Toxicology Reports 4 (2017) 391–398

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

# Neuropharmacological profile and chemical analysis of fresh rhizome essential oil of *Curcuma longa* (turmeric) cultivated in Southwest Nigeria

Idris A. Oyemitan[a,c,*], Christianah A. Elusiyan[b], Ayoola O. Onifade[a], Moses A. Akanmu[a], Adebola O. Oyedeji[c], Armando G. McDonald[d]

[a] Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State 220005, Nigeria
[b] Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State 220005, Nigeria
[c] Department of Chemical and Physical Sciences, Faculty of Natural Sciences, Walter Sisulu University, Nelson Mandela Drive Campus, Mthatha 5117, South Africa
[d] Renewable Materials Program, Department of Forest, Rangeland and Fire Sciences, University of Idaho, Moscow, ID 833844-1133, United States

ARTICLE INFO

*Keywords:*
Turmeric
Essential oil
Turmerone
Behavior
Acute toxicity
Sedative
Anticonvulsant

ABSTRACT

*Background:* *Curcuma longa* (turmeric) is commonly used as spice and also used to treat fever, cough and febrile convulsions in Nigeria. This study determined the chemical composition of the essential oil of *C. longa* and evaluated its neuropharmacological activity in mice.

*Methods:* Essential oil of *C. longa* (EOCL) fresh rhizome was obtained by hydrodistillation and its chemical composition determined by GC–MS. Acute toxicity ($LD_{50}$) profile of the essential oil was determined orally (p.o.) and intraperitoneally (i.p.); and the EOCL (50–200 mg/kg, i.p.) was evaluated for its behavioural, anxiolytic, sedative and anticonvulsant activities using appropriate models in Albino mice (Vom Strain, Jos, Nigeria).

*Results:* Analysis of the oil showed the presence of 23 compounds with turmerone (35.9%) being the major component. The $LD_{50}$ values obtained for the mice were 2154 mg/kg, p.o., and 693 mg/kg, i.p.

The EOCL (50–200 mg/kg, i.p.) caused significant ($p < 0.01$) inhibition of rearing {$F_{(4,20)} = 9$} and locomotor {$F_{(3,16)} = 42$} activity; decreased head dips in hole board {$F_{(4,20)} = 4$}; increased the time spent in the open arms of the elevated pus maze {$F_{(4,20)} = 9$}; prolonged total sleeping time {$F_{(4,20)} = 21$} induced by ketamine injection, and protected mice against pentylenetetrazol-induced convulsions.

*Conclusion:* The major component of the essential oil of this *C. longa* species was turmerone; the oil was slightly toxic orally but moderately toxic intraperitoneally in mice; exhibited significant anxiolytic, sedative and anticonvulsant activities in mice.

## 1. Introduction

The current trend in natural product research involves identifying and validating plant derived substances used ethno-medicinally in the management of various diseases. Incidentally, essential oils obtained from plant materials have been evaluated extensively for biological activities with promising results. Thus many compounds have been isolated from essential oils with proven potency and providing pharmacological basis for ethno-medicinal uses of several plants. *Curcuma longa*, commonly known as turmeric is a tropical herb, native to southern Asia and some parts of Africa. The aromatic yellow powder from its mature rhizomes is extensively used for imparting colour and flavor to food [1,2]. *Curcuma* spp. is used worldwide for its antioxidant, antispasmodic, anti-inflammatory and antimicrobial properties [3,4]. In Southwest Nigeria, the plant especially the rhizome is locally called *Ata ilẹ pupa* [5]. The rhizome of the plant is normally boiled and sipped but it is also added to other herbal agents to manage several ailments. Its folkloric uses includes the treatment of malaria, jaundice, gastric ulcer, skin diseases, rheumatoid arthritis, diabetes, hypertension, cold and flu symptoms, convulsions and emotional disorders [6,7].

Several pharmacological properties have been reported for curcumin, a major component of the alcohol extract of *C. longa* and *C. domestica* rhizomes [8]. The essential oil of *C. longa* has been reported to contain monoterpenes, sesquiterpenes, alcohols and ketones [9,3,10]. Terpinolene and α-phellandrene have been identified as the predominant constituents of leaf oil of a Nigerian grown *C. longa* [11], while [12] reported a different chemotype α-tumerone from Southwest Nigeria. The main focus of previous research study was on the anti-inflammatory, antimicrobial, anti-diabetic and other nutritional properties of the plant as well as on curcumin, ethanolic extract of the rhizome. However, there has been no report on the central nervous system effects of the plant, this study therefore aimed at evaluating the

* Corresponding author at: Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, 220005, Nigeria.
*E-mail addresses:* oyemix@yahoo.com, oyemitan@oauife.edu.ng, ioyemitan@wsu.ac.za (I.A. Oyemitan).

http://dx.doi.org/10.1016/j.toxrep.2017.07.001
Received 8 February 2017; Received in revised form 6 July 2017; Accepted 14 July 2017
Available online 17 July 2017

*in-vivo* neuropharmacological activity, determine the acute toxicity profile and investigate the chemical composition of the essential oil of the studied species since there were different chemotypes of the Nigerian *C longa*. Therefore this study reveals the contribution of the plant's volatile component to its central nervous system activities, toxicological profile and provides rationale for its ethnomedicinal uses.

## 2. Materials and methods

### 2.1. Plant material: identification and collection

Fresh rhizomes and leaves of *Curcuma longa* were collected at Ondo (Nigeria), identified and authenticated (specimen number Ife-16593) by Mr. G. Ibhanesebhor, a taxonomist, Herbarium Unit, Obafemi Awolowo University (OAU).

### 2.2. Extraction and analysis of the essential oil

The fresh rhizomes of *C. longa* (1.5 kg) were hydro-distilled for 4 h using a Clevenger-type apparatus to yield 10.5 g (0.7% w/w) brownish colour essential oil (EOCL) with characteristic pungent smell. The oil was dried over $Mg_2SO_4$ and refrigerated until use.

### 2.3. Gas chromatography–mass spectrometry analysis of the oils

EOCL (0.5 mg/mL in $CH_2Cl_2$) was analyzed by GC–MS (Focus-ISQ, Thermo Scientific). Separation was achieved using a ZB–5 ms capillary column (30 m × 0.25 mm Φ, Phenomenex) and a temperature program of 40 °C (2 min) ramped to 250 °C (10 m) at 5 °C/min. The eluted compounds were identified by determining the Kovat indices, spectral matching with the 2008 National Institute of Standard and Technology (NIST) spectral library and known standard and literatures [13,14].

