

**Title: Sedative effects of the essential oil from the leaves of *Lantana camara* occurring in the Republic of Benin via inhalation in mice**

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## **Abstract**

*Lantana camara* Linn. (Verbenaceae) is used traditionally for its numerous medicinal properties such as antimalarial, antibacterial, anticancer and anti-inflammatory. In the present study, we investigated the chemical composition of essential oil from the leaves of *L. camara* (LCEO) occurring in the Republic of Benin (West Africa) in comparison with LCEOs from other regions; evaluated its sedative effects in mice via inhalation administration; and identified the compounds responsible for activity. LCEO was extracted by hydrodistillation and chemical analyses of the oil were performed by GC and GC/MS. The oil was dominated by monoterpene hydrocarbons (60.58%) and oxygenated monoterpenes (33.39%), among which sabinene (38.81%) and 1,8-cineole (28.90%) were the most abundant. LCEO administered via inhalation to mice significantly decreased locomotor activity in a dose-dependent manner, mainly at the doses of 0.0004 and 0.04 mg per 400  $\mu$ L of triethyl citrate (TEC). The oil was fractionated to give two fractions, which were further investigated, and revealed that both sabinene and 1,8-cineole were the principal active compounds. The results of the present study indicated that via inhalation administration, LCEO and its main constituents could be considered as promising candidates for the management of dementia, insomnia, attention deficit hyperactivity disorder and other central nervous system-associated diseases.

**Keywords:** *Lantana camara* essential oil, vapor inhalation, GC/MS, sedative effect, sabinene, 1,8-cineole

## Introduction

*Lantana camara* Linn (Verbenaceae), the most widespread species of the *Lantana* genus, is an evergreen climbing aromatic shrub that can grow up to 2–4 m in height. The flowers are small, tubular and are various colors, from red to pink, white, yellow, and violet. The stems are quadrangular, and generally armed with hooked prickles. The fruits are green and turn black when ripe [1]. *L. camara* is native to tropical and sub-tropical regions of America and has now been introduced as an ornamental plant in various parts of the world. The main chemotypes reported in the literature are sesquiterpene hydrocarbons, namely  $\beta$ -caryophyllene, germacrene D, and bicyclgermacrene; monoterpenes are represented by limonene, sabinene, and  $\beta$ -phellandrene; and the oxygenated compounds are mainly davanone, 1,8-cineole, caryophyllene oxide, and (*E*)-nerolidol [2, 3].

*L. camara* is used in many parts of the world to treat a number of disorders. In Tanzania, it is used to treat malaria [4], whereas in India, essential oil from the leaves is used for its antiseptic and antifungal properties [5]. In the Republic of Benin, leaves are used in traditional medicine to treat skin diseases [6], and essential oil from the leaves of *L. camara* (LCEO) is claimed to demonstrate sedative effects via inhalation administration. Several aromatic natural medicines have been used as incense and perfume for their relaxing or energizing effects [7, 8], and excellent results were obtained using essential oils in young patients suffering from attention deficit hyperactivity disorder (ADHD) [9]. Also, inhalation administration of the vapor of herbal drug oil has caused significant decrease in mice locomotor activity and this supports the argument for their traditional usage as relaxing and possible anxiolytic or antidepressant agents via inhalation administration [10–14]. To our best knowledge, only a few data are available

concerning the chemical composition of LCEO occurring in Benin and its sedative effects remain not yet studied.

Therefore, in the present paper, the chemical composition of LCEO occurring in Benin was investigated as well as its sedative effects in mice via inhalation administration, and the components responsible for the sedative activity were identified.

## **Material and methods**

### **Materials**

Fresh leaves of *L. camara* (Supplementary data, Figure S1) were collected in October 2016 from Houedo in the Republic of Benin (West Africa) and air dried. GPS coordinates of the collection site were latitude 6°33'37.133" and longitude 2°22'26.567". Identification was confirmed by Gaudence Julien Djego of the Laboratory of Botany and Applied Ecology, University of Abomey-Calavi, and vouchers were deposited in the Herbarium of Experimental Station for Medicinal Plants, Graduate School of Pharmaceutical Sciences, Kyoto University, Japan (specimen number EST-5024) and the National Herbarium of Benin (specimen number AA 6677/HNB). Benzylacetone (purity: > 95%, Tokyo Kasei, Japan), a well-known sedative agent [10, 13, 14], was used as a positive control. Triethyl citrate (TEC; Merck, Darmstadt, Germany), a non-sedating odorless solvent, was used to dissolve the fragrant components. Sabinene (purity: ≥ 97%) was purchased from Chem Cruz Chemicals, Santa Cruz Biotechnologies (Dallas, USA). 1,8-Cineole (purity: > 85%) was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). All chemicals used were of the highest grade available.

### **Distillation of fresh leaves of *L. camara* and fractionation of their oil**

LCEO was extracted by hydrodistillation of *L. camara* leaves (56.60 g) for 2 h using a Clevenger apparatus as recommended in the Japanese Pharmacopoeia (JP17; <http://jpdbs.nihs.go.jp/jp17e/>). The oil was captured in hexane, dried over anhydrous sodium sulfate, and concentrated. The headspace of the oil was then analyzed by Solid Phase Micro Extraction (SPME)-GC/MS to confirm that the oil was free of hexane. The obtained oil was stored in sealed vials at 4 °C until analysis. LCEO was subjected to silica gel column chromatography for fractionation (column diameter: 16 mm; column height: 170 mm), and the column was eluted with hexane/acetone (4:1, about 250 mL) to give fractions 1 and 2. Both fractions were evaporated and subjected to GC/MS analysis to confirm that no remaining solvent was present before analysis of biological activity.

#### **GC and GC/MS analysis**

Qualitative analysis of LCEO was carried out using an Agilent 6850 series gas chromatograph connected to an MSD 5975 system (Agilent Technologies). The following operating conditions were employed: column, fused silica capillary column, DB-wax (HP), 60 m × 0.25 mm × 0.25 µm; column temperature, 60–200 °C, increasing at a rate of 2 °C/min, holding at 65 °C for 5 min, increasing at 0.5 °C/min until 77 °C then at 20 °C/min, and holding at 200 °C for 5 min. Injector temperature, 160 °C; carrier gas, helium, 25 cm/s; column head pressure, 100 kPa; ionization energy, 70 eV; injection volume, 1.0 µL; MS interface temperature, 150 °C/min; MS mode, electron impact (EI); detector voltage, 0.4 kV; mass range, 35–300 u; scan speed, 300 u/s.

