IBRO Neuroscience Reports 10 (2021) 66–74

Contents lists available at ScienceDirect

IBRO Neuroscience Reports

journal homepage: www.sciencedirect.com/journal/IBRO-Neuroscience-Reports

Research Paper

Behavioural and biochemical indications of the antidepressant activities of essential oils from *Monodora myristica* (Gaertn) seed and *Xylopia aethiopica* (Dunal) fruit in rats

Raphael Chukwuma Ekeanyanwu [a,*], Chinelo Chinenye Nkwocha [b], Chidinma Lynda Ekeanyanwu [c]

[a] Department of Biochemistry, Imo State University Owerri, Imo State, Nigeria
[b] Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria
[c] Department of Biochemistry, Federal University of Technology, Owerri, Imo State, Nigeria

ARTICLE INFO

Keywords:
Essential oils
Monodora myristica
Xylopia aethiopica
CUMS
Depression
Rat

ABSTRACT

Monodora myristica and *Xylopia aethiopica* are two underutilised spices that are hypothesized to be important in the management and treatment of certain stress-induced diseases such as depression. The present study was designed to test the anti-depressant effects of the essential oils of *Monodora myristica* (EOMM) and *Xylopia aethiopica* (EOXA) and the possible underlying mechanisms in a chronic unpredictable mild stress (CUMS) - induced depression in the rat. Forty-two male Wistar rats were assigned to seven groups (n = 6); group I received corn oil (p.o, unstressed control), group II (stressed control) administered corn oil, groups III-IV received EOMM (150 & 300 mg/kg, p.o), groups V – VI received EOXA (150 & 300 mg/kg, p.o) whereas group VII had fluoxetine (10 mg/kg, p.o in d/w). Corn oil served as the vehicle for the delivery of the essential oils and the doses were administered via gastric intubation to rat once daily for six consecutive weeks from the 2nd week. Open-field, tail suspension (TST), and forced swimming (FST) tests were used to evaluate the behavioural activity in addition to the biochemical parameters (catalase, superoxide dismutase, reduced glutathione, monoamine oxidase, corticosterone, protein carbonyl compound, malondialdehyde and nitric oxide). The result showed that the administration of EOMM (150 and 300 mg/kg b.wt.) and EOXA (150 and 300 mg/kg b.wt.) during CUMS significantly ameliorated these behavioural activities and some biochemical parameters in rats. EOMM and EOXA exhibited significant antidepressant-like effects in a rat model of CUMS. At treatment doses of especially 300 mg/kg b.wt, the antidepressant effects of EOMM and EOXA are comparable to a standard antidepressant drug, fluoxetine (Prozac ™). The EOXA especially at a dose of 300 mg/kg b.wt is more effective than EOMM even at 300 mg/kg dose level in ameliorating depression in stressed rats. In conclusion, the study revealed that both the EOXA and EOMM relieved depression-like states through the mitigation of oxidative stress with a reduction in serum Corticosterone (CORT) and brain Monoamine Oxidase-A (MAO-A) levels.

1. Introduction

Efforts to develop alternative depression treatments have recently been intensified, primarily due to the stigma associated with current therapies as well as the adverse effects of conventional depression treatment medications (APA, 2013), the slow progress of therapeutic results and the violence potential that may lead to suicide attempts (Nemeroff and Owens, 2002). Studies have shown that depression affects at least 121 million people worldwide (WHO, 2008). There is little data on depression rates and their associations, particularly among older adults in Africa (Thapa et al., 2015). A population-based World Mental Health Survey (WMHS) initiative documented lifetime and 12-month major depression at 3.1 per cent and 1.1 per cent respectively in Nigeria (Gureje et al., 2010). Major depression is spreading widely across the globe, and it is reported to be fewer than 21% in South America, 17% in Asia, 14% in North America, 12% in Europe and Asia respectively (Lim et al., 2018).