### 2.4. Drugs and reagents

Diazepam (Valium$^{(R)}$ Roche, Switzerland), pentylenetetrazole (Sigma, USA), strychnine (Sigma, Switzerland), ketamine HCL (Alpha Pharm. Nig.), normal saline (Unique Pharm. Nig. Ltd), Tween 80 and other reagents were of analytical grade.

### 2.5. Laboratory animals

Swiss-Webster mice (originally sourced from the National Veterinary Research Institute (NVRI, Vom, via Jos, Nigeria) of both sexes (18–25 g)) were bred and supplied from the Animal House, Department of Pharmacology, Faculty of Pharmacy, OAU. They were acclimatized to the laboratory environment for 1 week, maintained under 12 h light/dark circle at temperature of 25 ± 2 °C and housed 5 mice per cage in a Plexiglas cage with wood shavings as beddings. The procedures were carried out between 9.00 and 16.00 h. The animals were fed with standard laboratory food for rodents (Top Feed, Benin-City, Nigeria) and water was made available *ad libitum*, however, feed was withheld overnight prior to the test and during the experiment. For each experiment, a mixture of both sexes was chosen randomly for the treatment groups. The animal experiment was carried out in strict compliance with National Institute of Health NIH, 1985 as being implemented by the OAU Research Committee through the Faculty of Pharmacy Postgraduate Committee.

### 2.6. Acute toxicity test

Acute toxicity ($LD_{50}$) of EOCL was determined as described by [15] via both oral and intraperitoneal routes. Three doses (10, 100 and 1000 mg/kg) of emulsified EOCL were administered to three different groups of mice (n = 3). In the second phase, doses of 1000, 2000, 2900 and 5000 mg/kg (n = 1) were used for the oral route because there was no mortality at 1000 mg/kg in this route; while 400, 600, 800 and 1000 mg/kg (n = 1) were used for the intraperitoneal route because there was 100% mortality at 1000 mg/kg in the first phase for this route. The animals were monitored for 2 h and incidence of mortality recorded after 24 h for each group. The $LD_{50}$ values were calculated with the following formula:

$LD_{50} = \sqrt{(A \times B)}$,

Where A and B represent maximum non-lethal and minimum lethal doses respectively

### 2.7. Neuropharmacological studies

#### 2.7.1. General experimental design

Animals were randomly selected into 5 groups (n = 5). Group I serve as the negative control and received the vehicle (5% Tween 80, 10 ml/kg) only. Test groups II–IV were treated with the EOCL at doses of 50, 100 and 200 mg/kg respectively, while group V (positive control) received diazepam (1 mg/kg, i.p.). All treatments were by intraperitoneal (i.p.) route and each treatment was administered 30 min prior to test. The oil forms stable emulsion with Tween 80 and was subsequently used in the formulation of the oil while maintaining the concentration of Tween 80 at ≤5% for all the subsequent bioassays.

#### 2.7.2. Open field test (OFT)

The open field area was constructed of plexiglass box (30 × 30 × 15 cm), with one transparent wall for direct observation, and three opaque walls which prevent distractions and the floor divided into sixteen squares of equal area [16]. The OFT was used to evaluate the animal's exploratory activity. The method used was as described by [17] with slight modification. The novelty-induced behavioural effects scored were rearing (number of times the mouse raised its forepaws in the air or against the wall of the cage for 20 min) and locomotion (the number of squares crossed with all the fore and hind limbs within 10 min).

#### 2.7.3. Anxiolytic test

*2.7.3.1. Hole board test.* The hole-board is a flat space (field) of 25 × 25 $cm^2$ with 16 holes (each 3 cm in diameter). Each treated mouse was placed at the center of the hole-board and the number of head dips was recorded for a period of 5 min [18].

*2.7.3.2. Elevated plus maze.* The elevated plus maze (EPM) used was as described for mice [17,19] was used to evaluate anxiety in mice. Each mouse was observed after 30 min pre-treatment. Each mouse was placed at the center of the maze facing one of the enclosed arms. Number of entries into and time spent on closed and open arms were recorded for 5 min.

#### 2.7.4. Hypothermic test

The rectal temperature of each mouse in all the groups was taken with a digital thermometer (thermoprobe) by inserting the probe 2 cm deep into the anus of the mice shortly before pre-treatment, and after 30 and 60 min post-treatment for all the treatment groups [20].

#### 2.7.5. Sedative test

Animals in each group were pre-treated 30 min prior to ketamine (100 mg/kg, i.p.) injection and immediately dropped inside observation cage [21]. The parameters observed were sleep latency (SL), which is the time interval between injection time and loss of righting reflex; the second is the total sleeping time (TST), which is the time interval between loss and recovery of righting reflex [22].

#### 2.7.6. Anticonvulsant test

*2.7.6.1. Pentylenetetrazole (PTZ)-induced convulsions.* PTZ was used to

induce tonic-clonic convulsions [23]. PTZ (85 mg/kg, i.p.) was administered to different groups of mice (n = 5), which were pretreated with 5% Tween 80, oil (50,100 or 200 mg/kg) or diazepam (1 mg/kg) for 30 min. Each mouse in each group was individually monitored for incidence of convulsions, latency of convulsions, death and mortality. Mice that survived beyond 30 min after PTZ injection were considered protected [24]. Cut-off time of 1800 s was used for mice that did not convulse or died within 30 min for the purpose of statistical analysis.

*2.7.6.2. Strychnine induced convulsions*. Strychnine (2 mg/kg, i.p.) was used to induce tonic-clonic convulsions [25]. Strychnine was administered to different groups of mice (n = 5) as described in previous section. Each mouse in each group was individually monitored for incidence of convulsions, latency of convulsions, death and mortality. Mice that survived beyond 30 min after strychnine injection were considered protected.

*2.7.7. Statistical analysis of data*

The results were presented in Mean ± SEM and analyzed using one-way analysis of variance (ANOVA) followed by post hoc test using Dunnett's test using GraphPad InStat 3 and GraphPad Prism 5. The level of significance was set at 95% confidence interval ($p < 0.05$) for all treatment carried out compared to control group. The results of the anticonvulsant tests for percentage mortality or protection were analyzed with Chi square (non-parametric), while the latency to convulsion and time of death after chemo-convulsant treatment were analyzed with ANOVA followed by Dunnett's post hoc test.

## 3. Results

### 3.1. Chemical composition of the EOCL

Twenty-three compounds were detected while 21 compounds were fully characterized in the gas chromatogram of EOCL (Fig. 1). The most abundant components were oxygenated sesquiterpenes with tumerone (35.9%), curlone (12.9%), and *ar*-tumerone (10.0%). The monoterpene alcohol 1,8-cineole was found to be 10.3% while the monoterpene hydrocarbon α-phellandrene was 15.5%. The five compounds alone accounted for 84.5% of the oil (Table 1). Other minor constituents of EOCL that exceed 1% in composition includes two monoterpenes hydrocarbons; *p*-cymene (2.1%) and terpinolene (3.2%), and two sesquiterpenes hydrocarbons; α-zingiberene (2.0) and β-sesquiphellandrene (1.8%). The relative density of the oil was estimated to be 960 mg/mL.