Quantitative analysis was carried out on a gas chromatography (GC) system (G-5000, Hitachi) equipped with a flame ionization detector (FID) as follows: column, fused silica capillary column, TC-wax (HP), 60 m × 0.25 mm × 0.25 µm; column temperature,

as for GC/MS. Injector, 200 °C; detector, FID, 220 °C; carrier gas, helium, 0.8 mL/minute; split ratio, 100:1; column head pressure, 200 kPa; injection volume, 1 µL. The linear retention indices of each constituent were determined using n-alkanes as standards. Chemical compounds were identified by comparing their retention indices (RI) and mass spectra (MS) using NIST Special Database 2 (<https://www.nist.gov/srd/nist-special-database-2>) or by comparison with authentic samples.

### **Animals**

Four-week-old male ddY mice (20–30 g) were purchased from Japan SLC (Shizuoka, Japan). Animals were housed in colony cages under a 12 h/12 h light/dark cycle at 25 ± 2 °C and a relative humidity of 50–60%. They were fed pellet chow and water ad libitum and allowed to accommodate to these conditions for 1 week before experiments. Animal experiments were conducted following the recommendations of the Animal Research Committee of Kyoto University, Kyoto, Japan (approval number, 2014-14-3). Experimental procedures involving animals and their care were conducted in accordance with the institutional guidelines and in compliance with the Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology, Japan (2006). All experiments were conducted between 10:00 and 17:00 under identical conditions.

### **Open-field test**

The sedative effects of LCEO on mice were evaluated using an open-field test, as previously described [10]. The open-field arena is represented in Supplemental data,

Figure S2. Administered doses were expressed as milligrams of sample per 400  $\mu$ L of TEC, following previous experiments [11, 14–16]. Four pieces of filter paper were placed in the four corners of the inner walls of the glass cage (60 cm wide, 30 cm long, 34 cm high) using adhesive tape. A sample was deposited on each piece of filter paper and the cage was closed so that the vapor pervaded by natural diffusion. Sixty minutes after charging the sample, a mouse was placed in the center of the cage and subjected to video surveillance for another 60 min. During monitoring, the frequency each mouse crossed lines drawn at 10 cm intervals on the floor of the cage was counted every 5 min. The Area under the curve (AUC) of locomotor activity counts per 5 min (y-axis) and time (x-axis), representing total locomotor activity, was calculated by trapezoidal rule.

#### **Amount of compound evaporated in the cage before inserting the mice**

The amount of compound evaporated in the glass cage was measured using methodology previously described [8, 17]. Each compound was dissolved in TEC and dropped onto a filter paper. The filter paper was weighed and placed in a closed glass cage for 60 min, after what it was removed and weighed again. Weight difference from immediately after dropping the sample and after removal of the filter paper was regarded as the amount of compound evaporated per hour in the glass cage.

#### **Statistical analysis**

All values are expressed as the mean  $\pm$  standard error of mean (SEM). Statistical analyses were performed using Student's t-test or one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using GraphPad InStat software

(GraphPad Software, San Diego, CA, USA). A probability level of  $p < 0.05$  was considered to be statistically significant.

## **Results and Discussion**

### **GC and GC-MS analyses of LCEO**

The results of the GC and GC/MS analyses of LCEO are presented in Table 1 according to their retention indices on a DB-wax column and are compared in their ratio with LCEOs from other regions. In the present study, a total of 13 compounds were identified, with the main components being represented by monoterpene hydrocarbons (60.58%) and oxygenated monoterpenes (33.39%); sabinene and 1,8-cineole (38.81% and 28.90% respectively) were the main ones (Figure 1). Similar to our study, a previous work conducted in another region of the Republic of Benin (Seme) showed that the main compounds of LCEO were sabinene (21.5%) and 1,8-cineole (23.4%) [18]. LCEO is known to demonstrate an important quantitative and qualitative variability in the chemical contents of its oil. Therefore, we compared the results in our present study with previous studies on LCEOs from different regions.

Our results showed similar qualitative composition with LCEOs from Nigeria (wild), Cameroon, and Madagascar (cultivated), which all contained sabinene, 1,8-cineole,  $\alpha$ -pinene, and  $\beta$ -caryophyllene at various concentrations [19, 20], whereas LCEO from India (wild) showed notable difference with our study and contained no trace of sabinene [21]. The presence or absence of sabinene and 1,8-cineole in LCEO from northern Brazil (wild) depended on the collection site and time of collection [22, 23], whereas neither were found in LCEO from Cuba (cultivated) [24]. Citral was the major compound identified in five varieties of *L. camara* from Egypt (wild) [25]; however, it



was not detected in samples from Nigeria or Benin. More, a high content of sesquiterpene hydrocarbon compounds with mainly  $\beta$ -caryophyllene (13.26%) and  $\alpha$ -curcumene (24.69%) was reported in LCEO from Cameroon [20], while LCEO from China (wild) was represented by  $\alpha$ -humulene (9.3%) and germacrene-D (15.8%) [26]. Monoterpene hydrocarbons are mainly represented by  $\alpha$ -phellandrene (16.4%) and limonene (16.5%) in LCEO from northern Brazil [22], and sabinene (11.4%),  $\alpha$ -pinene (4.1%), and  $\beta$ -pinene (2.8%) in LCEO from Madagascar [27]. Among the oxygenated compounds, caryophyllene oxide (21.75%) in LCEO from Nigeria [28], 1,8-cineole (10.75%) in LCEO from India [21], and davanone (15.9%) in LCEO from Madagascar [20] have mainly been reported.

The chemical composition of parts of plants is reported to be affected by the developmental stage, genetic, geographical, and seasonal factors [3, 29, 30]. Thus, the differences in chemical composition of LCEO in the present study with LCEOs from other regions could be explained by variability in climate, altitude, time of collection and soil, or the method of extraction of the oil.

