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Sedative, anxiolytic and antidepressant-like effects of inhalation of the essential oil of *Ocimum gratissimum* L. from Cameroon in mice

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ABSTRACT

In this study, the behavioral effects of inhalation of the essential oil of *Ocimum gratissimum* from Cameroon were investigated. The open field test, light/dark box test, tail suspension test and Rota-rod test were used to assess the sedative, anxiolytic-like, antidepressant-like and motor coordination effects respectively, in mice. GC and GC/MS analyses were performed to investigate the chemical composition of *O. gratissimum* essential oil. Phytochemical analysis revealed thymol (68%) as main compound of *O. gratissimum* essential oil. Inhalation of *O. gratissimum* essential oil showed potent sedative, anxiolytic and antidepressant-like effects in mice, and did not cause any deleterious effects on motor coordination. It is suggested that a synergistic effect of the constituents of *O. gratissimum* essential oil might account for its sedative activity. In conclusion, inhalation of *O. gratissimum* essential oil might have aromatherapeutic potential.

Keywords: Sedative, Anxiolytic, Antidepressant, Inhalation, *Ocimum gratissimum*, Thymol.

1. Introduction

According to the World Health Organization (WHO) [1] “mental health is as important as physical health to the overall well-being of individuals, societies and countries. Yet only a small minority of the 450 million people suffering from a mental or behavioural disorder is receiving treatment”. WHO [2] also reported that 80% of the world’s population used natural remedies and traditional medicines for the treatment of their ailments. As such, it is very important to establish scientific validation of the effectiveness of these natural remedies. Aromatherapy is continuously gaining recognition as an alternative/conventional therapy for the management of several mental disorders especially in the US, UK, France and Germany [3]. Essential oils and fragrance compounds have been reported to elicit pharmacological effects on the CNS in experimental animals and humans [4-9]. Despite the widespread use of inhaled essential oils in aromatherapy for the treatment of anxiety, depression, insomnia, mental exhaustion etc, experimental data on psychopharmacological properties of inhaled essential oils are surprisingly scarce [4, 10].

In the search for new therapeutic products for the treatment of neurological or psychiatric disorders, medicinal plant research worldwide has demonstrated the pharmacological effectiveness of different plant species [11, 12]. The African continent and Cameroon in particular holds an enormous resource in terms of floral biodiversity and its medicinal plants have remained a main reservoir of phytochemicals for pharmaceutical drug development [13-16]. However, documentation and scientific validation of the medicinal potentials of plants commonly used in traditional medicine is lacking [13, 15].

O. gratissimum (Lamiaceae) is an aromatic medicinal plant found in the wild or cultivated throughout the tropics and subtropics. In West Africa, it is commonly found around village huts and gardens and cultivated for medicinal and culinary purposes [17]. It is commonly known as “masepo” in Cameroon and is used for flavouring a local well known dark fish sauce called “Bongo Tjobi” [18].

O. gratissimum is used in African Traditional Medicine for the treatment of several diseases including epilepsy, fever, diarrhea, mental illness, fungal infections, cold and convulsion [19]. Leaf extracts of *O. gratissimum* [17] and the essential oil administered orally [20-22] have been reported to show anticonvulsant, anxiolytic, CNS depressant, and antinociceptive activities respectively. However, there are no reports on the aromatherapeutic effects of *O. gratissimum* essential oil. In this study, psychopharmacological effects of inhalation of the essential oil of *O. gratissimum* from Cameroon were investigated, and the chemical constituents and active compounds responsible for its activity were identified. The anxiolytic activity and antidepressant-like activities were also evaluated.

2. Materials and Methods

2.1 Animal care

Four-week-old male ddY mice (20-30 g) purchased from Japan SLC (Shizuoka, Japan) were used for this study. They were kept under an ambient temperature of 25 ± 2 °C and a relative humidity of 50–60% with a light-dark cycle of 12 h. The animals were fed pellet chow and water *ad libitum*. All animal experiments were designed following the recommendations of the Animal Research Committee of Kyoto University, Kyoto, Japan (Approval numbers 2011-19, 2012-18, 2013-17). Experimental procedures involving animals and their care were conducted in conformity with institutional guidelines that complied with the Fundamental Guidelines for Proper Conduct of Animal Experiments 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 10:00–17:00 h under the same conditions.

2.2 Plant materials

Dried aerial parts of *O. gratissimum* were used for this study. The plant material originated from the wild in Batibo, Northwest region Cameroon. A voucher specimen of *O. gratissimum* (specimen number: EST-4975) was deposited in the herbarium of Experimental Station for Medicinal Plants, Graduate School of Pharmaceutical Sciences, Kyoto University.

2.3 Drugs and reagents

Benzylacetone (Tokyo Kasei), Diazepam (Wako Pure Chemical Industries Ltd., Osaka, Japan), and Fluoxetine (Nacalai Tesque Inc., Kyoto, Japan) were used as positive controls. Thymol was purchased from Nacalai Tesque. Triethyl citrate (TEC; Merck, Darmstadt, Germany), a non-sedating odorless solvent, was used to dissolve the essential oil and fragrant compounds. All chemicals used in this study were of the highest grade available.