Depression is caused by neurotransmitter metabolism defects (e.g., serotonin and dopamine) that are subsequently impaired by enzymes involved in their degradation (e.g., monoamine oxidase) or tryptophan precursor synthesis (Vaváková et al., 2015). Inflammatory mechanisms

* Corresponding author.
E-mail address: ekeanyanwuraphael@yahoo.com (R.C. Ekeanyanwu).

https://doi.org/10.1016/j.ibneur.2021.01.001
Received 26 August 2020; Accepted 18 January 2021

Nomenclature

CAT	Catalase
CORT	Corticosterone
CUMS	Chronic Unpredictable Mild Stress
d/w	distilled water
EOMM	Essential oil of *Monodora myristica*
EOXA	Essential oil of *Xylopia aethiopica*
FST	Forced swim test
MAO-A	Monoamine Oxidase-A
MDA	Malondialdehyde
NO	Nitric Oxide
PC	Protein Carbonyl compound
Red. Glut	Reduced Glutathione
SOD	Superoxide dismutase
TST	Tail suspension test
Vit C	Vitamin C

and elevated levels of pro-inflammatory cytokines are also correlated with significant depression, reduced neurogenesis and subsequent neuroprogression (pathological central nervous system reorganization), mitochondrial dysfunction and hypothalamic-pituitary-adrenal axis dysfunction. Decreased antioxidant concentrations and elevated levels of oxidative stress have also been observed (Vaváková et al., 2015). Persons with depression have difficulty in thinking, suffer from loss of pleasure, a headache, disturbed sleep, loss of energy, lowered mood and reduced libido (Mann, 2005; Ekeanyanwu and Njoku, 2015).

Some classes of medications, such as tricyclic antidepressants, monoamine oxidase (MAO) inhibitors, and selective serotonin uptake inhibitors, are existing therapies for depression. Any of the issues associated with the existing treatment protocol for depression are the stigma associated with these treatments in some societies and religions, as well as the adverse effects, the sluggish onset of therapeutic effects and the potential for substance misuse, which can lead to suicide attempts. For just about half of those who seek treatment, existing treatments can perform well even when the disease is diagnosed. To resolve such issues, new therapeutic approaches are needed. Several documented reports on the application of essential oils of different plants in the management of mood and emotions essentially due to their observed anticonvulsive and antidepressant effect (De Sousa et al., 2017) as well as their sedative and anxiolytic effects (Lehrner et al., 2000). The beneficial effect of certain essential oils on symptoms of anxiety and depression has generated interest in the antidepressant effect of essential oils, as they can be a valuable replacement for current depression treatment, which is known to cause a wide range of adverse side effects, such as nausea, increased appetite and weight gain, loss of sexual desire and other sexual issues, tiredness and drowsiness, insomnia, dry mouth, blurry vision, constipation and potential interactions with other medications (Gumnick and Nemeroff, 2000).

M. myristica and *X. aethiopica* are two underutilized spices used as food in Africa and most of the Asian countries. There are several documented reports on the usefulness of their seeds and fruits in traditional medicine to relieve a toothache, dysentery, diarrhoea, dermatitis, a headache, cough, stomach ache, dizziness, amenorrhea, bronchitis, neuralgia, carminative, female infertility, purgative, biliousness, and skin infections (Okwu, 2001; Ishola et al., 2016). *M. myristica* and *X. aethiopica* have also been reported to be used as a vermifuge (Okwu, 2001; Ishola et al., 2016). Previous studies on essential oils of *M. myristica* seed have reported on the chemical compositions (Onyenekwe et al., 1993), as well as antimicrobial and antihypertensive activities (Koudou et al., 2007). There are also several documented reports on the biochemical composition of the essential oils of *X. aethiopica* (Noudjou and Kouninki, 2007; Bakarnga-Via et al., 2014; Konan et al., 2009) as well as antifungal activity (Asekun and Adeniyi, 2004; Tegang et al., 2018), antimicrobial activity (Hassan et al., 2014) and antineoplastic potencies (Bakarnga-Via et al., 2014).

These spices are known to possess components with potent antioxidant activity and are hypothesized to be important in the management and treatment of certain stress-induced diseases such as depression (Biney et al., 2016; Karioti et al., 2004; Konan et al., 2009; Njoku et al., 2012; Akinwunmi and Oyedepo, 2015; Moukette et al., 2015; Tegang et al., 2018). There is no recent report on the antidepressant effect of the essentials oils from these spices. This study aimed to explore and compare the biochemical effects and antidepressant potentials of the essential oils from *M. myristica* seeds and *X. aethiopica* fruits in experimental rats.