### 3.2. Acute toxicity effect of EOCL in mice

The oil caused zero and 100% mortality at 2000 and 2900 mg/kg, p.o., while zero and 100% death was observed at 600 and 800 mg/kg, i.p. Hence, the $LD_{50}$ values estimated for EOCL were 2154 mg/kg, p.o., and 693 mg/kg, i.p.

**Table 1**
Chemical Composition of essential oil of fresh rhizome of *Curcuma longa* L.

| Compounds | MW | KI | % Area |
|---|---|---|---|
| α-pinene | 136 | 933 | 0.6 |
| Sabinene | 136 | 975 | 0.2 |
| Myrcene | 136 | 992 | 0.4 |
| α-phellandrene | 136 | 1004 | 15.5 |
| δ-3-carene | 136 | 1010 | 0.3 |
| α-terpinene | 136 | 1016 | 0.5 |
| p-cymene | 134 | 1024 | 2.1 |
| D-limonene | 136 | 1028 | 1.0 |
| 1,8-cineole | 154 | 1031 | 10.3 |
| γ-terpinene | 136 | 1060 | 0.7 |
| Terpinolene | 136 | 1089 | 3.2 |
| terpinen-4-ol | 154 | 1179 | 0.2 |
| α-terpineol | 154 | 1192 | 0.4 |
| β-caryophyllene | 204 | 1425 | 0.2 |
| Unknown | – | 1450 | 0.3 |
| *ar*-curcumene | 204 | 1487 | 0.7 |
| α-zingiberene | 204 | 1498 | 2.0 |
| β-curcumene | 204 | 1512 | 0.2 |
| β-sesquiphellandrene | 204 | 1528 | 1.8 |
| Unknown | – | 1608 | 0.6 |
| *ar*-turmerone | 216 | 1670 | 10.0 |
| Turmerone | 218 | 1674 | 35.9 |
| Curlone | 218 | 1697 | 12.9 |

KI: kovats index; MW: molecular mass.

### 3.3. Effects of EOCL on novelty-induced behaviours in mice

The EOCL at 200 mg/kg, i.p. significantly ($p < 0.01$) decreased rearing {$F_{(4,20)} = 6.4$} and at 50–200 mg/kg, significantly {$p < 0.01$; $F_{(4,20)} = 24$} decreased spontaneous locomotive activity in mice compared to vehicle. Diazepam also caused significant ($p < 0.01$) decrease in rearing and locomotor activity when compared to the negative control (Fig. 2A–B).

### 3.4. Effects of EOCL on anxiety test in mice

*3.4.1. Effect of EOCL on head-dipping in mice*

The EOCL (50–100 mg/kg, i.p.) dose dependently and significantly ($p < 0.05$) increased exploratory head dipping behaviour {$F_{(4,20)} = 4.3$} in mice when compared to negative control. However, at 200 mg/kg, i.p., it caused a significant ($p < 0.05$) reduction in the number of head-dips when compared to the vehicle. Diazepam (1 mg/kg, i.p.) used as positive control similarly caused increase in head dipping activity (Fig. 3).

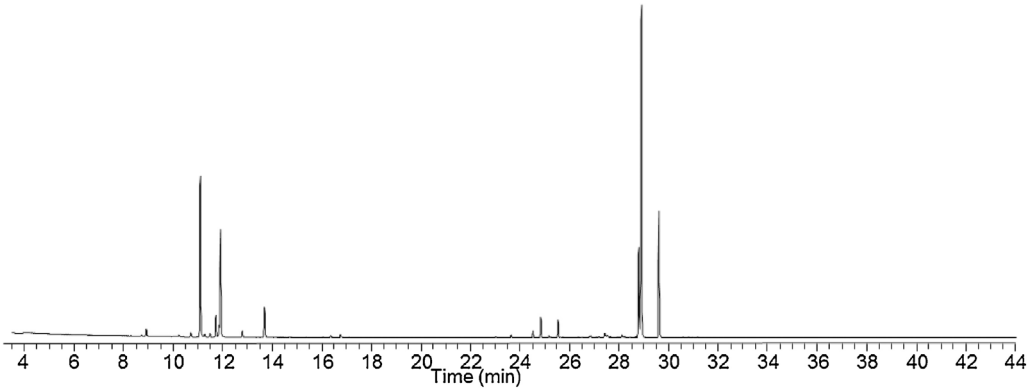


**Fig. 1.** Chromatogram of essential oil of fresh *Curcuma longa* rhizome.

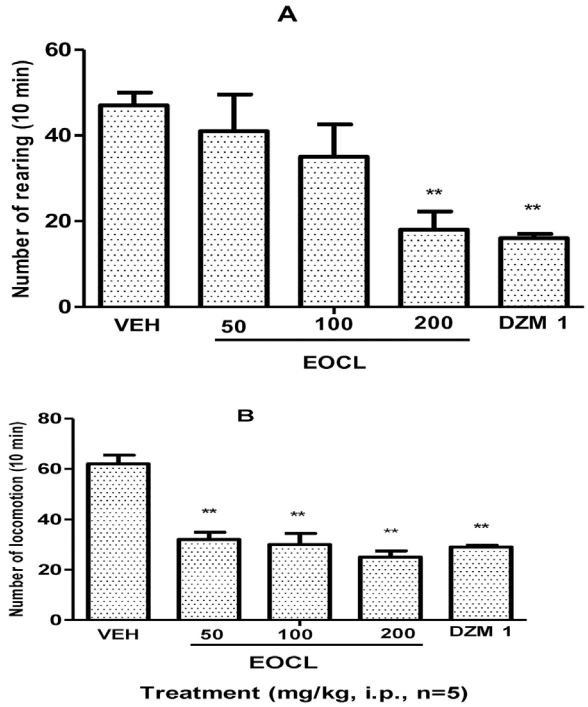


**Fig. 2.** Effects of the EOCL on rearing (Panel A) and locomotor activity (Panel B) in mice. Each bar represents mean ± SEM. VEH, EOCL and DZM represent vehicle (5% Tween 80), essential oil of *C. longa* and diazepam respectively. N = 5.
*p < 0.01; compared to control group (ANOVA, Dunnett's test).