2.4 Isolation of OGEO and fractionation

The essential oil of *O. gratissimum* (OGEO) was prepared by hydrodistillation of dried aerial parts for 2 h using a Clevenger apparatus, as designated in the Japanese Pharmacopeia (JP XVI). The oil was captured in hexane, dried with anhydrous sodium sulfate and concentrated. Headspace of the oil was analysed by solid phase micro extraction (SPME) and gas chromatography/mass spectrometry (GC/MS) to confirm that the oil was void of substantial amount of hexane. The pure essential oil obtained was stored in sealed vials at 4 °C until analysis. Fractionation of OGEO was carried out using preparative thin layer chromatography (TLC). The TLC plates were developed with petroleum ether: acetone (5:1)

and partitioned to obtain fractions 1–3. Fractions 1 and 3 were evaluated for their biological activity.

2.5 Behavioral testing apparatus

2.5.1 Open field test

The sedative effect of OGEO was evaluated based on mouse spontaneous locomotor activity in an open field test. The open field test apparatus used was described previously [23]. The doses administered are expressed as milligrams of OGEO in 400 μ L TEC per cage. The administration procedure was as follows: 4 filter-paper discs were adhered to 4 corners of the inner walls of the glass cage. OGEO was charged on the filter paper discs and the cage was closed, so that the vapor pervaded the cage by natural diffusion. Sixty minutes after charging the sample, a mouse was placed in the center of the cage and monitored by a video camera for 60 min. The frequency that the mouse crossed the lines drawn on the floor of the cage (at 10 cm intervals) was counted every 5 min for 60 min. The area under the curve (AUC) of locomotor activity counts per 5 min (X-axis) and time (Y-axis) which represented total spontaneous locomotor activity was then calculated. Benzylacetone, a fragrant compound which had been previously reported to show a sedative effect upon inhalation was used as positive control [23, 24]. Benzylacetone was administered by inhalation at a dose of 4.0×10^{-3} mg per cage.

2.5.2 Light/dark transition test

The light/dark box test is a widely used behavioral test for evaluating the effect of anxiolytic agents [25]. The light/dark transition apparatus consisted of 2 equally sized compartments; a light area (30 cm \times L 30 cm \times H 34 cm) illuminated by a 6.5 W desk LED lamp, and a dark area (30 cm \times L 30 cm \times H 34 cm) blackened with black plastic sheets. The two compartments were separated by a black wall with an aperture (small doorway) in its center (5 cm \times 5 cm) to allow passage from one compartment to the other. OGEO was charged in both compartments for 60 min in accordance with the open field test. Thereafter, a mouse was placed in the center of the lit area facing the tunnel and the following parameters were recorded using a video camera during a 15 min test period: (1) the number of crossings between the light and dark compartments; and (2) the total time spent in the illuminated area. A mouse was considered to have entered the new area when all 4 legs crossed the threshold of the compartment. Diazepam was used as a positive control [26].

2.5.3 Tail suspension Test

The tail suspension test is a widely used behavioral model for testing the effect of antidepressant agents [27]. Tail suspension test was carried out by a method described by Steru and colleagues (1985) [28]. Mice were suspended from the edge of a table (63 cm high) by an adhesive tape placed approximately 1 cm from the tip of the tail. The mice were considered immobile when they stopped to make any struggling movements and hung passively. Immobility time was recorded for a period of 6 min. A reference compound fluoxetine was administered at a dose of 20 mg/kg p.o 1 h prior to testing, to confirm the validity of the apparatus. Fluoxetine was dissolved in physiological saline (vehicle) and administered at an injection volume of 10 ml/kg. The dose of fluoxetine administered was determined based on literature report [29] and on observations made in a preliminary experiment. OGEO was administered to mice by inhalation in the open field arena for 30 min before the tail suspension test.

2.5.4 Rota-rod Test

The effect of active doses of OGEO on motor coordination was assessed using a Rota-rod Treadmill 660C (Muromachi Kikai CO; LTD), 5 cm diameter, 20 cm high, at accelerating speed (40 rpm; 5 mins). The latency to fall from the rotating treadmill was recorded for 5 min. OGEO was administered to mice by inhalation in the open field arena for 30 min before the Rota-rod test.

2.5.5 Qualitative and quantitative analyses of OGEO

Qualitative analysis of OGEO was carried out on an Agilent 6850 series gas chromatograph connected to an MSD 5975 with the following operation conditions: column: fused silica capillary column, DB-wax (HP), 60 m × 0.25 mm × 0.25 μm; column temperature program: 40–200 °C, increasing at a rate of 4 °C/min, holding at 40 °C for 2 min and at 190 °C for 20 min; injector temperature: 110 °C; carrier gas: helium, 25 cm/s; column head pressure: 100 kPa; ionization energy: 70 eV; injection volume: 1.0 μL. Quantitative analysis was carried out on a Hitachi G-5000 equipped with a flame ionization detector with the following conditions: column: fused silica capillary column, TC-wax (HP), 60 m × 0.25 mm × 0.25 μm; column temperature program: same as GC/MS; injector: 180 °C, detector: 200 °C, FID; carrier gas: helium, 0.8 mL/min; split ratio: 100:1; column head pressure: 200 kPa; injection volume: 1 μL. The linear retention indices of the constituents were determined using a series of *n*-alkanes as standards. The chemical compounds were identified by use of NIST 2 and flavors libraries and the identities of most compounds were confirmed by comparison of their retention indices and mass spectra with those of reference standards or published data [30].