2. Materials and methods

2.1. Animals

The animal experimentation committee of the Department of Biochemistry, Imo State University, Nigeria approved the protocol for all animal experiments carried out in this study with ethical number IMSU/EC/02/2019. Processes involving experimental animals were carried out following the National Institute of Health Care Guide for the Care and Use of Laboratory Animals (NIH publication #85 – 23, revised in 1985). All efforts were made to minimalize the animal's pain and to reduce the number of animals used in the experiment. Forty-two male Wistar rats (150–180 g) were used in carrying out the studies. Due to the need for rats with good health, rats were obtained from the Veterinary Research Institute, Vom, Jos, Nigeria. The rats were transported from Vom, Jos to Owerri, Imo State using commercial carrier vehicles. The rats were carried in plastic animal housing cages placed in secondary containers. On arrival in Owerri, Imo State, the rats were housed in the animal house of the department of biochemistry, Imo State University for 2 weeks before being moved to the experimentation room to reduce the impact of the transportation. To allow for free and unhindered access to diet and water, the animals were housed individually under standard colony and specific pathogen-free conditions and maintained with a 12 h light/dark cycle (lights on at 6.00 a.m.) at 25–27 °C and relative humidity of 40–60% which was measured using a CEM hydrometer (DT-615, Shenzhen, China).

2.2. Essential oils

The fresh seeds of *M. myristica* and fresh fruits of *X. aethiopica* were procured from the local farmers in Imo State and authenticated at the Department of Plant Science and Biotechnology, Imo State University Owerri by comparing with voucher specimen present in their herbarium. The seeds and fruits were shade dried for 72 h and pulverised using a manual blender (Corona, Landers Colombia). The ground seeds of *M. myristica* (0.90 kg) and fruits *X. aethiopica* (0.65 kg) were maintained with distilled water (5 L) at 35 ± 2 °C for 7 days to properly macerate them and separately subjected to hydrodistillation using an all-glass Clevenger apparatus (Deschem Equipment Co. Ltd) for 4 h according to the method described by Oliveira et al. (2017) with slight modifications. To ensure high quality of the essential oil extracted, extraction was done from one batch each of the *X. aethiopica* fruit and *M. myristica* seed harvested from the same plant in the same month. The essential oils were separated from hydrolyte by liquid-liquid partitioning, removed with a micropipette and stored in a sterile amber glass vial and kept in a refrigerator (Haier Thermocool) at 4 °C without any further treatment until used for analysis. The purity of the essential oil was determined using a Refractometer by comparing the refractive index with available data.

2.3. Drug and extract administration

Following seven (7) days of acclimatization allowed for the rats to adapt to their new environment, the rats were randomly divided into equal 7 (seven) groups according to their body weight; rats in group I were unstressed and were not exposed to CUMS but administered 2 ml corn oil (vehicle used for diluting the essential oils); rats in group II was treated orally with 2 ml of corn oil; rats in group III to group VI were treated orally with corn oil and different doses of EOMM (150 mg/kg and 300 mg/kg), EOXA (150 mg/kg and 300 mg/kg) all of which were diluted in 2 ml of corn oil. The doses were selected based on the previous studies of the acute and sub-acute toxicity studies on EOXA and EOMM (Ekeanyanwu et al., 2020; Bakarnga-via et al., 2014). The doses selected were below the toxic dose. The Positive control (group VII) was administered with 10 mg/kg of fluoxetine hydrochloride (Prozac®) dissolved in distilled water. Fluoxetine was selected since it's the commonly prescribed antidepressant drug and is used in various kinds of psychiatric disorders (Bhardwaj et al., 2009). The administration of CUMS, antidepressant drugs and the essential oils was done daily for 5 weeks starting on the second day of the CUMS procedure.

2.4. Chronic unpredictable mild stress (CUMS) procedure

Animal model of depression was used to assess the antidepressant effects of EOMM and EOXA in the rats. The chronic depressive disorder model was induced by chronic unpredictable mild stress (CUMS) according to the methods described by Zhao et al. (2018) and Okoh et al. (2020) with minor modifications to the protocol. Briefly, stressors were administered once daily between 08:30 a.m. and 10:30 a.m., except for the 24-h duration stressors. The stressors consisted of the following mild stressors such as:

- 5-min of forced swimming in warm water (at 37 °C) – *Day One;*
- 24-h wet litter (100 ml of water and 50 g of litter mixed) – *Day Two;*
- 24 h of feed denial – *Day Three*;
- 90-s tail pinch – *Day Four*;
- 24 h of water denial – Day Five;
- 5 min of forced swimming in cold water (at 4 °C), after which they were towelled dry then – *Day Six*;
- 24-h cage tilt (we tilted the cages to 45° from the plane) – *Day Seven*.