### **Sedative effects of LCEO administered to mice via inhalation**

To investigate the effects of LCEO on mice locomotor activity, mice were administered via inhalation doses of 0.000004, 0.00004, 0.0004, 0.004, 0.04 or 0.4 mg LCEO dissolved in 400  $\mu$ L of TEC. Benzylacetone, a well-known sedative agent [10, 13, 14], was used as positive control and it significantly decreased mice locomotor activity by 61% ( $p < 0.05$ ) as compared to the control group. This indicated that the experimental model for sedative activity in this study was validated. A decrease in locomotor activity was observed at all doses of LCEO in a dose-dependent manner and AUC values were significantly reduced

at doses of 0.0004, 0.004 and 0.04 mg by 68%, 48% and 67% respectively ( $p < 0.05$ ), when compared to the control (Figure 2a). The sedative effects produced by the doses of 0.0004 mg and 0.04 mg LCEO were the most effective and locomotor activity dropped nearly to zero after 20-30 min of inhalation administration (Figure 2b). Previous studies on essential oils from *Ocimum gratissimum*, *Piper guineense* and *Zingiber zerumbet* have also reported sedative effects in a dose-dependent manner, supporting the argument that, similar to LCEO, there is a great variety of plant essential oil showing sedative activity, and possibly useful for the treatment of central nervous system-related diseases [11, 13, 14].

The LD<sub>50</sub> value for 1,8-cineole, one of the main components of LCEO, was 3849 mg/kg [31]. Regarding sabinene, the other main component of LCEO, there were no adverse effects on nidation, reproduction, fetal development, or maternal survival when 224 mg/kg/day sabinene was administered orally for 6-15 days to pregnant mice and rats [32]. In our study, the highest administered dose, 0.4 mg LCEO, contains 0.15 mg sabinene and 0.11 mg 1,8-cineole, which are much lower than the concentrations required to induce toxicity. More, during all the experiments, abnormalities, such as an increase in urination or defecation, were not noticed.

### **The most effective fraction contained sabinene and 1,8-cineole**

To identify the components responsible for activity, LCEO was fractionated to give two fractions which were further investigated for sedative activity. The main components of fraction 1 (367 mg, yellowish) were sabinene (33.95%), 1,8-cineole (32.95%),  $\beta$ -caryophyllene (15.65%), and  $\alpha$ -pinene (8.75%), while fraction 2 (129 mg, pale yellow) consisted of terpinen-4-ol (61.63%) and (+)-2-bornanone (38.37%). The structures of the

different components are represented in Figure 1. Both fractions were administered individually to the mice by inhalation at doses of 0.000004, 0.00004, 0.0004, 0.004, 0.04 or 0.4 mg per 400  $\mu$ L of TEC. As shown in Supplementary data, Figure S3a, fraction 2 induced a significant decrease in locomotor activity at 0.004 mg ( $p < 0.05$ ). Terpinen-4-ol, one of the major compounds of fraction 2, causes a decrease in spontaneous locomotor activity in mice [33], and its sedative effects are greater than or equal to that of eugenol when administered at the same concentration in silver catfish [34]. The other major component of fraction 2, (+)-2-bornanone, has sedative, anesthetic, and analgesic properties [35, 36]. These two compounds therefore seem to explain the sedative activity of fraction 2.

Fraction 1 caused a significant decrease in mouse locomotor activity at doses of 0.0004, 0.004 and 0.04 mg, with the strongest effect observed at 0.004 mg (Supplementary data, Figure S3b,  $p < 0.05$ ). Fraction 1 appeared more effective than fraction 2, suggesting that it contained the most active components of LCEO. Of the main compounds of fraction 1,  $\alpha$ -pinene is an antidepressant previously found to have sedative activity upon inhalation in mice [37, 38], whereas  $\beta$ -caryophyllene has been reported to possess sedative, local anesthetic, antidepressant, and anxiolytic activity via a CB2 receptor agonist [14, 39].  $\beta$ -Caryophyllene and  $\alpha$ -pinene are present in relatively high amounts in fraction 1 and could therefore be responsible for the sedative effects. However, sabinene and 1,8-cineole comprised a larger portion of fraction 1 and LCEO, and therefore their sedative effects on mice were examined further.

### **Effects of sabinene and 1,8-cineole on locomotor activity in mice**

The weight of the total administered compounds on filter paper was measured before and after the experiment for sabinene and 1,8-cineole. The weight of each compound was found to be reduced by 80-90% after administration for 1h, indicating that a large portion of the administered sample had effectively evaporated in the air before the mice were placed in the glass cage.

Sabinene and 1,8-cineole were administered individually to the mice by inhalation at doses of 0.000004, 0.00004, 0.0004, 0.004, 0.04 or 0.4 mg per 400  $\mu$ L of TEC. Sabinene decreased locomotor activity at all doses, with a significant effect at the doses of 0.0004 and 0.004 mg (Figure 3a,  $p < 0.05$ ). 1,8-Cineole showed a sedative effect similar to that of LCEO (Figure 3b); however, only 0.0004 mg ( $p < 0.05$  when compared to control) demonstrated true sedative activity, because 0.04 mg did not induce a significant effect and the mice displayed excitation behavior such as jumping and rearing. Similar effects have been observed for hexahydroxyzerumbone derivatives [14] and basil essential oil [40].

Sabinene, a natural bicyclic monoterpene, is one of the chemicals that contribute to the flavor of black pepper and is a major constituent of carrot and nutmeg seed oils [41]. It has also been reported to act as an anti-inflammatory agent [42]. Sabinene is a double-bond isomer of thujene, a major constituent of *Hedyosmum brasiliense*, which is also used as a sedative[43]; this suggested a possible sedative activity of sabinene. 1,8-Cineole is a naturally occurring saturated monoterpene found mainly in the essential oils of *Eucalyptus* and has been reported to have smooth muscle relaxant, anti-inflammatory, and antinociceptive effects [44, 45]. Also, the sedative effect of two *Eucalyptus* species was suggested to be due to their high 1,8-cineole content [46]. In the present study, we found evidence that sabinene and 1,8-cineole could be used as a potent sedative agent via

inhalation. A future study should examine the relationship between the structure and activity of each sabinene, 1,8-cineole and their derivative compounds in order to elucidate the structural features with significant sedative activity.

### **Effects of a mixture of sabinene and 1,8-cineole administered to mice via inhalation**

Both of the main compounds of LCEO (sabinene and 1,8-cineole) showed sedative activity, and a mixture (ratio 1:1) of these compounds was tested to understand their role in this activity. A mixture of sabinene and 1,8-cineole was administered to mice via inhalation at doses of 0.00008, 0.0008, 0.008, 0.08 or 0.8 mg per 400  $\mu$ L of TEC, which resulted in a decrease in locomotor activity in a dose-dependent manner. Analysis of AUC values showed a significant decrease at doses of 0.0008, 0.008 and 0.08 mg by 65%, 52% and 63% respectively ( $p < 0.05$ ) as compared to the control group (Figure 4a). Mice in the 0.0008 and 0.08 mg administered groups calmed within 20-30 min (Figure 4b), as it was observed in LCEO. More, comparison of locomotor activity transition of mice treated with the mixture of sabinene and 1,8-cineole with that of mice treated with LCEO showed that their sedative activity described a similar decrease in the time course. This result suggested that both sabinene and 1,8-cineole were the principal components responsible for sedative activity of LCEO. However, it is important to observe that LCEO contained other compounds than sabinene and 1,8-cineole, and these compounds though present only in a small amount might have combined to cause a sedative activity in LCEO. Similarly, some authors have previously reported a synergistic action of various constituents present in essential oils from plant material [13, 47].