2.5.6 Statistical analysis

Data are expressed as the mean ± standard error of the mean. Statistical analyses were performed using Student's *t*-test or one way analysis of the variance (ANOVA) followed by Dunnett's test using GraphPad Instat (GraphPad Software, San Diego, CA, USA). A probability level of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1 Phytochemical analysis of OGEO

The dried aerial parts of *O. gratissimum* afforded 0.7% (w/w) EO with an amber color and sharp thyme-like odor. Table 1 shows the 22 identified constituents listed in their order of elution from the DB-wax column. Thymol was found to be the principal constituent of OGEO (68.0%). Phytochemical analyses of the fractions of OGEO revealed that thymol was the main component of Fraction 3 (95.2%). The main components of Fraction 1 were β-selinene, γ-terpinene, *trans*-caryophyllene and *p*-cymene (31.0, 28.4, 14.8 and 9.7%, respectively), whereas thymol was not detected in this fraction.

3.2 Effect of OGEO on mouse spontaneous locomotor activity

Figure 1 shows the locomotor activity following the administration of OGEO via inhalation at doses ranging from 4.0×10⁻¹⁰ to 4.0×10⁻¹ mg per cage. A biphasic dose response pattern was observed. Mouse spontaneous locomotor activity was enhanced at low doses ranging from 4.0×10⁻¹⁰ to 4.0×10⁻⁶ mg per cage, whereas higher doses (4.0×10⁻⁵ to 4.0×10⁻¹ mg per cage) suppressed mouse spontaneous locomotor activity.

Table 1: Phytochemical constituents of *Ocimum gratissimum* essential oil

Compound	RI	Peak area %
α-pinene	1032	trace
myrcene	1168	0.5
α-terpinene	1188	0.5
γ-terpinene	1222	3.0
<i>p</i> -cymene	1316	5.9
dihydro- <i>p</i> -cymene	1449	0.5
1-octen-3-ol	1457	0.8
α-copaene	1506	0.5
linalool	1552	0.6
terpinene-4-ol	1619	4.3
<i>trans</i> -caryophyllene	1619	2.6
<i>o</i> -cresol	1663	0.6
α-humulene	1690	0.4
α-terpineol	1711	0.6
borneol	1719	0.6
β-selinene	1744	4.0
α-selinene	1748	1.2
α-panasinen	1788	0.6
<i>p</i> -cymen-8-ol	1864	0.4
caryophyllene oxide	2010	1.9
thymol	2146	68.0
carvacrol	2177	2.5

RI: Retention indices. Compounds are listed in order of their elution from DB-Wax column.

A significant decrease in locomotor activity was observed at doses 4.0×10⁻⁴ to 4.0×10⁻¹ mg per cage in a dose dependent manner. The AUC values of the 4.0×10⁻⁴ to 4.0×10⁻¹ mg OGEO-treated groups were significantly smaller than the control values, and the decrease in locomotor activity produced by these concentrations was statistically significant ($P < 0.05$ and $P < 0.01$), suggesting a potential sedative effect of OGEO. The most effective concentrations were 4.0×10⁻³ mg and 4.0×10⁻² mg which showed a reduction in locomotor activity that was comparable to that of benzylacetone. Transitions of total locomotor activity per 5 min were compared among administered doses. Mice administered OGEO at the dose of 4.0×10⁻³ to 4.0×10⁻² mg became calm from the 20th minute. By the 30th minute, mouse locomotor activity dropped to nearly zero (data not shown). This indicated that an administration period of about 30 minutes was necessary for OGEO to be effective at the active doses.

3.3 Effects of OGEO fractions on mouse locomotor activity

Fractions 1 and 3 of OGEO were administered individually to mice by inhalation at doses ranging from 4.0×10⁻⁸ to 4.0×10⁻¹ mg per cage. Fraction 2 was not tested because the composition of the fraction was a mixture of fractions 1 and 3, and the amount of the fraction was insufficient for the tests using mice. Both Fraction 1 and 3 induced a significant decrease in locomotor activity compared to the control groups. This suggested that these two fractions contained active ingredients of OGEO. Fraction 3 appeared to be more potent than Fraction 1. Fraction 1 showed a significant decrease in locomotor activity at a dose of 4.0×10⁻¹ mg while Fraction 3 showed a significant decrease in locomotor activity at doses of 4.0×10⁻⁵ and 4.0×10⁻⁴ mg per cage (Fig. 2A and 2B). The active doses of fraction 3 (4.0×10⁻⁵ and 4.0×10⁻⁴ mg per cage) were lower than those of OGEO (4.0×10⁻⁴ and 4.0×10⁻¹ mg per cage). Thus, the effect of the main compound of Fraction 3 (thymol) was further examined to confirm its role in the sedative effect of OGEO.