The stressors were distributed randomly with intervals of at least 7 days and then administered three times within 6 weeks precisely on week 2, week 4 and week 6. At the end of the period of administration, the animals were subjected to behavioural tests (forced swim test and tail suspension test on day 1 whereas the Open field test on day 2). Immediately after the forced swim test and tail suspension test, the rats were briefly exposed to stress by denying them food for 24 h before being subjected to the open field test. About 5 ml of blood was collected from the retro-orbital plexus without the use of topical anaesthesia after overnight fasting and sera were prepared by centrifugation at 640 *g* and 4 °C for 10 min (Ekeanyanwu and Njoku, 2015) and then stored at − 20 °C for different biochemical analysis. Immediately after blood collection, the rats were sacrificed by decapitation with a rodent guillotine (Harvard Apparatus, USA) and their brains carefully and quickly dissected out and rinsed in ice-cold saline (0.9% NaCl). The hippocampus of both sides of the brain was carefully removed following the established technique explained by Spijker (2011) and homogenised as stated by Shukkoor et al. (2016). The supernatant we obtained after homogenisation was collected and stored in dry ice. The experimental protocol is shown in Fig. 1.

2.5. Behavioural Evaluation

Animals were taken to the experimentation room in a clean cage, approximately 2 h before behavioural evaluation and all behavioural tests were performed following a double-blinded procedure. The behavioural changes were evaluated through the forced swim test, open field test, and tail suspension test.

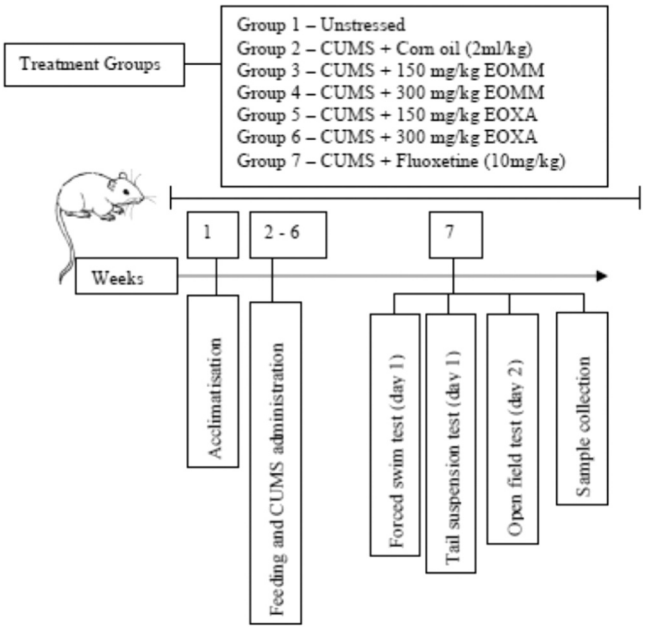

Fig. 1. The experimental design. Abbreviations: CUMS, chronic unpredictable mild stress, EOMM, essential oil of *M. myristica*, EOXA, essential oil of *X. aethiopica*.

2.5.1. Forced swim test and measurement of immobility

we used the modified standard method of Lucki (1997) for the forced swim test and measurement of immobility on week seven of the experiment. For this study, a test apparatus consisting of a vertical cylindrical glass container (46 cm high, 21 cm in diameter) filled with 25 ± 0.5 °C tap water to a depth of 30 cm was used. This depth was adequate to ensure that animals with their hind paws or their tails did not touch the bottom of the container. The rats were subjected to a 15-min swimming test session and then removed, cleaned, towelled, and returned to their cage. For each rat, the water was changed. All the animals were subjected to 4 min of forced swimming the next day, and the three behaviours we reported are (1) climbing behaviour, described as upward-directed forepaw movements along the side of the swim chamber; (2) swimming behaviour, the (usually horizontal) movement in the swim chamber, which also involves crossing into another quadrant; and (3) immobility, assigned if no additional activity is observed other than that necessary to hold the head of the rat above the water.