The identification of active components is an important step to investigate the effects of plant material. The doses applied to rodents are known to be very different from

the effective doses in humans. However, the findings of the present study indicate that LCEO and its related compounds might be useful for the sedation of individuals with dementia, attention deficit hyperactivity disorder, insomnia, or other psychological disorders [8]. Previous studies on *O. gratissimum*, *P. guineense*, *Microtoena patchoulii*, *Zingiber zerumbet*, and *Heracleum afghanicum* [8, 11, 13–15], as well as the present paper, have shown that a number of volatile components from the essential oils of various plants potently reduce the locomotor activity in mice following inhalation administration. Studies are currently underway to investigate the relationship between the structure and activity of LCEO components, and their possible beneficial actions for the treatment of anxiety and depression. The results of these studies could lead to the use of LCEO as a natural medicine in the management of diseases related to the central nervous system.

## **Conclusion**

A great variability in the chemical composition of LCEOs from different regions was demonstrated in the present paper. We also showed that LCEO collected during the rainy season in Houedo (Republic of Benin), possesses strong sedative activity when administered via inhalation, and the major components of the oil, namely sabinene and 1,8-cineole, were identified as principal active compounds. The reduction of locomotor activity may indicate relaxing, anxiolytic or antidepressant effects. Therefore, further investigations using animal models of anxiety and depression are now required to better understand the activities of LCEO and its related compounds.

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**Conflict of Interest:** The authors declare that they have no conflict of interest.

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**Table 1. Phytochemical constituents of Beninese LCEO and comparison of their ratio in LCEOs of different origins**

Compound <sup>a</sup>	Retention index		Peak area (%)							
	Calculated <sup>b</sup>	Literature <sup>c</sup>	Present study <sup>d</sup>	Ref. 1	Ref. 2	Ref. 3	Ref. 4	Ref. 5	Ref. 6	Ref. 7
$\alpha$ -Pinene	1025	1025	5.38	-	1.90	0.31	-	3.68	-	-
$\beta$ -Pinene	1105	1104	3.74	3.40	2.10	0.56	-	2.59	-	-
<b>Sabinene</b>	1117	1119	<b>38.81</b>	<b>21.50</b>	<b>14.70</b>	0.15	-	<b>9.02</b>	-	-
3-Carene	1146	1147	6.06	-	1.70	-	-	2.26	-	-
Myrcene	1159	1160	3.21	2.10	-	0.02	-	0.63	-	-
Limonene	1199	1198	3.38	-	-	0.11	-	1.62	-	-
<b>1,8-Cineole</b>	1215	1212	<b>28.90</b>	<b>23.40</b>	<b>15.80</b>	0.12	<b>10.75</b>	2.83	-	0.78
(+)-2-Bornanone	1532	1529	2.59	-	-	-	-	-	-	-
(Z)- <i>p</i> -Menth-2-en-1-ol	1566	1565	1.57	0.10	-	-	-	-	-	-
<b><math>\beta</math>-Caryophyllene</b>	1609	1608	4.69	<b>10.90</b>	<b>8.90</b>	<b>13.26</b>	<b>16.37</b>	<b>11.98</b>	4.80	<b>9.90</b>
Terpinen-4-ol	1612	1612	0.33	2.50	1.70	0.27	-	tr	0.10	0.90
$\alpha$ -Humulene	1696	1689	0.13	4.40	-	1.38	<b>8.22</b>	6.17	4.90	-
Bicyclogermacrene	1736	1734	0.21	-	2.80	-	3.65	2.59	-	-
<b>ar-Curcumene</b>		1777	-	-	-	<b>24.69</b>	-	0.78	0.60	-
<b>Caryophyllene oxide</b>		1987	-	-	-	-	2.98	-	-	<b>21.75</b>
<b>(E)-Nerolidol</b>		2031	-	1.30	5.90	0.25	-	2.28	<b>43.40</b>	<b>10.39</b>
<b>Davanone</b>		2040	-	-	-	-	2.88	<b>15.94</b>	-	-
<b>Spathulenol</b>		2133	-	-	3.40	1.48	-	0.68	-	<b>14.95</b>

<sup>a</sup>Order of elution determined using a DB-wax column.

<sup>b</sup>Retention index, calculated against C10–C26 *n*-alkanes on a DB-wax column.

<sup>c</sup>Retention index, taken from the NIST library.

<sup>d</sup>Peak area percentage was determined by calculating the peak area of the FID chromatogram in GC analyses.

Ref. 1: Seme (Rep. of Benin) [18]; Ref. 2: Nigeria [3]; Ref. 3: Cameroon [20]; Ref. 4: India [21]; Ref. 5: Madagascar [20]; Ref. 6: Cuba [24]; Ref. 7: Nigeria [28]; tr: trace; -: not detected.

## Figure Legends

### **Fig. 1 Chemical structures of the major constituents of LCEO and fractions**

Sabinene (1), 1,8-Cineole (2),  $\beta$ -Caryophyllene (3),  $\alpha$ -Pinene (4), Terpinen-4-ol (5), and (+)-2-Bornanone (6)

### **Fig. 2 Total spontaneous motor activity (a) and locomotor activity transition (b) of mice treated with LCEO (0.000004, 0.00004, 0.0004, 0.004, 0.04 or 0.4 mg)**

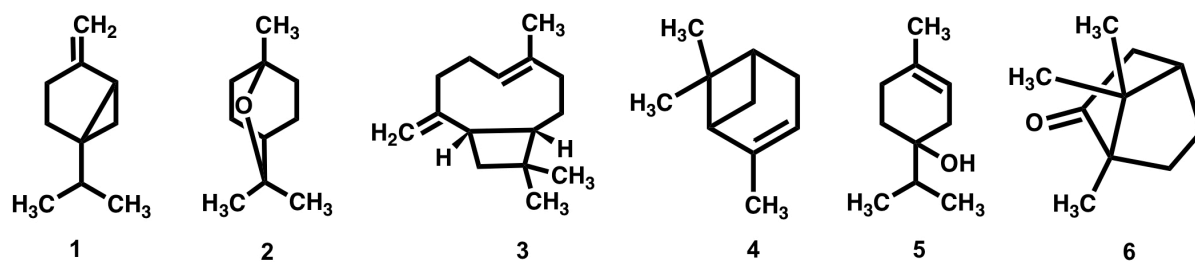
Data represent the mean  $\pm$  SEM of 6 mice. Statistical differences vs. the control group were calculated using ANOVA followed by Dunnett's test. \* $p < 0.05$ .