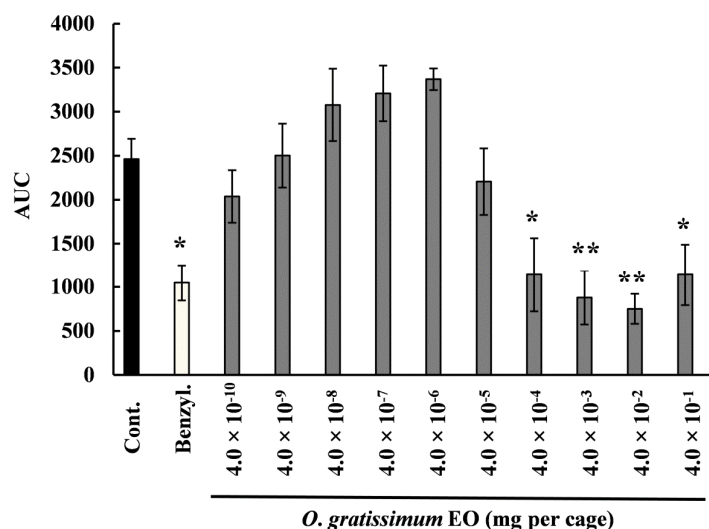


Fig 1: Sedative activity of *Ocimum gratissimum* essential oil. Total spontaneous locomotor activity of mice that received vehicle (triethyl citrate, 400 μ L) and *Ocimum gratissimum* essential oil (4.0×10^{-10} – 4.0×10^{-1} mg per cage; EO). Data are shown as the mean \pm standard error of the mean of 6 mice. Statistical differences vs. the control group (Cont.) were calculated using analysis of the variance followed by Dunnett's test. * $p < 0.05$, ** $p < 0.01$. Benzyl.; Benzylacetone (4.0×10^{-3} mg per cage).

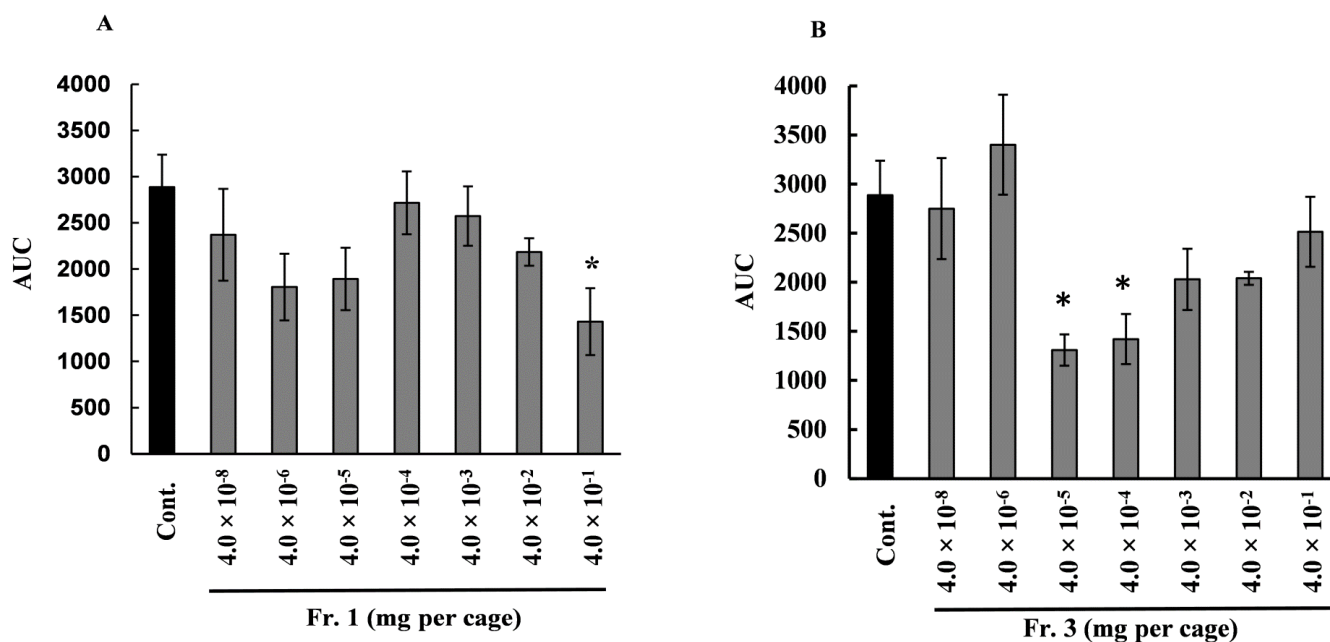


Fig 2: Effects of OGEO fractions on mouse locomotor activity. Total spontaneous locomotor activity of mice treated with *Ocimum gratissimum* essential oil fractions; A: Fraction (Fr.) 1 and B: Fraction 3. Data are shown as the mean \pm standard error of the mean of 6 mice. Statistical differences vs. the control group (Cont.) were calculated using analysis of the variance followed by Dunnett's test.

* $p < 0.05$.