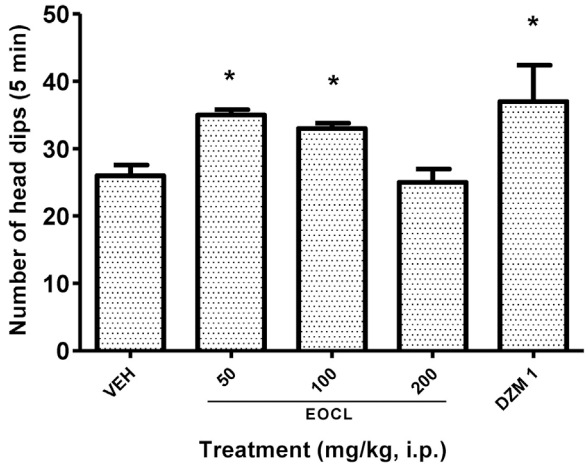


**Fig. 3.** Effect of the EOCl on head-dipping behaviour on the hole board test.
Each bar represents mean ± SEM. VEH, EOCL and DZM represent vehicle (5% Tween 80), essential oil of *C. longa* and diazepam respectively. N = 5.
*p < 0.01, compared to control group, (ANOVA, Dunnett's test).

### 3.4.2. Effects of EOCL on the elevated plus-maze (EPM)

The time spent in the open arms of the EPM increased significantly ($p < 0.05$; $F_{(4,20)} = 14$) with EOCL administration. EOCL at 50, 100 and 200 mg/kg, i.p., caused 49, 55 and 56% increase in the time spent in the open arms compared to 33 and 72% for negative and positive controls respectively. The EOLC at 50 mg/kg caused significant ($P < 0.01$) increase in the% number of entries into the open arms compared to negative group (Fig. 4A–B).

### 3.5. Effect of EOCL on normal rectal temperature of mice

In the EOCL (100 and 200 mg/kg, i.p.) treated groups the oil caused significant ($p < 0.05$) decrease in rectal temperature at 30 and 60 min post-treatment. Diazepam, 1 mg/kg, i.p., only caused significant

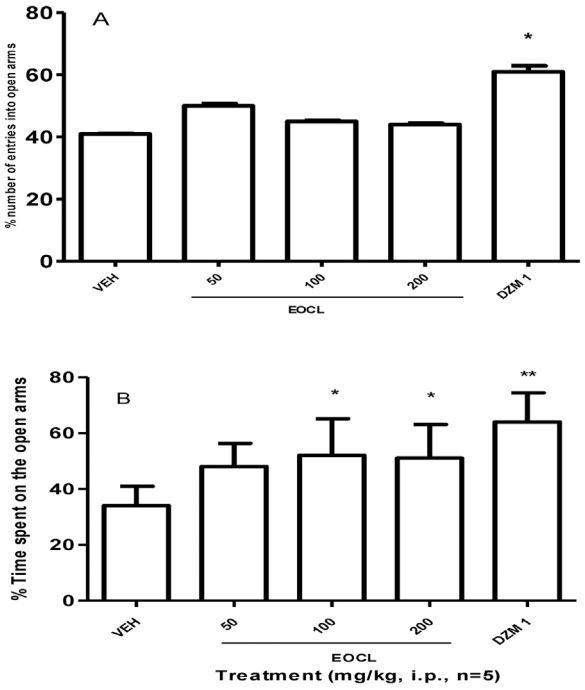


**Fig. 4.** Effect of the EOCL on percentage number of entries into open arms (Panel A) and percentage time spent on open arms (Panel B) of the epm.
VEH, EOCL and DZM represent vehicle (5% Tween 80), essential oil of *C. longa* and diazepam respectively.
*p < 0.05, **p < 0.01; significant compared to vehicle group (ANOVA, Dunnett's test).

**Table 2**
The effect of EOCL on rectal temperature variation in mice.

| Treatment (mg/kg, i.p.) (n = 5) | Variation in rectal temperature at (°C) | | |
|---|---|---|---|
| | 0 min | 30 min | 60 min |
| Control 5%, Tween 80, (10 ml/kg) | 0.0 | −0.70 | −0.12 |
| EOCL 50 mg/kg | 0.0 | 0.48 | 0.30 |
| EOCL 100 mg/kg | 0.0 | −1.70* | −3.12** |
| EOCL 200 mg/kg | 0.0 | −4.16** | −2.98** |
| Diazepam 1 mg/kg | 0.0 | 0.02 | −1.24* |

Each value represents the difference in rectal temperature at different time intervals. EOCL represents essential oil of *C. longa*.
*p < 0.05, statistically different from vehicle.
**p < 0.05, statistically different from other groups (ANOVA, Dunnett's test).

(p < 0.05) reduction in rectal temperature at 60 min post treatment (Table 2).

### 3.6. Effect of EOCL on ketamine-induced hypnosis

The EOCL (50 mg/kg, i.p.) caused significant ($p < 0.05$) decrease and at 100 and 200 mg/kg, i.p., it caused significant decrease ($p < 0.01$) in sleep latency {$F_{(4,20)} = 22.89$} compared to control; Similarly, at the same dose levels, EOCL induced significant [$p < 0.05$–$0.01$; $F_{(4,20)} = 21$] prolongation of ketamine-induced total sleeping time (Fig. 5A–B).

### 3.7. Effect of EOCL chemically-induced convulsions in mice

#### 3.7.1. Effect of EOCL on PTZ-induced convulsions

EOCL (100 and 200 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) completely blocked the PTZ-induced convulsions, prolonged the latency for convulsions and decreased mortality rate (Table 3). However, at the lowest dose of 50 mg/kg, i.p., there was about 20% protections but

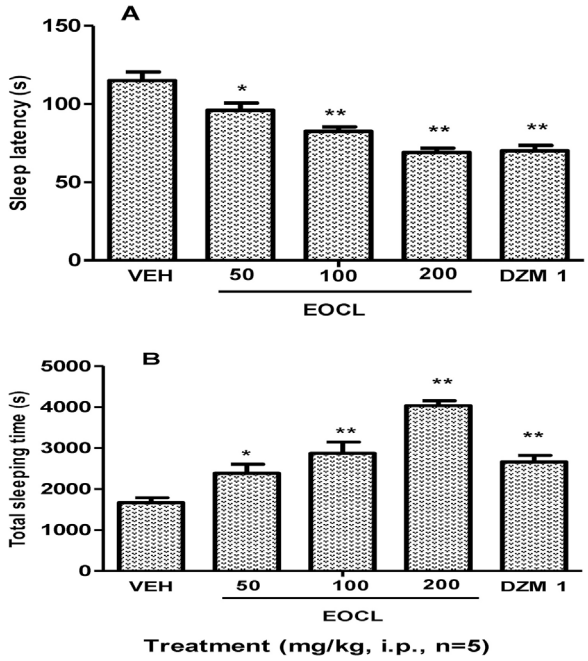


**Fig. 5.** Effect of the EOCL on ketamine-induced sleep latency (Panel A) and total sleeping time (Panel B) in mice.
VEH, EOCL and DZM represent vehicle (control), essential oil of *C. longa* and diazepam respectively. N = 5.
*p < 0.05 compared to control; **p < 0.01compared to other groups (ANOVA, Dunnett's test).