### **Fig. 3 Total spontaneous motor activity of mice treated with sabinene and 1,8-cineole (0.000004, 0.00004, 0.0004, 0.004, 0.04 or 0.4 mg) (a and b respectively)**

Data represent the mean  $\pm$  SEM of 6 mice. Statistical differences vs. the control group were calculated using ANOVA followed by Dunnett's test. \* $p < 0.05$ .

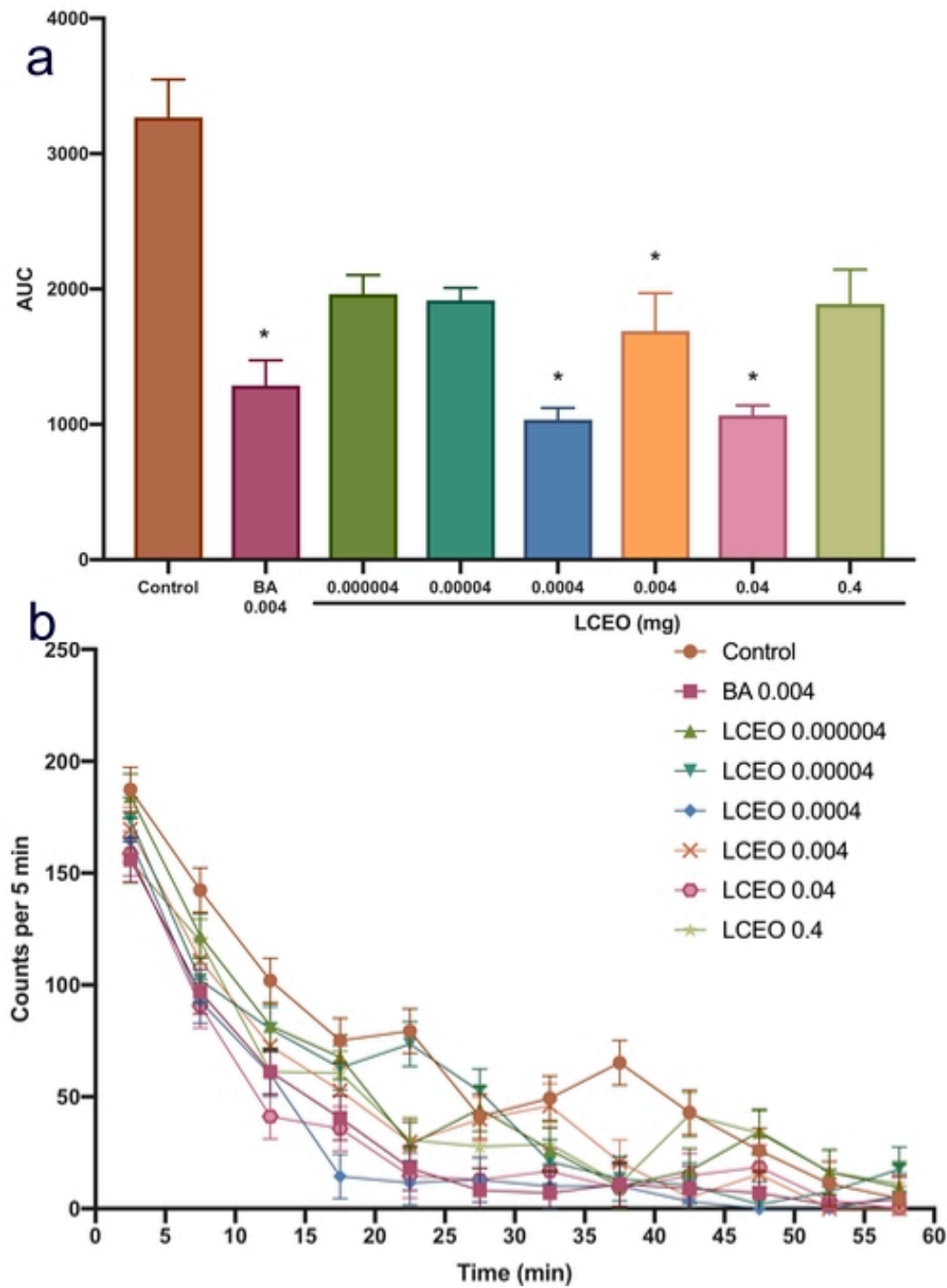
### **Fig. 4 Total spontaneous motor activity (a) and locomotor activity transition (b) of mice treated with a mixture of sabinene and 1,8-cineole (0.00008, 0.0008, 0.008, 0.08 or 0.8 mg)**

Data represent the mean  $\pm$  SEM of 6 mice. Statistical differences vs. the control group were calculated using ANOVA followed by Dunnett's test. \* $p < 0.05$ .