3.4 Effects of thymol on mouse locomotor activity

Figure 3 shows the effect of thymol on mouse spontaneous locomotor activity. Thymol was administered at concentrations ranging from 4.0×10^{-10} to 4.0×10^{-1} mg per cage. It significantly decreased the locomotor activity of mice at concentrations of 4.0×10^{-5} and 4.0×10^{-4} mg. This indicated that thymol might possess a sedative activity. Thymol also significantly decreased mouse spontaneous locomotor activity at a dose of 4.0×10^{-2} mg. However, the inhibition of locomotor activity at the 4.0×10^{-2} mg dose was considered to be as a result of muscle relaxation because the performance of mice that were administered this dose of thymol showed shorter latency to fall off the Rota-rod treadmill compared

to the control group (data not shown). A comparison of the effect of thymol and OGEO on mouse spontaneous locomotor activity was made to elucidate their relative potencies. Both thymol and OGEO showed a biphasic dose response pattern. At low doses (phase 1), the dose patterns of thymol and OGEO were quite similar with a linear increase in locomotor activity from the dose of 4.0×10^{-10} to 4.0×10^{-6} mg per cage. However, at higher doses (4.0×10^{-5} to 4.0×10^{-1} mg per cage), thymol and OGEO showed different dose patterns. The therapeutic window of OGEO was wider and the potency of OGEO was greater than that of thymol. The dose dependent decrease in locomotor activity observed with OGEO at higher doses was not observed in thymol.

3.5 Anxiolytic-like activity of OGEO

In the light/dark transition test, anxiolytic-like agents typically increase the time spent in the light area and the movements between the two compartments [25]. The validity of the experimental system was confirmed using diazepam as positive control. Diazepam, dissolved in corn oil (0.5 mg/kg) and administered intraperitoneally at 30 min prior to testing, significantly increased

the total time spent in the light area and the number of transitions between the two compartments compared to the vehicle (corn oil) [26]. The administration of OGEO at a concentration of 4.0×10^{-4} mg per cage significantly increased the total time spent in the light area as well as the number of transitions between the light and dark compartments (Fig. 4A and 4B). This suggested that OGEO might induce an anxiolytic-like effect.

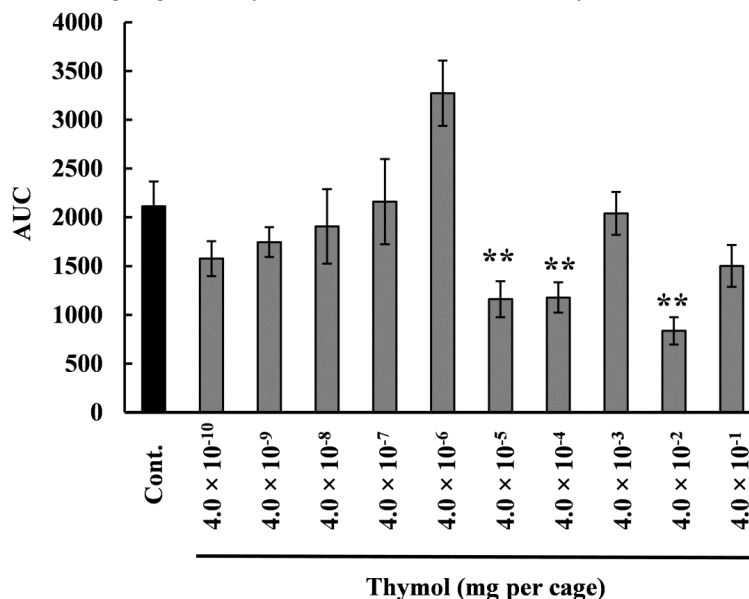


Fig 3: Effects of thymol on mouse locomotor activity. Total spontaneous locomotor activity of mice treated with vehicle (triethyl citrate 400 μ L; Cont.) and thymol. Data are shown as the mean \pm standard error of the mean of 6 mice. Statistical differences vs. the control group were calculated using analysis of the variance followed by Dunnett's test. * $p < 0.05$, ** $p < 0.01$.