**Table 3**
Effect of the EOCL on pentylene tetrazol-induced convulsions in mice.

| Treatment (mg/kg, i.p., n = 5) | Incidence of convulsions | % Mortality | % Protections |
|---|---|---|---|
| Vehicle (5% Tween 80) | + | 100 | 0 |
| EOCL 50 | + | 80 | 20 |
| EOCL 100 | − | 0 | 100* |
| EOCL 200 | − | 0 | 100* |
| Diazepam 1 | − | 0 | 100* |

(+) means incidence of convulsions; (−) means absence of convulsions.
*p < 0.01, statistically different from the control group (Chi square).

there was statistically significant (P < 0.01) delay in death time of the mice compared to control (Table 4).

### 3.7.2. Effect of EOCL on strychnine-induced convulsions

In the strychnine-induced convulsions, the test materials including EOCL (50,100 and 200 mg/kg, i.p.), 5% Tween 80, and diazepam (1 mg/kg, i.p.) all failed to protect the mice which resulted in mortality.

## 4. Discussion

The chemical composition and the chemotype of the essential oil of *C. longa* were established and its neuropharmacological profile assessed for novelty induced behaviour (NIB), anxiolytic, hypothermic, sedative and anticonvulsant activities in mice. The oil was dominated by oxygenated sesquiterpenes, turmerone and manifested remarkable central effects.

The $LD_{50}$ values of the oil were 693 mg/kg (i.p.) and 2154 mg/kg, p.o. The results of the acute toxicity study revealed that the oil is moderately toxic intraperitoneally but slightly toxic orally [26]. Low oral bioavailability could be responsible for the wide variation in the lethal effect of this oil compare to its effect after intraperitoneal administration as observed in this study and similarly to previous report [27,28]. The implication of this acute toxicity result is that at high doses, the oil could be harmful due to accumulative toxic effect, therefore, its consumption as a spice, condiment, medicinal recipes and flavouring agents should be with caution and closely monitored [29]. The intraperitoneal route was used for further tests because the test agent could be subjected to unpredictable metabolism in the gastrointestinal tract [27,30] and low systemic bioavailability through oral route has been reported for extract of *C. longa* [28]. Furthermore, it has been suggested that the parenteral route is preferable in neuropharmacological tests in order to prevent the biodegradation or inactivation of the test compounds if given through the oral route [31]. There is no pharmacokinetic or toxicokinetic data from human studies on the essential oil component of this plant. However, in previous animal study, the essential oil of *L. longa* was found to cause 20% mortality in mice at ≥28 mg/kg/day for 28 days [27]. Low oral bioavailability could be responsible for the wide variation in the lethal effect of this oil compare to its effect after intraperitoneal administration as observed in this study and similarly to previous report [27,28]. In a clinical trial study, 0.6 ml (≈0.5 g) of Turmeric oil administered orally thrice daily for 12 weeks produce no clinical, hematological, renal or hepatic-toxicity in the volunteers [32]. This report however has the shortcomings of using very few subjects (7) and short duration (12 weeks), which are grossly inadequate statistically and fell short of the minimal standard for chronic studies in humans. Future studies are imperative to evaluate the long term toxicological profile of oral administration of this extract in preclinical and clinical studies.

Dose-dependent inhibitory effects were observed for all exploratory behaviours. The novelty-induced behaviours including rearing and spontaneous locomotor activity were found to decrease significantly (p < 0.01) at all the doses used (Fig. 2A–B). It is has been reported in previous studies that CNS depressant suppresses exploration or inquisitiveness of animals and common sedative drugs have inhibitory effects on exploratory behaviours of animals [33]. Locomotion is believed to be mediated through dopaminergic pathway and other neural mechanisms [34]. It can be suggested that the inhibitory effect of the oil of *C. domestica* on novelty-induced behaviour could be mediated through augmentation of GABA neurotransmission in the CNS as reported for some medicinal plants [20,35]. Rearing is also associated closely with the excitability of the CNS [36] and since this oil significantly decreased rearing and locomotor activity in this study, central inhibitory activity is suggested.

It has been reported that anxiolytic drugs increase exploration of the holes [37]. It was observed that the EOCL at 50 and 100 mg/kg, i.p

**Table 4**
Effect of the EOCL on latency of convulsions and time of death in the pentylene tetrazol – induced convulsions.

| | Vehicle | EOCL mg/kg, i.p., n = 5, | | | diazepam 1 mg/kg, |
|---|---|---|---|---|---|
| | | 50 | 100 | 200 | |
| Onset of Convulsions (s) | 180.0 ± 6.5 | 240.0 ± 6.3 | – | – | – |
| Death time(s) | 162.0 ± 7.2 | 614.0 ± 26.0* | 1800.0# | 1800.0# | 1800.0# |

(−) means absence of convulsions.
* p < 0.01, statistically different from control.
# p < 0.01, statistically different from other groups at the 30 min cut-off time (ANOVA, Dunnett's).

increased the exploratory head dipping behaviour of the animals, however, at 200 mg/kg, i.p., it decreased head dipping. This observation might likely be due to increased sedative effect of EOCL at higher doses compared to diazepam (standard sedative drug) has been shown to be effective in these models (Rabbani et al., 2003. EOCL also increased% time spent by the animals in the open arms to 49, 55 and 56% as the concentration increases compared to negative control (33%), (Fig. 3) indicating anxiolytic effect [38].

The EOCL at 100 and 200 mg/kg, i.p., significantly ($p < 0.05$) caused reduction in rectal temperature at 30 and 60 min post treatment compared to negative control or standard agent, diazepam (1 mg/kg) which caused significant effect at 60 min only (Table 2). Hypothermia is an effect usually observed with benzodiazepine receptor agonists that produce this effect at relatively low doses hence the use of diazepam [39]. Hypothermia has been linked with decrease metabolic heat production and/or vasodilation. The hypothalamus has been reported to regulate body temperature, hence it could be suggested that oil may be mediating its hypothermic effect in the hypothalamus through activation of DA-D2 receptor sites [40].