2.5.2. Tail suspension test

For the evaluation of behaviour despair a feature of depression, the tail suspension test model was used as described by Can et al. (2012) of depressive-like behaviour. In this method, at a height of 23.5 cm from the ground, rats were suspended upside down on a metal rod in a tail suspension box using an adhesive tape placed approximately 1 cm from the tip of the tail. The total time of immobilisation during the 6-minute test was recorded through direct observation and video recording. When not making any struggling movements, the rat was considered immobile, trying to catch the adhesive tape, body torsion or jerks for at least 5 s

2.5.3. Open field test

Open field test is a commonly used model of anxiety-like behaviour developed to measure animal emotionality and is focused on subjecting an animal to an unfamiliar area whose escape is prevented by

surrounding walls on week 7 of the experiment (Gogas et al., 2007). We presented the animals as defined by Wang et al. (2012). The open-field box is used in this, which is a rectangular area consisting of a hard floor measuring 60 cm × 60 cm × 40 cm and made of white painted wood. The floor was split into 16 equal squares at the bottom using permanent read markings, placed each rat individually in one corner of the field, and recorded the total locomotion and rearing frequency for each 10-minute cycle. After each of these assays, to remove olfactory bias, the area was cleared with 70 per cent alcohol and the area allowed drying out before adding a fresh rat.

2.5.4. Biochemical analysis

Biochemical parameters were analysed using standard laboratory kits for the different parameters according to their various manufacturers' guidelines. For the assay of Brain Monoamine oxidase (Mono-A) activity and determination serum Corticosterone levels, Monoamine oxidase A assay kit (Sigma Aldrich) and serum Corticosterone ELISA kit (DRG Diagnostics, Marburg, Germany) for rat respectively were used. For the quantitative determination of antioxidant system markers in the brain homogenate, superoxide dismutase was assayed using superoxide dismutase colourimetric assay kit (Bioassay Systems, Hayward, USA), catalase was assayed using the catalase colourimetric assay kit (Bioassay Systems, Hayward, USA), reduced glutathione (GSH) level was determined using the reduced glutathione assay kit (Cepharm Life Sciences, Baltimore, USA) and serum ascorbic acid (vitamin C) was determined using the ascorbic acid assay kit (Bioassay Systems, Hayward, USA). The protein content of the supernatant obtained from the Hippocampi of rats was determined using the Bradford protein assay kit (Cepharm Life Sciences, Baltimore, USA). For the measurement of oxidative stress markers, malondialdehyde (MDA) an indication of lipid peroxidation in animal tissues was assayed using thiobarbituric acid reacting substance (TBARS) assay kit (Bioassay Systems, Hayward, USA). As a characteristic of protein oxidation, total protein carbonyl (PC) content was determined using the Protein Carbonyl assay kits (Sigma Aldrich). Nitric oxide (NO) level was estimated by nitrate/nitrite Greiss reaction after conversion of nitrate to nitrite using the Nitric Oxide assay kit (Bioassay Systems, Hayward, USA).

2.6. Statistical analysis

Changes in all behavioural and biochemical parameters for all rats were determined using one way ANOVA followed by Bonferroni post hoc comparison test. A p-value of less than 0.01 was taken as significant. All data obtained were expressed as Mean ± Standard Error Mean (Mean ± SEM). SPSS 19.0 was used to analyze the data.

3. Results

3.1. Behavioural results

The results of the behavioural analysis were presented in Figs. 2 and 3. Generally, there was a significant increase ($p < 0.01$) in immobility duration and immobility time in the tail suspension test in rats exposed to CUMS when compared to the unstressed rats. Similarly, there was a significant reduction ($p < 0.01$) in the number of squares crossed and the number of rearing instances in the open field test in rats exposed to CUMS when compared to the unstressed rats.

Analysis of data showed differences between EOMM and EOXA and Fluoxetine in forced swim test (FST) and tail suspension test (TST) parameters. Particularly, a trend towards a statistically significant decrease ($p < 0.01$) in immobility duration (in FST) is observed after oral administration of EOMM and EOXA when compared to the stressed group and corn oil (vehicle) group. However, statistical analysis indicates a significant increase ($p < 0.01$) in immobility duration in rats treated with EOMM and EOXA versus fluoxetine. No significant difference was observed in the immobility duration and immobility time in rats treated with EOMM compared to EOXA treated rats.