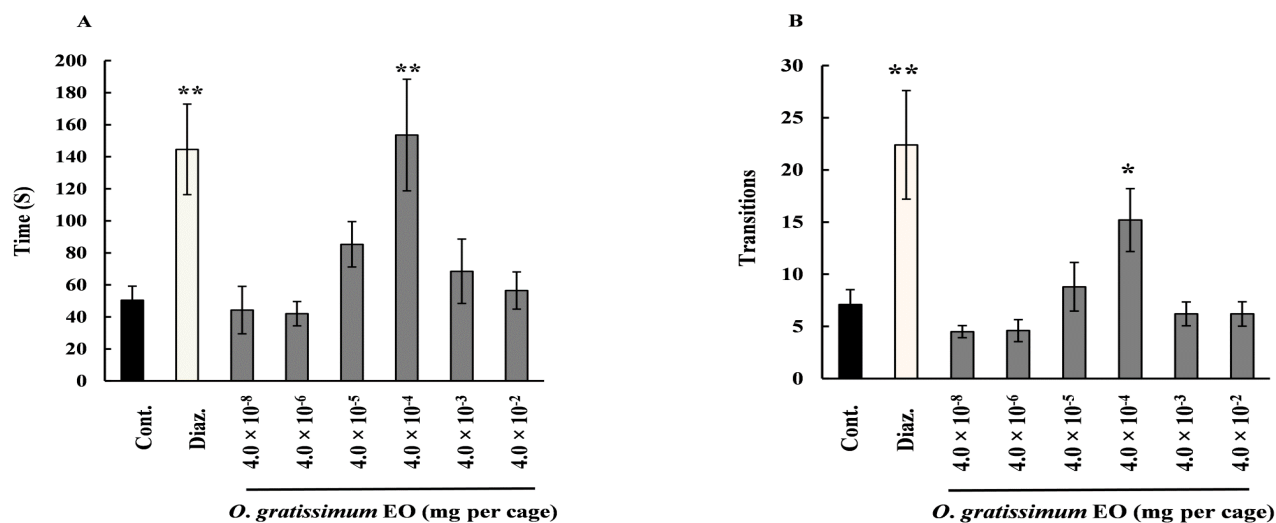


Fig 4. Anxiolytic-like activity of mice that received control (triethyl citrate 400 μ L; Cont.), diazepam (0.5 mg/kg; Diaz.), and *Ocimum gratissimum* essential oil (EO); A: time spent in the light area, B: number of transitions between the compartments. Data are shown as the mean \pm standard error of the mean of 10 mice. Statistical differences were calculated using analysis of the variance followed by Dunnett's test. * $p < 0.05$, ** $p < 0.01$.

3.6 Antidepressant-like activity of OGEO

In the tail suspension test, antidepressant-like activity is represented by a decrease in immobility time [27]. Fluoxetine, dissolved in 0.9% physiological saline (20 mg/kg) and administered orally 1 h prior to testing, significantly shortened the immobility time compared to the vehicle (saline). This confirmed that the experimental system was valid. OGEO was administered at doses of 4.0×10^{-10} to 4.0×10^{-1} mg per cage. At doses of 4.0×10^{-3} to 4.0×10^{-1} mg per cage, OGEO

significantly decreased the immobility time compared to the control group (Fig. 5). This suggested that OGEO might induce an antidepressant-like effect. OGEO at a dose of 4.0×10^{-6} mg per cage caused an increase in immobility time compared to control group.

3.7 Effect of OGEO on motor coordination

OGEO was administered to mice by inhalation at doses of 4.0×10^{-3} to 4.0×10^{-1} mg per cage and 30 minutes later, the Rota-rod test was

performed. There were no significant differences in the latency to fall off the treadmill at the tested doses of OGEO treated group compared to control group (Fig. 6). This indicated that OGEO had no deleterious effects on motor coordination at the tested doses. Fraction 1, at the dose of 4.0×10^{-1} mg per cage and thymol at the dose of 4.0×10^{-2} mg per cage were also tested in the Rota-rod test.

Fraction 1 showed no deleterious effects on motor coordination at the tested dose. However, thymol at a dose of 4.0×10^{-2} mg per cage showed a relatively short latency to fall off the Rota-rod treadmill, although this effect was not significant (Fig. 6). This indicated that the decrease in locomotor activity exhibited by thymol at a concentration of 4.0×10^{-2} mg might not be as a result of sedation.

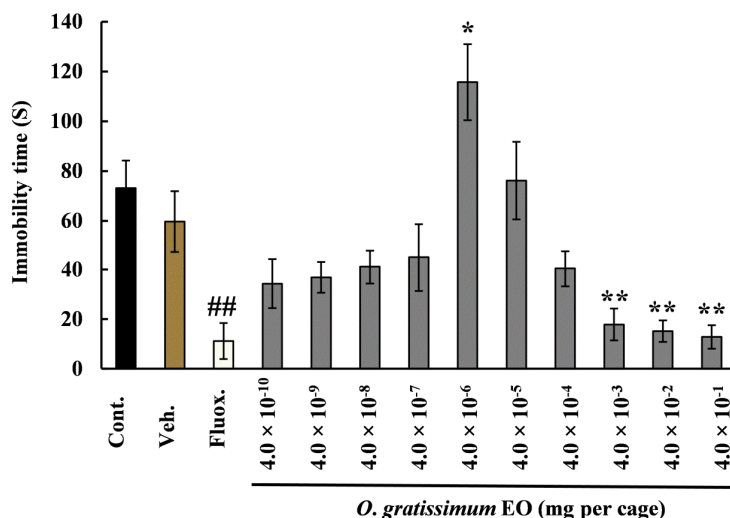


Fig 5: Effect of inhalation of OGEO on immobility time in Tail Suspension Test. Data are shown as the mean \pm standard error of the mean of 6 mice. Statistical differences vs. the control group were calculated using analysis of the variance followed by Dunnett's test. * $p < 0.05$, ** $p < 0.01$ compared to the control group (triethyl citrate), ## $p < 0.01$ compared to vehicle. Veh.; Vehicle (Saline), Fluox.; Fluoxetine (20 mg/kg p.o., 30 min before test).