Results in Fig. 5(A–B) showed that the EOCL (50–200 mg/kg) and diazepam (1 mg/kg) caused significant ($p < 0.05$–$0.01$) reduction in sleep latency (SL), and caused significant ($p < 0.05$–$0.01$) increase in ketamine-induced total sleeping time (TST), indicating sedative effect [21,22]. Ketamine is known to act primarily in the thalamus and limbic systems acting as a non-competitive antagonist of *N*-methyl-*D*-aspartate receptor *(NMDA)* receptor. Thus the EOCD may be mediating its sedative effect through augmentation of the inhibitory activity on the NMDA receptors in addition to general CNS depression. *Curcuma longa* is a component of herbal preparations used in the treatment of fever and mental disorders in Southwest Nigeria thus giving an insight into the rationale for its use in this region.

Furthermore, it was observed that EOCL failed to protect against PTZ-induced convulsions at the lowest dose (50 mg/kg, i.p), but there was significant ($p < 0.05$) delay in time of death compared to negative control (Table 4). However, EOCL higher doses (100 and 200 mg/kg, i.p.) prevented episodes of convulsions and offered 100% protections comparable to diazepam (1 mg/kg, i.p.). This result indicates that this oil demonstrated significant anticonvulsant effect and may be effective in the management of generalized convulsion such as tonic-clonic and myoclonic convulsions as suggested in other studies [41]. The mechanism of action of the anticonvulsant effect of the oil was not evaluated but enhancement or augmentation of $GABA_A$ neurotransmission in CNS may be involved [42]. Strychnine-induced convulsions are closely linked with glycine inhibition in the spinal cord and the brain stem. The ability to reduce percentage mortality after seizures is suggestive of some potential usefulness of a drug against neurotoxicity which normally causes death following extensive seizures. However, the oil at all the tested doses failed to protect the mice against the strychnine-induced convulsions indicating that the oil or its constituent (s) may not have significant facilitatory effect on the glycine neural circuit [43]. The anticonvulsant result obtained in this study provides additional justification for the use of this plant in treating epilepsy by the traditional medical practitioners. Generally, this study gives further evidence for the contribution of *C. longa* to several CNS activities that have been reported for the plant.

The chemical analysis of the essential oil by GC–MS (Table 1) showed the presence of twenty-three (23) components. Four major classes of compounds were displayed in the chemical profile of the oil which are monoterpenes (24.6%), monoterpenoids (10.9%), oxygenated sesquiterpenes (58.7%) and sesquiterpene hydrocarbons (4.8%). The chemical composition of our rhizome oil seems unique when compared to those in literature [9]. Our results showed marked differences in composition of rhizomes collected from North Central Nigeria in which β-bisabolene (13.9%), *trans*-ocimene (9.8%) and myrcene (7.6%) were identified as the major constituents [9]. Interestingly, the rhizome oil showed a close similarity with an earlier study conducted on a Southwest, Nigerian grown *C. longa* spp. leaf and rhizome essential oils. However, the lower monoterpenes α-pinene and *p*-cymene and the monoterpene alcohol 1,8-cineol present in our oil were not detected in the previous report [12].

Although Nigeria has similar climate with India, reports show that the leaf and rhizome oils from India have varying chemical composition [44]. Studies on *C. longa* from other regions also confirmed substantial variability in their chemical composition which may be associated with climatic and geographical factors [45]. There were also global variation in the oil composition of rhizomes from different regions, for instance, α-turmerone (45.3%), linalool (14.9%) and β-turmerone (13.5%) were reported as the main constituent compounds in the oil of the fresh rhizome of Malaysian *C. domestica* [46]. While α-phellandrene (24.5%), 1,8-cineole (15.9%), *p*-cymene (13.2%) and β-pinene (8.9%) were the major ones in found in the leaf oil of the Vietnam species [47]. It is however noteworthy that turmerone or its isomers appears to be present in substantial quantity in most species so far reported but the isomeric curlone is being reported here as a constituent of the essential oil of fresh rhizome from Nigeria for the first time. This report therefore confirms that the Nigerian spp. *C. longa* is unique in its chemical composition compare to either *C. longa* from other regions where α-turmerone (20.1%) and ar-turmerone (68.9%) have been reported to be the major constituents of an Iranian *Curcuma* species [48]. The constituent(s) of the oil responsible for the observed neuropharmacological effects could not be ascertained in this study. However, α-phellandrene has been reported to possess both antinociceptive and anti-inflammatory effect [49]. Also, 1,8-cineol which is the major constituent of many essential oils especially, *Eucalyptus globulus*, has been reported to possess CNS inhibitory activity [50], hypotensive activity in rat [45] and anti-inflammatory activity [51,52]. Moreover, *E. tereticornis* and *E. globulus* essential oils have been reported to contain 60–90% of 1,8-cineole [53] which have also been shown to possess both analgesic and antiinflammatory activities in animals [50]. Therefore, the prominent components of the oil in the present study, contributed majorly to the central inhibitory activities observed for the oil. In view of the remarkable potentials displayed by the plant, its use in ethnomedicine should further be harnessed. We do not have data concerning the pharmacokinetic of this oil in systemic circulation in humans; hence it is imperative to carry out further studies to unravel this.

## 5. Conclusion

The major constituents of essential oil from the fresh rhizome of *C. longa* in this study included turmerone, α-phellandrene, 1,8-cineole, *ar*-turmerone and curlone. The neuropharmacological studies showed that the essential oil of the plant displayed significant CNS depressant activity, and exhibited hypothermic, sedative, anxiolytic and anticonvulsant properties in mice, thus providing evidence that may support the folkloric application of this extract.

## Competing interest

The authors declare that they have no competing interest.

## Authors' contributions

Author IAO designed the project in collaboration with authors CAE and MAA; directly carried out all the animal experiments with author AOO; analyzed all the results; prepared the first draft with author AOO and coordinated the editing, revision and processing of the manuscript for publication. Author CAE carried out the oil extraction; analyzed the oil and interpreted the GC/MS results, edited, revised and processed the manuscript for publication. Author AOO carried out all the animal experiments in conjunctions with author IAO; prepared the first draft and analyzed the results together with author IAO. Author MAA participated in the design of the work; crosschecked and proofread the results

analyses and the manuscript. Authors AOO edited the manuscript. Author AGM provided both the standard and the facility for the analysis of the oil, and also edited the manuscript. All authors read and approved the final manuscript.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Acknowledgements

IAO is grateful to Obafemi Awolowo University for study leave and Walter Sisulu University for post-doctoral fellowship.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.toxrep.2017.07.001.