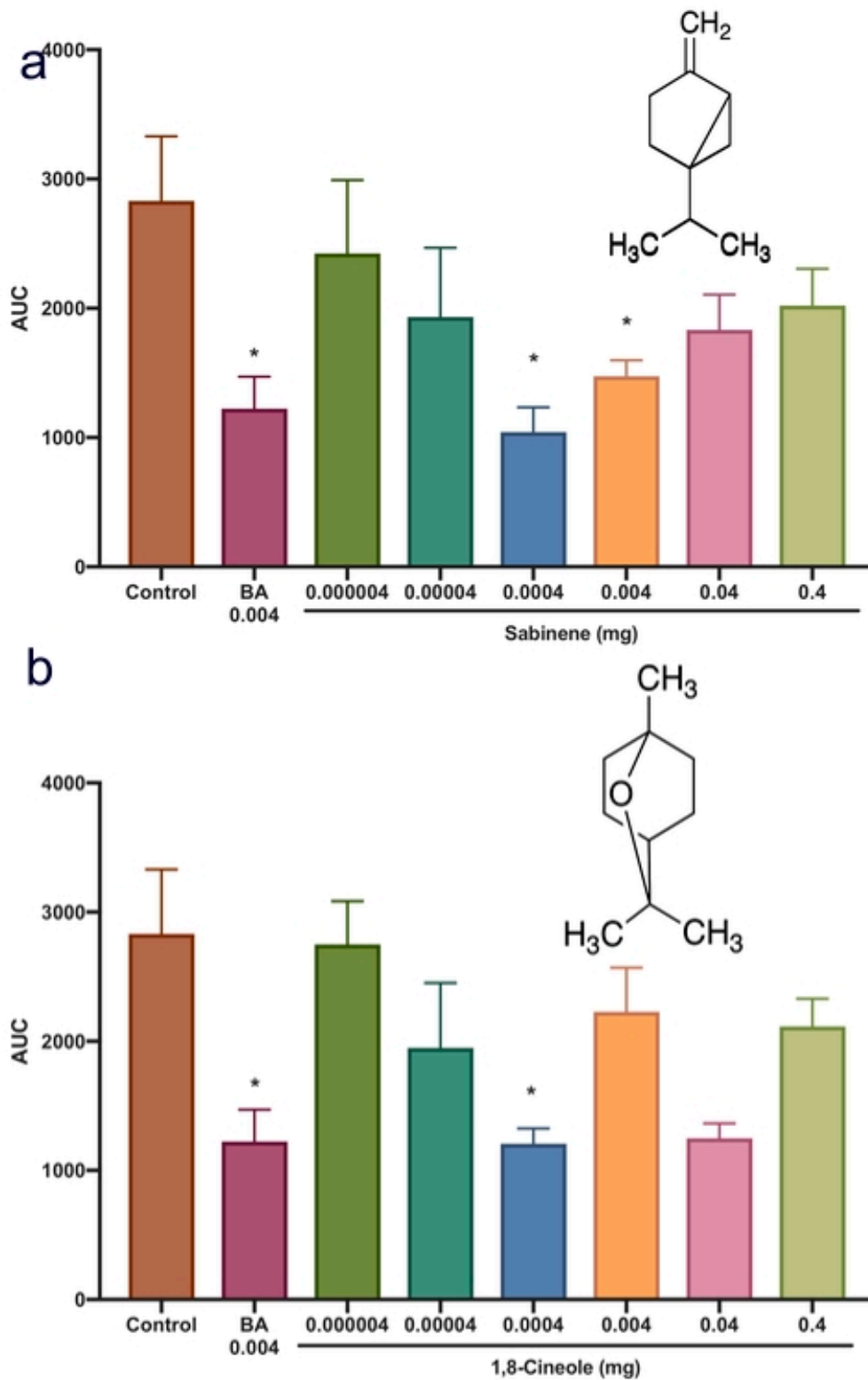
**Fig. 1** Chemical structures of the major constituents of LCEO and fractions

Sabinene (1), 1,8-Cineole (2),  $\beta$ -Caryophyllene (3),  $\alpha$ -Pinene (4), Terpinen-4-ol (5), and (+)-2-Bornanone (6)



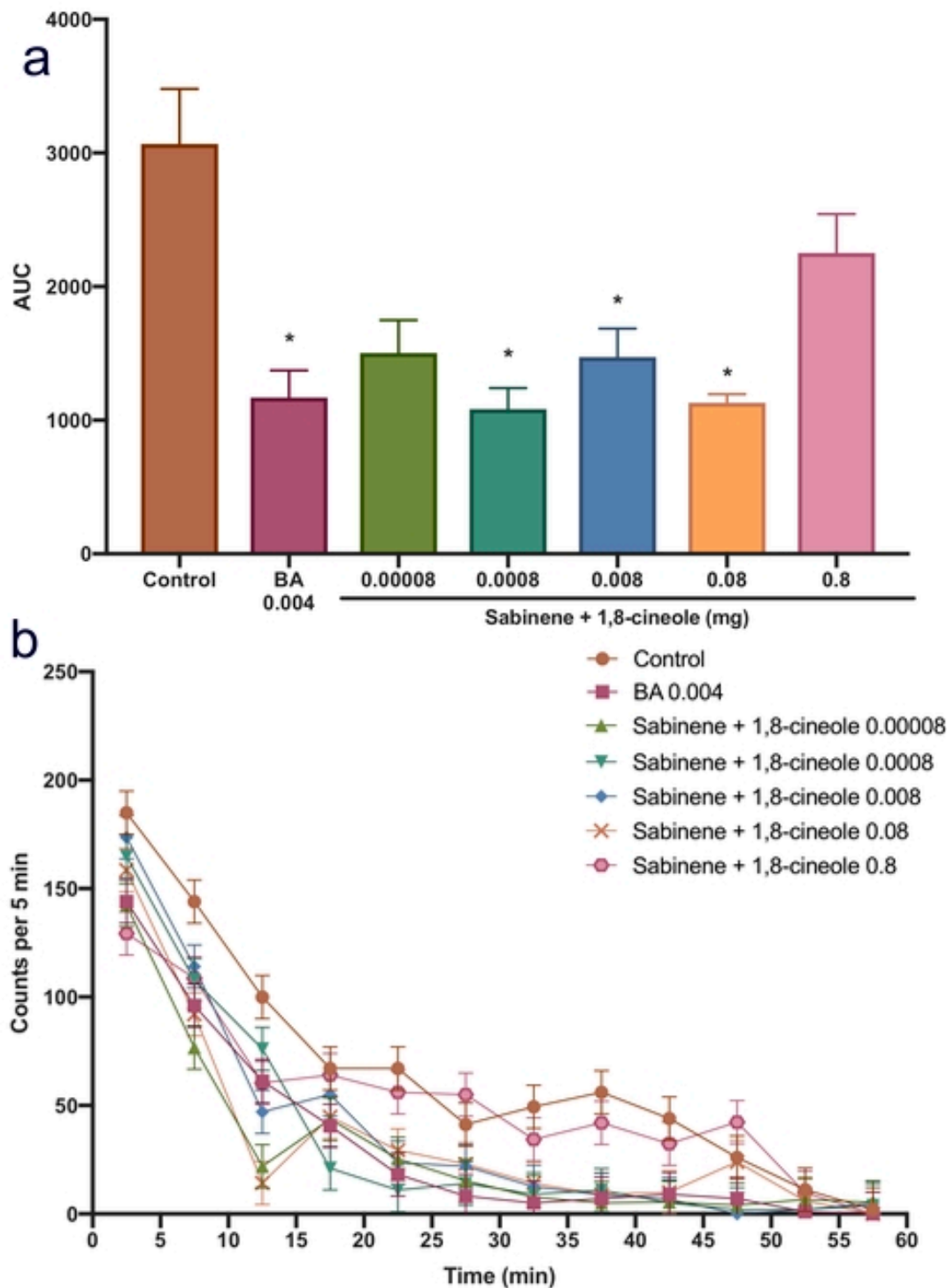
**Fig. 2 Total spontaneous motor activity (a) and locomotor activity transition (b) of mice treated with LCEO (0.000004, 0.00004, 0.0004, 0.004, 0.04 or 0.4 mg)**