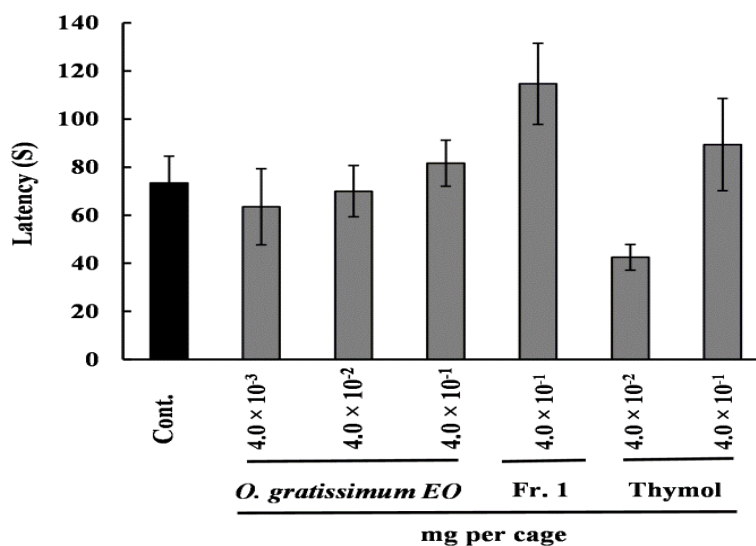


Fig 6: Effects of OGEO, fraction 1 and thymol on motor coordination in Rota-rod test. Data are shown as the mean \pm standard error of the mean of 6 mice. Statistical differences vs. the control group were calculated using analysis of the variance followed by Dunnett's test.

4. Discussion

4.1 Behavioral effects of OGEO

In the current study, the psychopharmacological effects upon inhalation of the essential oil of *Ocimum gratissimum* were demonstrated. *O. gratissimum* appeared to have potent sedative and anxiolytic-like activities in mice without producing motor impairments. These results suggest that inhaled OGEO might elicit a tranquilizing effect. *O. gratissimum* also appeared to show potent antidepressant-like activity at doses of 4.0×10^{-3} to 4.0×10^{-1} mg per cage. These doses did not cause increase in locomotor activity in the open field test, indicating that OGEO indeed possessed

antidepressant properties. This suggests that inhalation of OGEO might exert a thymoleptic effect. Taken together, these results indicate that inhalation of the essential oil of *O. gratissimum* might be effective in the management of affective disorders.

4.2 Active principles of OGEO

Several chemotypes of OGEO such as the eugenol, geraniol and thymol types have been reported [31]. Although the eugenol type has been extensively studied, very few studies have focused on the geraniol or thymol types. OGEO used in this study was the thymol type (68%). Assays with fractions of OGEO and single compound

revealed that thymol is an active compound in the inhalative sedative effect of OGEO. However, other monoterpene compounds present in OGEO such as linalool, *p*-cymene, 1-octen-3-ol, have been previously reported to show sedative effect upon inhalation in mice [26, 32-34]. It is suggested that other active compounds, though present only in small amounts in OGEO might have worked in synergy with thymol to contribute to the inhalative sedative effects of OGEO. Furthermore, comparison of the sedative activities of OGEO and thymol indicated that OGEO was more potent than thymol, suggesting that the whole essential oil of *O. gratissimum* might be more beneficial than its active compounds. This finding is consistent with that of Galindo *et al.* 2010 [41] who reported that the anticonvulsant and sedative effects of oral administration of the eugenol type essential oil of *O. gratissimum* might be due to a synergistic interaction of its components.

4.3 Selectivity and dose response patterns of psychoactive agents

According to Brunton *et al.* 2011 [36] drugs that selectively modify the CNS function (e.g. anti-depressants, tranquilizers, sedatives, hypnotics, certain stimulants, anti-psychotics etc) may cause either depression or excitation. In some instances, a drug may produce both effects simultaneously on different systems. Although selectivity of action may be remarkable, it is usually over-estimated as a drug usually affects several CNS functions to varying degrees [36]. For example, Brunton *et al.* [36] also stated that although the public often considers alcoholic beverages as a stimulant, ethanol is a CNS depressant. However, mild doses of alcohol appear to disinhibit the CNS function. Although this view is controversial, Harvey 1962 [37] asserted that "the disinhibition hypothesis is more than plausible and has actually proved most useful in ordering the effects of a wide range of doses of depressant drugs. To think of depressant drugs as acting simple along the vertical axis of the CNS, and not to consider the possible actions of these drugs along non-vertical dimensions of the CNS system, is to disregard the empirical findings." According to Calabrese 2008, [38] the non-linear hormetic-like biphasic dose response pattern (inverted U-shape or low dose stimulation, high dose inhibition) is known to be quite predominant in anxiolytic drug screening tests. It is assumed to result from a mixed agonist/antagonist effect [38]. Published data indicate that while several essential oils and fragrance compounds have been reported to have CNS depressant effects, others such as chamomile essential oil, peppermint essential oil and its constituents have been shown to have psychomotor stimulant effects on the CNS [4, 39, 40]. These psychomotor stimulating essential oils are suggested to be potentially useful in the treatment of mental fatigue or Attention Deficit Hyperactivity Disorder (ADHD) [40, 41]. Interestingly, some pure compounds such as eugenol and 1, 8-cineole have been simultaneously reported to show stimulatory and sedative effects on the CNS [35]. It is therefore important that the pharmacological effects of essential oils and fragrance compounds be tested over a wide dose range in order to elucidate their effects.