Analysis of data indicates that oral administration of EOMM and EOXA induces significant differences in the frequencies of crossing indicated in the number of squares crossed (Fig. 3A) and rearing indicated in the number of rearing instances (Fig. 3B) when compared to the corn oil (vehicle) group. Specifically, the rats treated with the higher dose of EOMM and EOXA showed a higher increase of crossing and rearing that reach statistically significant when compared to CUMS rats (Fig. 3). Conversely, fluoxetine administration to stressed rats significantly ($p < 0.01$) increased the frequency of crossing and rearing when compared to the vehicle group.

3.2. Hippocampal enzymatic/nonenzymatic antioxidant defence systems and markers of oxidative stress

The results presented in Figs. 4 and 5 demonstrated that; 1. A significant decrease ($p < 0.01$) in the enzymatic defence system parameters (CAT and SOD) in rats administered CUMS, however, administration of different doses of EOMM and EOXA significantly ($p < 0.01$) increased the CAT and SOD activities in the stressed rats. 2. A

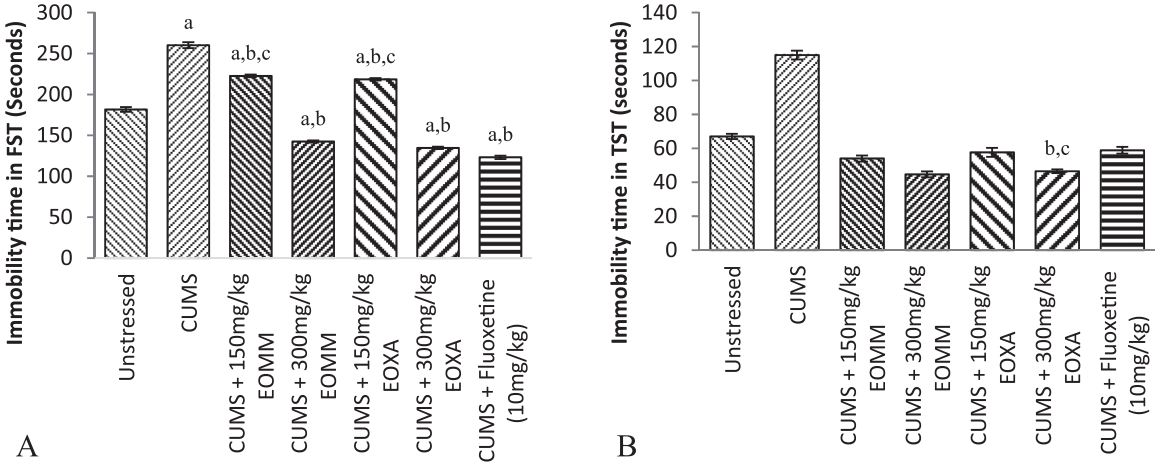

Fig. 2. The effect of the essential oils on Immobility time in FST (A) and immobility time in TST (B) in rats after oral administration of EOMM and EOXA, Fluoxetine or Corn oil (one way ANOVA followed by multiple post hoc comparisons of means). a) $p < 0.01$ compared to an unstressed group, b) $p < 0.01$) compared to the stressed group. c) ($p < 0.01$) compared to fluoxetine group. CUMS: Chronic Unpredictable Mild Stress; EOMM: Essential oil of *M. myristica*; EOXA: Essential oil of *X. aethiopica*.