Data represent the mean  $\pm$  SEM of 6 mice. Statistical differences vs. the control group were calculated using ANOVA followed by Dunnett's test. \* $p < 0.05$ .



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Data represent the mean  $\pm$  SEM of 6 mice. Statistical differences vs. the control group were calculated using ANOVA followed by Dunnett's test. \* $p < 0.05$ .



**Fig. 4** Total spontaneous motor activity (a) and locomotor activity transition (b) of mice treated with a mixture of sabinene and 1,8-cineole (0.00008, 0.0008, 0.008, 0.08 or 0.8 mg) Data represent the mean  $\pm$  SEM of 6 mice. Statistical differences vs. the control group were calculated using ANOVA followed by Dunnett's test. \* $p < 0.05$ .

# Supplementary data

## **Sedative effects of the essential oil from the leaves of *Lantana camara* occurring in the Republic of Benin via inhalation in mice**

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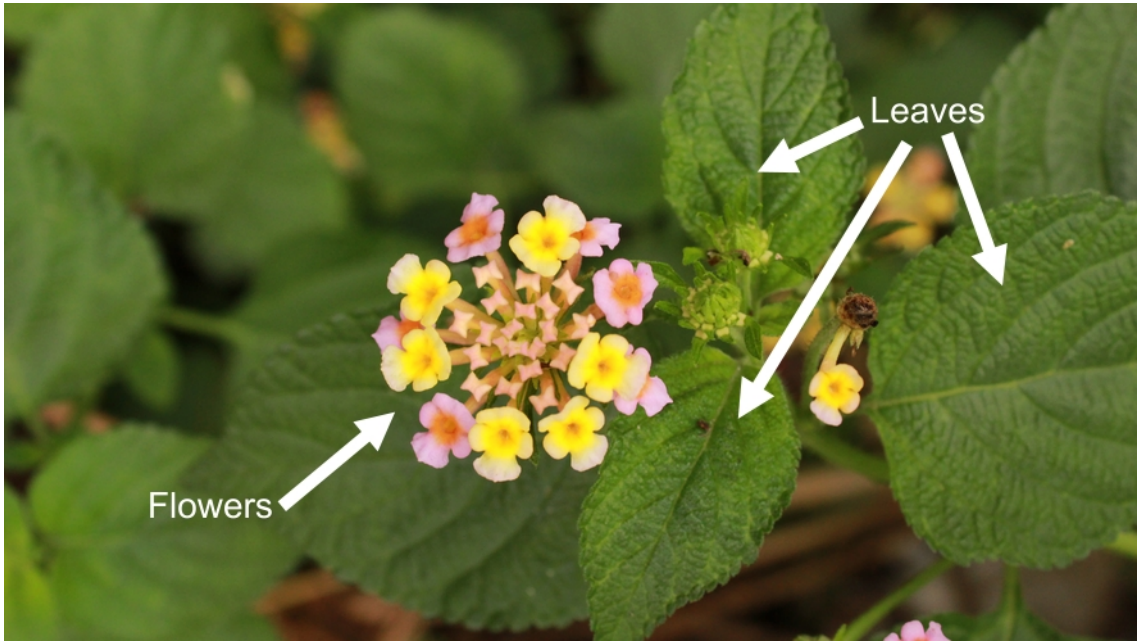
E-mail: [michihoi@pharm.kyoto-u.ac.jp](mailto:michihoi@pharm.kyoto-u.ac.jp)

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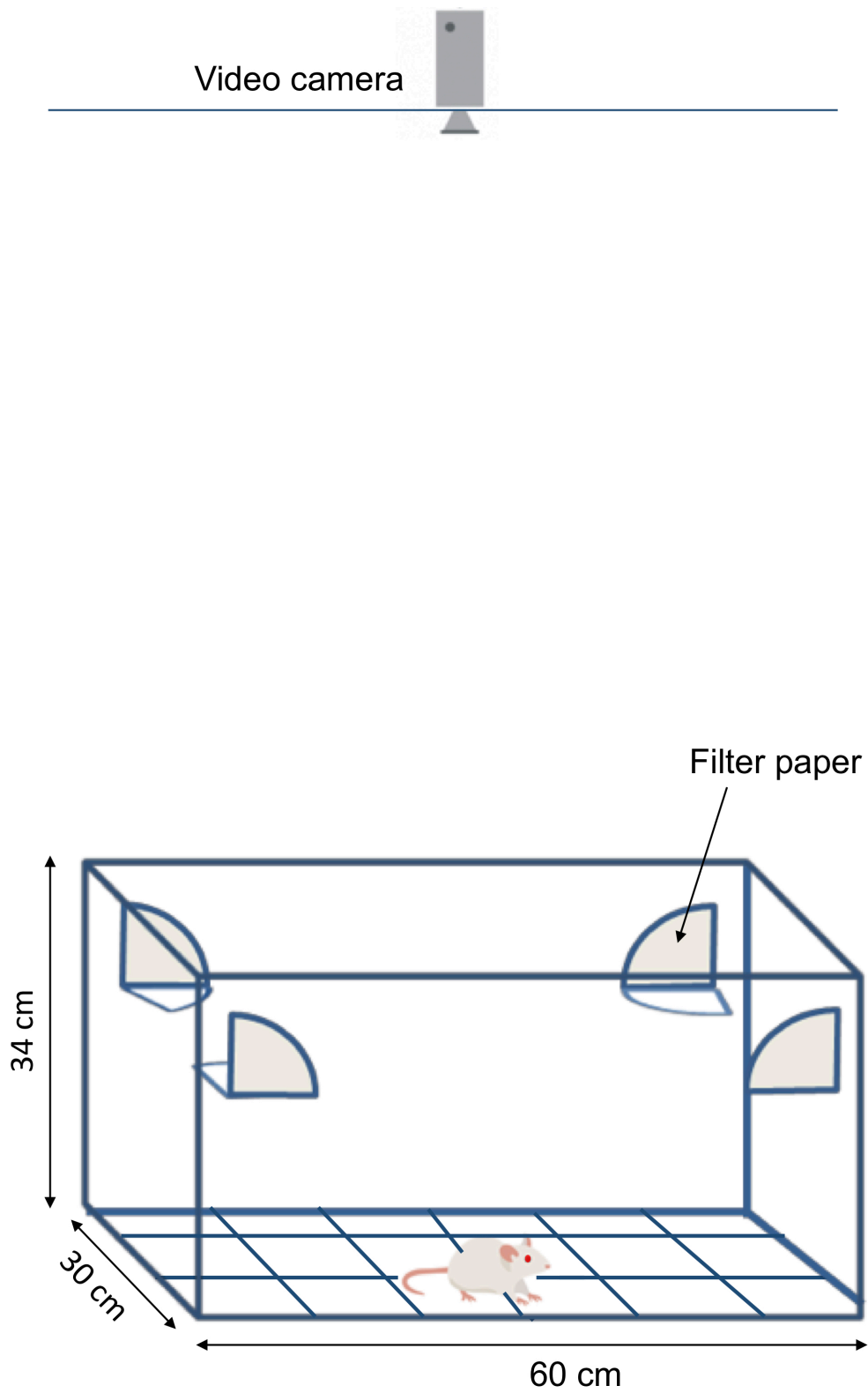


