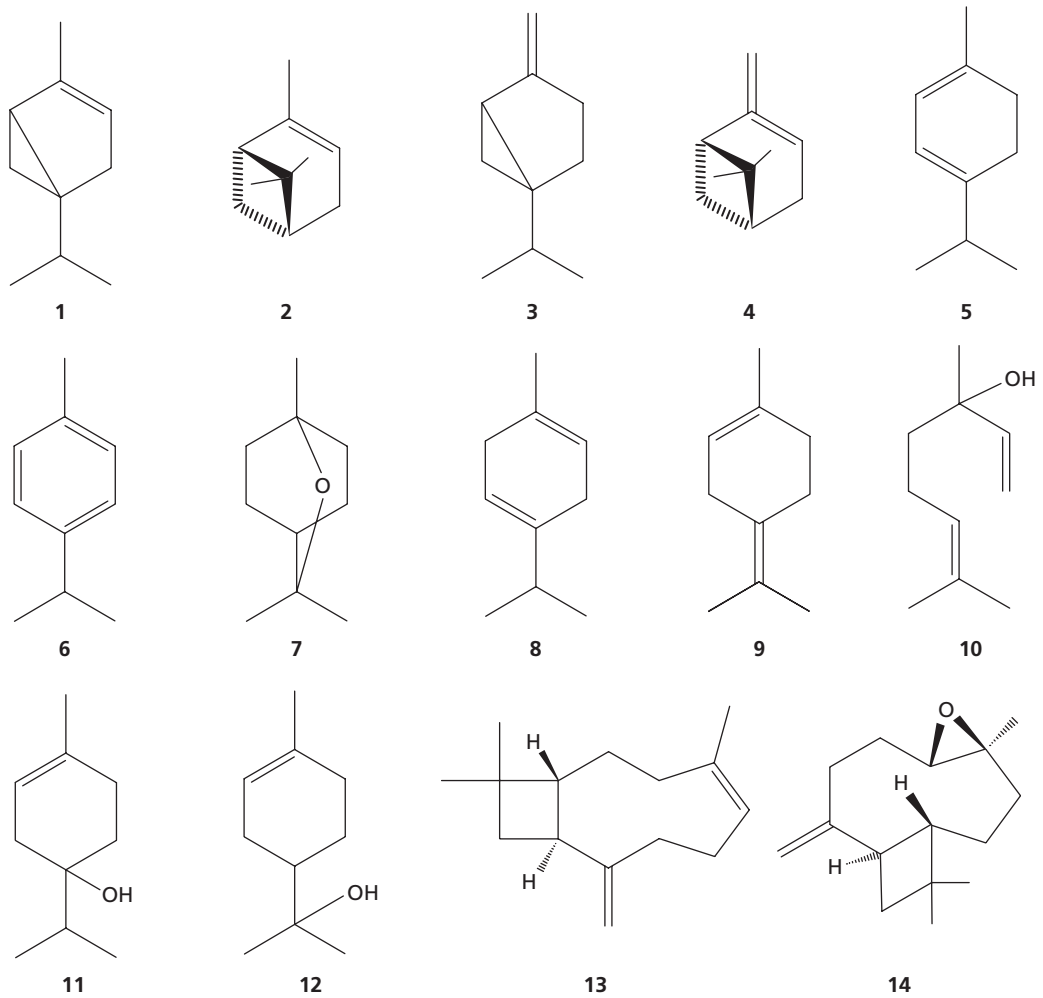

Figure 1 Chemical structure of the constituents of the essential oil of *Alpinia zerumbet* leaves. **1**, α-thujene; **2**, α-pinene; **3**, sabinene; **4**, β-pinene; **5**, α-terpinene; **6**, ρ-cimene; **7**, 1,8-cineole; **8**, γ-terpinene; **9**, terpinolene; **10**, linalool; **11**, terpinen-4-ol; **12**, α-terpineol; **13**, cariophilene; **14**, cariophilene oxide.