## References

[1] N.M. Khanna, Turmeric-nature's precious gift, Curr. Sci. 76 (1999) 1351–1356.
[2] H.P. Ammon, M.A. Wahl, Pharmacology of *Curcuma longa*, Planta Med. 57 (1) (1991) 1–7.
[3] B.B. Aggarwal, C. Sundaram, N. Malani, H. Ichikawa, Curcumin: the Indian solid gold, Adv. Exp. Med. Biol. 595 (2007) 1–75.
[4] I. Chattopadhyay, K. Biswas, U. Bandyopadhyay, R.K. Banerjee, Turmeric and curcumin: biological actions and medicinal applications, Curr. Sci. 87 (2004) 44–53.
[5] Z.O. Gbile, Vernacular Names of Nigerian Plants (Yoruba), Caxton Press, Great Britain, 1984.
[6] M.E. Osawaru, R.E. Eholor, Ethnotaxonomy and traditional use of turmeric (*Curcuma longa* L.) among some Nigerian tribes in South Edo State, Nigeria, Plant Arch. 10 (1) (2010) 181–189 http://www.plantarchives.org/.
[7] L.O. Shosan, O.O. Fawibe, A.A. Ajiboye, T.A. Abeegunrin, D.A. Agboola, Ethnobotanical survey of medicinal plants used in curing some diseases in infants in Abeokuta South Local Government Area of Ogun State, Nigeria, Am. J. Plant Sci. 5 (2014) 3258–3268, http://dx.doi.org/10.4236/ajps.2014.521340.
[8] E. Adel-Lateef, F. Mahmoud, O. Hammam, E. El-Ahwany, E. El-Wakil, S. Kandil, H.B. Taleb, M. El-Sayed, H. Hassenein, Bioactive chemical constituents of Curcuma longa L. rhizomes extract inhibit the growth of human hepatoma cell line (HepG2), Acta Pharm. 66 (2016) 387–398.
[9] L.A. Usman, A.A. Hamid, O.C. George, O.M. Ameen, N.O. Muhammad, M.F. Zubair, A. Lawal, Chemical composition of rhizome essential oil of Curcuma longa L. growing in North Central Nigeria, World J. Chem. 4 (2) (2009) 178–181.
[10] R.K. Sharma, B.R. Misra, T.C. Sarma, A.K. Bordoloi, M.G. Pathak, R.A. Leclercq, Essential oils of Curcuma longa L. from Bhutan, J. Essent. Oil Res. 9 (1997) 589–592.
[11] B.O. Oguntimehin, P. Weyerstah, H. Marschall-Weyerstah, Essential oil of *Curcuma longa* L. leaves, Flavour Frag. J. 5 (1990) 89–90.
[12] E.O. Ajaiyeoba, W. Sarna, E.E. Essien, I.O. Olayemi, O. Ekundayo, T.M. Walker, W.N. Setzer, Larvicidal activity of turmerone-rich essential oils of *Curcuma longa* leaf and rhizome from Nigeria on *Anopheles garnbiae*, Pharm. Biol. 46 (2008) 279–282.
[13] R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/ Quardrupole Mass Spectrometry, Allured Publishing Corporation, Illinois, 2004, p. 456.
[14] V.I. Babushok, P.J. Linstrom, G.I. Zenkevich, Retention Indices for frequently reported compounds of plant essential oils, J. Phys. Chem. 40 (4) (2011) 1–35.
[15] D. Lorke, A new approach to practical acute toxicity testing, Arch. Toxicol. 54 (1983) 275–287.
[16] M.A. Akanmu, O.S. Adeosun, O.R. Ilesanmi, Neuropharmacological effects of Oleamide in male and female mice, Behav. Brain Res. 182 (2007) 88–94.
[17] P.B. Gomes, E.C. Noronha, C.T. de Melo, J.N.S. Bezerra, M.A. Neto, C.S. Lino, et al., Central effects of isolated fractions from the root of *Petiveria alliacea* L (tipi) in mice, J. Ethnopharmacol. 120 (2008) 209–214.
[18] H. Takeda, M. Tsuji, T. Matsumiya, Changes in head-dipping behaviour in the hole-boardtest reflect the anxiogenic and/or anxiolytic state in mice, Eur. J. Pharmacol. 350 (1998) 21–29.
[19] S. Pellow, P. Chopin, S.E. File, M. Briley, Validation of open: closed arm entries in an elevated-plus as a measure of anxiety in the rat, J. Neurosci. Methods 14 (1985) 149–167.
[20] T.B. Al-Naggar, M.P. Gomez-Serranillos, M.E. Carretero, A.M. Villar, Neuropharmacological activity of *Nigella sativa* L. extracts, J. Neuropharmacol. 88 (2003) 63–68.
[21] M. Mimura, A. Namiki, R. Kishi, T. Ikeda, H. Miyake, Antagonistic effect of physostigmine on ketamine-induced anaesthesia, Psychopharmacology (Berl.) 102 (1990) 399–403.
[22] M. Rabbani, S.E. Sajjad, H.R. Zareitt, Anxiolytic effects of *Stachys lavandufolia* Vahl on the elevated plus-maze model of anxiety in mice, J. Ethnopharmacol. 89 (2003) 271–276.
[23] E.A. Swinyard, J.H. Woodlead, H.S. White, M.R. Franklin, Experimental selection, quantification and evaluation of anticonvulsants, in: R. Levy, R. Mattson, B.S. Meldrum, J.K. Perry, F.E. Dreifuss (Eds.), Antiepileptic Drugs, Ravens Press, New York, 1989, pp. 85–102.
[24] M.H. Pourgholami, M. Kamalinejad, M. Javadi, S. Majzoob, M. Sayyah, Evaluation of the anticonvulsant activity of the essential oil of *Eugenia caryophyllata*, in male mice, J. Ethnopharmacol. 64 (1999) 167–171.
[25] G.J. Amabeoku, C.G. Kinyua, Evaluation of the anticonvulsant activity of *Zanthoxylum capense* (Thunb.) Harv. (Rutaceae) in mice, Int. J. Pharmacol. 6 (2010) 844–853.
[26] V.J. Rodricks, Calculated Risks; Understanding the Toxicity and Human Risks of Chemicals in Our Environment, Cambridge University Press Cambridge, 1992, pp. 49–64.
[27] J.L. Funk, J.B. Frye, J.N. Oyarzo, H. Zhang, B.N. Timmermann, Anti-arthritic effects and toxicity of the essential oils of Turmeric (*Curcuma longa* L.), J. Agric. Food Chem. 58 (2) (2011) 842–849.
[28] J.S. Jurenka, Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: a review of preclinical and clinical research, Altern. Med. Rev. 14 (2009) 141–153.
[29] European Medicines Agency, Report on Curcuma longa L. Rhizoma, Committee on Herbal Medicinal Products (HMPC) Assessment, European Medicines Agency: Evaluation of Medicines for Human Use 7 Westferry Circus, Canary Wharf, London, 2010 http://www.emea.europa.eu . Doc. Ref.: EMEA/HMPC/456848/2008.