## Contents

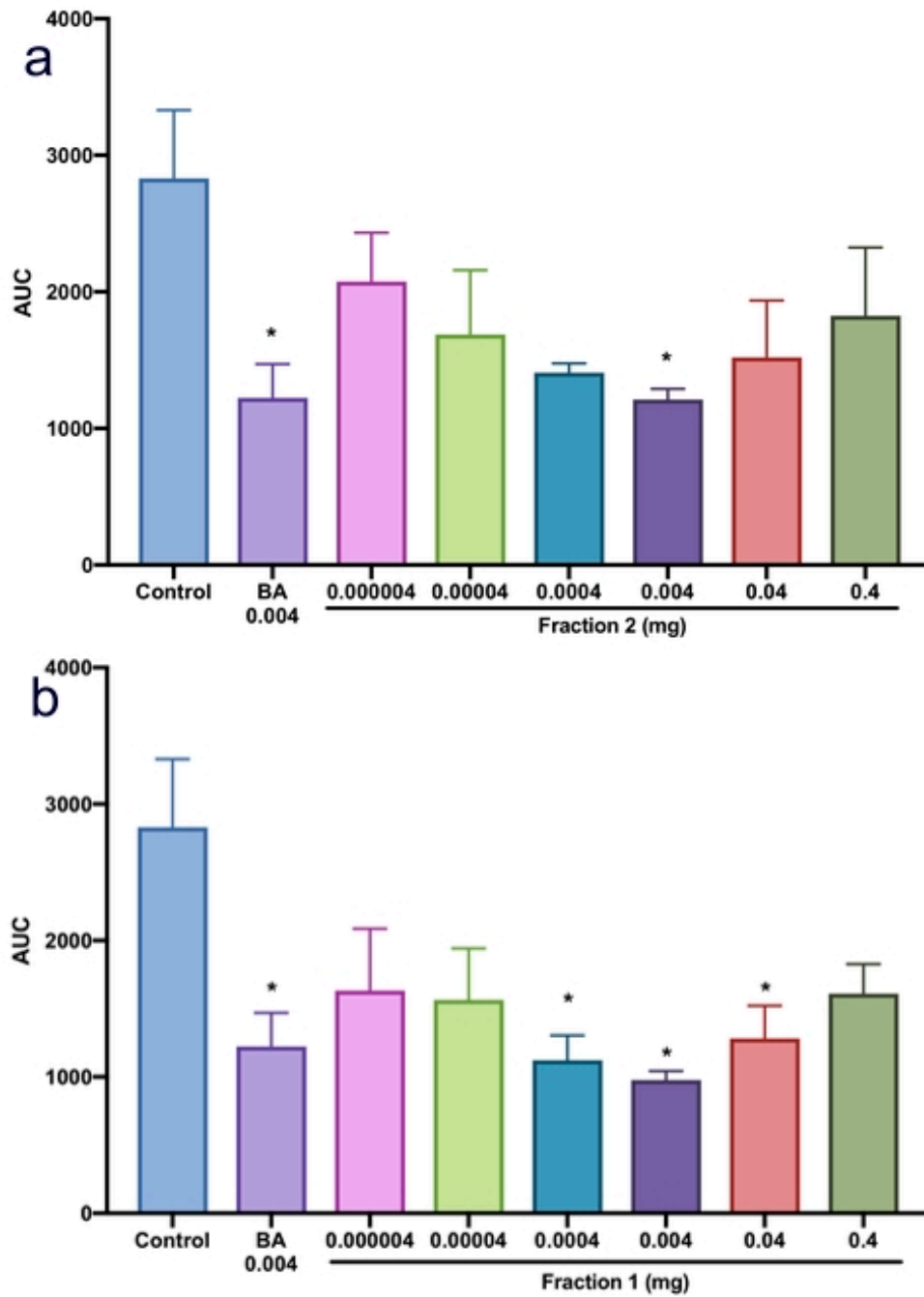
<b>Fig. S1</b> Leaves of <i>Lantana camara</i> in their natural environment .....	<b>3</b>
<b>Fig. S2</b> The open-field arena .....	<b>4</b>
<b>Fig. S3</b> Total spontaneous motor activity of mice treated with fraction 2 and fraction 1 (0.000004, 0.00004, 0.0004, 0.004, 0.04 or 0.4 mg) (a and b respectively).....	<b>5</b>



**Fig. S1** Leaves of *Lantana camara* in their natural environment



**Fig. S2 The open-field arena**



**Fig. S3 Total spontaneous motor activity of mice treated with fraction 2 and fraction 1 (0.000004, 0.00004, 0.0004, 0.004, 0.04 or 0.4 mg) (a and b respectively)**

Data represent the mean ± SEM of 6 mice. Statistical differences vs. the control group were calculated using ANOVA followed by Dunnett's test. \* $p < 0.05$