4.4 Biphasic effect of OGEO on spontaneous locomotor activity

In the present study, OGEO evaluated at doses ranging from 4.0×10^{-10} to 4.0×10^{-1} mg per cage showed a biphasic effect on mouse spontaneous locomotor activity. At low doses, OGEO enhanced mouse locomotor activity while at higher doses, a dose dependent decrease in locomotor activity was observed. The enhancement of locomotor activity showed by OGEO might have

been brought about by thymol (main compound of OGEO) since thymol showed a similar increase in locomotor activity at low doses. Elhabazi *et al.*, (1996) [42] reported that the aqueous extract of *Thymus broussonetii* had anxiolytic effects in the light/dark box test, meanwhile the ethyl acetate extract enhanced locomotor and exploratory activities. Thymol and thyme essential oil on the one hand, have been reported to show stimulating effects upon inhalation in mice [4, 39]. On the other hand, *in vitro* reports have revealed that thymol is a potent GABA agonist, implying that it might have sedative properties [43, 44]. We might therefore conclude that OGEO and thymol might be CNS depressants but might exert stimulant-like effects at mild doses. To the best of our knowledge this is the first report elucidating the dual effect (dose-related) of OGEO or thymol on locomotor activity *in vivo*. These results suggest that the doses at which OGEO or other thymol-rich essential oils would be administered need to be studied carefully with respect to the desired effect targeted.

4.5 Dose-effect relationship in the antidepressant-like effect of OGEO

The antidepressant-like effect of OGEO was also investigated at doses ranging from 4.0×10^{-10} to 4.0×10^{-1} mg per cage in the tail suspension test. While a significant anti-depressant-like effect was observed at doses of 4.0×10^{-3} to 4.0×10^{-1} mg per cage, mice showed a depressed-like behavior with a significant increase in immobility time at the dose of 4.0×10^{-6} mg. In the open field test, OGEO showed a biphasic dose pattern with locomotor activity increase from 4.0×10^{-10} mg dose up to 4.0×10^{-6} mg dose and then a decrease in locomotor activity from this dose downward. This implied that the 4.0×10^{-6} mg dose represented the peak for locomotor activity enhancement. In addition to marked increase in ambulation, mice administered OGEO at this dose (4.0×10^{-6} mg) showed excited-like behavior in the open field test such as jumping, increased rearing and grooming (data not shown). This further confirmed the stimulant-like effect of this dose. Stimulation of the CNS by stimulant drugs is known to be followed by a period of mental depression [45]. Although adequate scientific data about the relationship between depression and the use of recreational drug is lacking, many recreational drugs can cause depression or anxiety [46]. According to Mulholland, [47] in depression, the level of neurotransmitters which control our emotions are altered, and recreational drugs affect these neurochemicals. For example; dopamine is affected by cocaine, amphetamine and ecstasy, serotonin is affected by ecstasy and LSD and noradrenaline is affected by amphetamines and opiates, and it is largely these three chemicals on which antidepressant medicines work. [47] This might explain why the 4.0×10^{-6} mg OGEO dose induced a depressed-like behavior in the tail suspension test. Furthermore, the inverted U-shape dose response has been reported for several psychoactive drugs such as benzodiazepines, amphetamine, cocaine, nicotine and morphine. [38, 48] Although further studies are required to elucidate the underlying mechanisms for the activities of OGEO at different doses, our results highlight the importance of testing the effects of psychoactive essential oils over a wide dose range. It is worth mentioning that the doses administered in this study are only given as the concentration of samples administered per cage. Due to the low concentration of the compounds and the simplicity of our apparatus, it was not feasible to measure the true concentration of compounds in the vapor phase that saturated the cage. However, in a previous study, [32] headspace measurement of compounds in the vapor phase (using an SPME/GCMS technique) revealed that

the dose administered correlates positively with the amount of compound in the vapor state.

5. Conclusion

The use of essential oils for the treatment of CNS related disorders seems to be a promising aspect in the field of complementary and alternative medicine. We previously reported that inhalation of the essential oil of *Piper guineense* from Cameroon shows sedative and anxiolytic effects in mice [26]. It is hoped that the aromatherapeutic potentials of indigenous African aromatic plants would be explored further as they could be valuable resources and beneficial in complementary and/or alternative therapy for the management of mental disorders. In conclusion, this study demonstrated that inhalation of the essential oil of *O. gratissimum* shows potent sedative, anxiolytic-like and antidepressant-like effects in mice. These results could be useful in the development of complementary and/or alternative therapies for the management of CNS-related disorders with less invasiveness. Furthermore, *O. gratissimum* has a good potential for exploitation because it is a commonly used spice, cheap, accessible and can be easily cultivated.

6. References

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