[30] K.E. Stine, T.M. Brown, Principles of Toxicology, 2nd ed., Taylor & Francis Group, CRC Press, Boca Taton, USA, 2006, pp. 1–48.
[31] R.S.M. de Carvalho, F.S. Duarte, T.C. de Lima, Involvement of GABAergic non-benzodiazepine sites in the anxiolytic-like and sedative effects of the flavonoid baicalein in mice, Behav. Brain Res. 22 (1995) 75–82.
[32] J. Joshi, S. Ghaisas, A. Vaidya, R. Vaidya, D.V. Kamat, A.N. Bhagwat, S. Bhide, Early human safety study of turmeric oil (Curcuma longa oil) administered orally in healthy volunteers, J. Assoc. Physicians India 51 (2003) 1055–1060.
[33] M.C. Hellion-Ibarrola, D.A. Ibarrola, Y. Montalbetti, O. Heinichen, E.A. Ferro, Acute toxicity and general pharmacological effect on central nervous system of the crude rhizome extract of *Kyllinga brevifolia* Rottb, J. Ethnopharmacol. 66 (1999) 271–276.
[34] D.L. Jones, G.J. Mogenson, M. Wu, Injections of dopaminergic, cholinergic, serotonergic and GABAergic drugs into the nucleus accumbens: effects on locomotor activity in the rat, Neuropharmacology 20 (1981) 29–37.
[35] G. Zapata-Sudo, T.C.F. Mendes, M.A. Kartnaller, T.O. Fortes, N.F.B. Freitas, M.A.C. Kaplan, et al., Sedative and anticonvulsant activities of methanol extract of *Dorstenia arifolia* in mice, J. Ethnopharmacol. 130 (2010) 9–12.
[36] F.S. Labella, C. Punsky, V. Havlicek, Morphine derivatives with diminished opiate receptor potency showed enhanced central activity, Brain Res. 174 (1979) 263–271.
[37] A.L. da Silva, E. Elisabetsky, Interference of Propylene glycol with the hole board test, Braz. J. Med. Biol. Res. 34 (2001) 545–547.
[38] A.A. Walf, C.A. Frye, The use of the elevated plus maze as an assay of anxiety-related behaviour in rodents, Nat. Protocols 2 (2007) 322–328.
[39] H.C. Jackson, D.J. Nutt, Body temperature discriminates full and partial benzodiazepine receptors agonist, Eur. J. Pharmacol. 185 (1990) 243–246.
[40] M.R. Zarindast, S.A. Tabatabai, Involvement of dopamine receptors subtypes in mouse thermoregulation, Psychopharmacology (Berl.) 107 (1992) 341–346.
[41] B.E. Bastidas-Ramez, R.N. Navvaro, J.D. Quezada-Arellano, M.B. Ruiz, M.T. Villanueva- Michael, P. Garzon, Anti-convulsant effects of Magnolia grandifolia L. in the rat, J. Ethnopharmacol. 61 (1998) 143–152.
[42] R.L. McDonald, K.M. Kelly, Antiepileptic drugs: mechanisms of action, Epilepsia 34 (1993) S1–S8.
[43] R.Q. Huang, C.L. Bell-Horner, M.I. Dibas, D.F. Covey, J.A. Drewe, G.H. Dillon, Pentylene-tetrazole-induced inhibition of recombinant gamma-aminobutyric acid type A ($GABA_A$) receptors: mechanism and site of action, J. Pharmacol. Ther. 298 (2001) 986–995.
[44] V.K. Raina, S.K. Srivastava, N. Jain, A. Ahmad, K.V. Syamasundar, K.K. Aggarwal, Essential oil composition of *Curcuma longa* L. cv. Roma from the plains of northern India, Flavour Fragr. J. 17 (2) (2002) 99–102.
[45] M. Lahlou, Methods to study the phytochemistry and bioactivity of essential oil, Phytother. Res. 18 (2004) 435–448.
[46] I.B. Jantan, A.S. Ahmad, N.A.M. Ali, A.R. Ahmad, H. Ibrahim, Chemical composition of the rhizome oils of four Curcuma species from Malaysia, J. Essent. Oil Res. 11 (6) (1999) 719–723.
[47] N.X. Dung, N.T.B. Tuyet, P.A. Leclercq, Constituents of the leaf oil of *Curcuma domestica* L. from Vietnam, J. Essent. Oil Res. 7 (6) (1995) 701–703.
[48] G.I. Asghari, A. Mostajeran, M. Shebli, Curcuminoid and essential oil components of turmeric at different stages of growth cultivated in Iran, Res. Pharm. Sci. 4 (2009) 55–61.
[49] D.F. Lima, M.S. Brandão, J.B. Moura, J.M.R.S. Leitão, F.A.A. Carvalho, L.M.C.V. Miúra, Antinociceptive activity of the monoterpene α-phellandrene in rodents: possible mechanisms of action, J. Pharm. Pharmacol. 64 (2) (2012) 283–292.
[50] F.A. Santos, V.S. Rao, Antiinflammatory and antinociceptive effects of 1,8-cineole a terpenoid oxide present in many plant essential oils, Phytother. Res. 14 (2000) 240–244.
[51] V.P. Bastos, A.J. Gomes, F.J. Lima, T.S. Brito, P.M. Soares, J.P. Pinho, et al., Inhaled 1,8 cineole reduces inflammatory parameters in airways of ovalbumin-challenged Guinea Pigs, Basic Clin. Pharmacol. Toxicol. 108 (2010) 34–39.

[52] U.R. Juergens, M. Stober, H. Vetter, Inhibition of cytokine production and arachidonic acid metabolism by eucalyptol (1,8-cineole) in human blood monocytes in vitro, Eur. Med. Res. 3 (1998) 508–510.
[53] J. Silva, W. Abebe, S.M. Sousa, V.G. Duarte, M.I.L. Machado, F.J.A. Matos, Analgesic and anti- inflammatory effects of essential oils of Eucalyptus, J. Ethnopharmacol. 89 (2003) 277–283.

**IAO** is a Senior Lecturer, Department of Pharmacology, Faculty of Pharmacy, OAU and a Postdoctoral Fellow, Walter Sisulu University.

**AOO** holds a Bachelor of Pharmacy, OAU and presently engaged in private practice.

**CAE** is a Senior Research Fellow, Drug Research and Production Unit, Faculty of Pharmacy, OAU.

**MAA** is a Professor of Pharmacology, Faculty of Pharmacy, OAU.

**AOO** is a Professor of Organic Chemistry, Walter Sisulu University.

**AGM** is a Professor of Renewable Materials Chemistry, University of Idaho.