Discussion

Behavioural manifestation after essential oil administration indicated that it could significantly alter some parameters associated with motor activity in the open-field test. In this study, the essential oil of *A. zerumbet* leaves reduced locomotion and grooming. From the results obtained, the decrease in the locomotor activity occurred in a dose-related manner. Grooming is a 'maintenance' behaviour that is specifically elicited in situations in which an animal is under stress-induced conflict or frustration. Under this situation, grooming may play a deactivating role in restoring homeostasis.[28] Grooming behaviour in rodents has long been related to dopamine receptors in the brain. Drago *et al*.[29] showed that neuropeptide-induced excessive grooming was reduced in dopamine D_1 receptor-deficient mice compared

Table 1 Effects of essential oil of *Alpinia zerumbet* on the open-field test and catalepsy in mice

Experimental group	Dose (mg/kg)	Number of crossings (5 min)	Rearing frequency (5 min)	Grooming frequency (5 min)	Catalepsy time (s)
Control	–	64.90 ± 5.30	8.13 ± 1.32	5.10 ± 0.84	10.8 ± 2.20
Essential oil	50	39.60 ± 4.50*	5.60 ± 1.06	2.30 ± 0.47*	6.1 ± 1.60
Essential oil	100	24.36 ± 1.73*,†	6.85 ± 1.26	2.20 ± 0.42*	28.83 ± 3.15*
Haloperidol	0.2	46 ± 4.1*	15.8 ± 2.6*	2.4 ± 0.6*	22.00 ± 2.10*

Results are expressed as mean ± SEM of 6–11 animals/group. *P< 0.05 compared with control, †P < 0.05 compared with the essential oil 50 mg/kg (analysis of variance and Student–Newman–Keuls as *post hoc*).

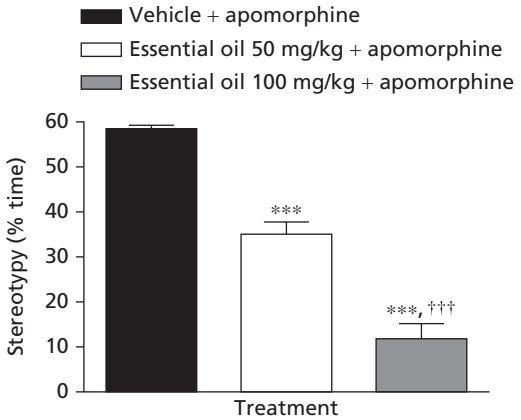

Figure 2 The essential oil of *Alpinia zerumbet* leaves attenuated apomorphine-induced stereotyped behaviour in mice. Animals were treated intraperitoneally (i.p.) with vehicle (2% Tween 80) + apomorphine 20 mg/kg or the essential oil of *A. zerumbet* leaves (50 or 100 mg/kg) followed by an injection of apomorphine (20 mg/kg, i.p.) 30 min later. Results were expressed as mean ± SEM % time showing stereotypy in all observation intervals (1 min at 10 min intervals during 1 h). n = 7–8 animals per group; $^{***}P < 0.001$ compared with vehicle + apomorphine-treated animals, $^{\dagger\dagger\dagger}P < 0.001$ compared with the essential oil 50 mg/kg + apomorphine 20 mg/kg.

with that of their mate, while in contrast dopamine D_2 receptor-null mice showed a normal level of behavioural activation. They were able to establish that D_1-like receptors (D_1 and D_5 receptors) were highly involved in grooming behaviour, but not the dopamine D_2-like (D_2–D_4) receptors. Based on the facts cited above, the significant decrease in grooming behaviour stimulated by the essential oil may have been related to a possible inhibitory effect on dopamine D_1-like receptors.

Table 2 Effect of the essential oil of *Alpinia zerumbet* on the elevated plus maze test in mice

Experimental group	Dose (mg/kg)	*n*	Number of entries in the open arms (5 min)	Time spent in the open arms (s)
Control	–	10	5.38 ± 0.98	119.90 ± 10.20
Essential oil	50	10	4.10 ± 0.48	153.0 ± 18.42
Essential oil	100	10	5.10 ± 0.75	122.90 ± 17.37
Diazepam	1	8	9.60 ± 1.30*	168.0 ± 16.0*

*$P < 0.05$ compared with control. Analysis of variance followed by Student–Newman–Keuls *post hoc*.

Table 3 Effect of the essential oil of *Alpinia zerumbet* on the forced swimming test in mice

Experimental group	Dose (mg/kg)	*n*	Immobility time (s)
Control	–	6	101.3 ± 8.90
Essential oil	50	7	104.30 ± 13.15
Essential oil	100	6	167.0 ± 16.45*
Imipramine	10	8	18.1 ± 2.74*

*$P < 0.05$ compared with control. Analysis of variance followed by Student–Newman–Keuls *post hoc*.

Table 4 Effect of the essential oil of *Alpinia zerumbet* on the tail suspension test in mice

Experimental group	Dose (mg/kg)	*n*	Immobility time (s)
Control	–	11	80.59 ± 8.97
Essential oil	50	9	85.07 ± 7.54
Essential oil	100	8	113.4 ± 10.32*
Haloperidol	0.2	10	197.6 ± 9.98*

*$P < 0.05$ compared with control. Analysis of variance followed by Student–Newman–Keuls *post hoc*.

It has been suggested that some drug-induced hyperlocomotion is mediated by the dopaminergic system especially by D_2-receptors, since the prenatal administration of the selective D_2 receptor antagonist, sulpiride, produced long-lasting effects on the locomotion of rats.[30] Taken together, our results obtained in the open-field test suggested an involvement of dopaminergic receptors in the actions of the essential oil of *A. zerumbet* leaves. This led us to investigate the effects of the oil on the apomorphine-induced stereotypy behaviour, since this behaviour was dependent on the activation of dopamine receptors.[31,32] The essential oil was able to reverse in a dose-dependent manner the climbing and sniffing induced by apomorphine administration, a fact that strongly indicated the participation of dopamine receptors in the mechanism of action for this oil.

As catalepsy is a side effect related to D_2/D_3 receptor occupancy, this behaviour was evaluated in this study. The results showed that the higher dose of the essential oil of *A. zerumbet* leaves (100 mg/kg) was able to increase this activity. The same happened to haloperidol, a typical antipsychotic (a D_2-receptor antagonist) known to induce catalepsy in animals and humans.[33]

To elucidate the behavioural effects of the essential oil of *A. zerumbet* leaves, models of anxiety (elevated plus maze) and depression (forced swimming and tail suspension tests) were used. The elevated plus maze is the most popular test to search for new benzodiazepine-like anxiolytic agents.[34] In this study, the essential oil did not alter the performance of mice in the elevated plus maze test, suggesting that it did not seem to have anxiolytic/anxiogenic activity at the doses tested. Diazepam, as expected, reduced the animal's natural aversion to the open arms of the maze, and promoted the exploration thereof.

On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity access stress-precipitated behaviour. The forced swimming and tail suspension tests are the most widely used animal models for antidepressant screening. It has been well demonstrated that drugs with antidepressant activity reduce the time during which the animals remain immobile.[35] In our results, a significant increase in the immobility time for mice treated with 100 mg/kg essential oil was observed in both tests. In this way, the overall results seem to predict depressant properties of this oil. Based on the fact that neuroleptics increased immobility in an automated version of the tail

suspension test, it has been suggested that this test is not only sensitive to antidepressants but could also be useful for generating activity profiles for different kinds of psychotropic agents.[36] Thus we observed the behaviour of mice treated with haloperidol and compared the results with the results from those treated with the essential oil of *A. zerumbet* leaves. The results we obtained with haloperidol confirmed those obtained by Steru *et al.*[36]

The two major constituents of the essential oil, terpinen-4-ol (200 mg/kg, p.o.) and 1,8-cineole (100, 200 and 400 mg/kg, p.o.) caused a significant decrease in the spontaneous motor activity of mice and potentiated the pentobarbital sleeping time.[37,38] Therefore, both compounds had a plausible depressant effect on the CNS, as did the essential oil of *A. zerumbet* leaves.

Conclusions

The essential oil of *A. zerumbet* leaves reduced locomotor activity and grooming with both doses studied. It increased the time spent on the bar (cataleptic activity) and immobility in the forced swimming and tail suspension tests only at the high dose (100 mg/kg). This indicated a depressant activity of this oil, compared with results obtained with haloperidol, suggesting a possible antipsychotic activity for this substance (probably through a blockage of dopaminergic receptors), although further studies are needed to elucidate its mechanism of action. This is important if the essential oil of *A. zerumbet* leaves is to be exploited, since it has been reported that the administration of an extract of *Ginkgo biloba* may have enhanced the efficiency of the classic antipsychotic haloperidol in patients with schizophrenia, especially on their positive symptoms and chronic refractory schizophrenia,[39] and this may be a possible outcome also for *Alpinia zerumbet.*

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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