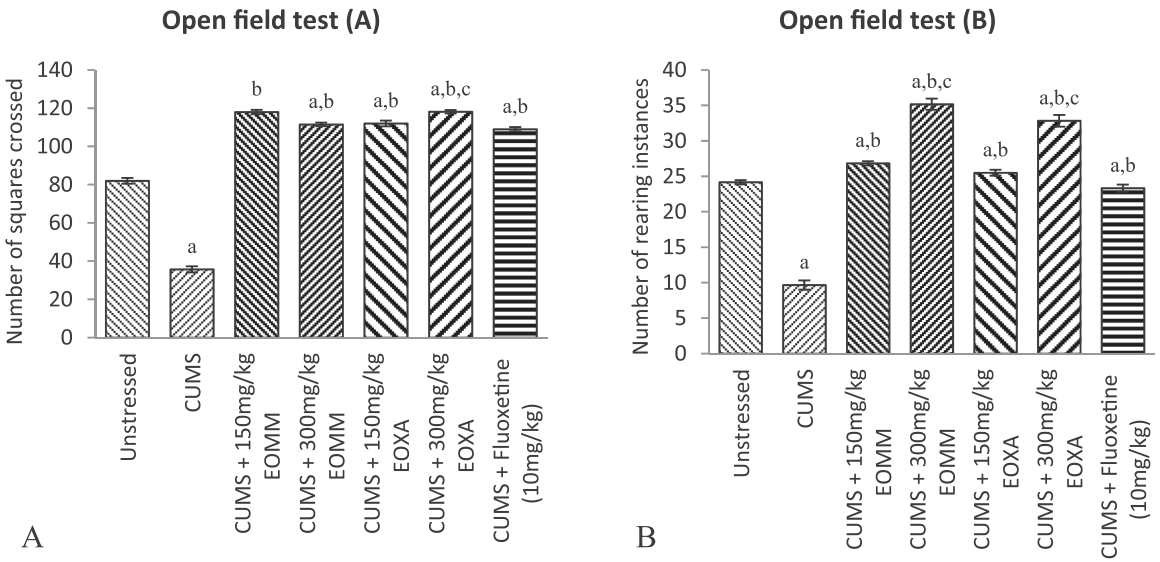

Fig. 3. Number of squares crossed (A) and the number of rearing instances (B) in rats after oral administration of EOMM and EOXA, Fluoxetine or Corn oil (one way ANOVA followed by an LSD test for multiple post hoc comparisons of means). a) $p < 0.01$ compared to an unstressed group, b) $p < 0.01$) compared to the stressed group. c) ($p < 0.01$) compared to fluoxetine group. CUMS: Chronic Unpredictable Mild Stress; EOMM: Essential oil of *M. myristica*; EOXA: Essential oil of *X. aethiopica*.

significant increase ($p < 0.01$) in the non-enzymatic antioxidant defence system parameters (Red GSH and Vit C) were observed in rats administered CUMS. It was also observed that EOMM and EOXA significantly ($p < 0.01$) lowered the GSH and Vit C levels in stressed rats. 3. As expected, fluoxetine significantly ($p < 0.01$) increased enzymatic (CAT and SOD) and non-enzymatic (Red GSH and Vit C) defence system parameters while significantly ($p < 0.01$) lowering the various doses of the markers of oxidative stress (PC, MDA and NO) in stressed rats.

3.3. Enzymatic defence and Non-enzymatic system

See Fig. 4.

3.4. Markers of oxidative stress

The result of the effect of different treatment of EOMM, EOXA and fluoxetine on some markers of oxidative stress such as protein carbonyl, malondialdehyde and nitric oxide is presented in Fig. 5. Oral administration of EOMM and EOXA significantly ($p < 0.01$) increased PC, MDA and NO levels in the stressed rats. Fluoxetine administration to stressed rats expectedly decreased significantly ($p < 0.01$) the levels of PC, MDA and NO in stressed rats.

3.5. Monoamine oxidase activity

A significant increase ($p < 0.01$) in brain MAO-A activity was observed in the Hippocampi after administration of CUMS. Interestingly, oral administration of EOMM and EOXA significantly reduced brain monoamine oxidase activity in the stressed rats. As expected administration of Fluoxetine significantly decreased ($p < 0.01$) the brain monoamine oxidase activity in stressed rats.

3.6. Corticosterone level

A significant increase ($p < 0.01$) in the serum Corticosterone level was observed after administration of CUMS. As evident from Fig. 7 there was a significant decrease ($p < 0.01$) in the Corticosterone level in rats administered different concentrations of EOMM and EOXA as well as Fluoxetine.

4. Discussion

The major findings from this research work can be summarised as follows: (a) Administration of EOXA, EOMM and CUMS to rats significantly suppressed any changes in behavioural parameters in the stressed rats (b) Significant alteration in the enzymatic defence system parameters (CAT and SOD), non-enzymatic defence system parameters (Red. GSH and Vitamin C) and markers of oxidative stress (PC, MDA and NO) were suppressed in the stressed rats after administration of EOXA, EOMM and the antidepressant drug fluoxetine. (c) Significant decrease in the serum levels the stress hormone CORT as well as a reduction in the activity of MAO-A which mediates the production of reactive oxygen species in the stressed rats after administration of EOXA, EOMM and the antidepressant drug fluoxetine. (d) Generally, it was observed that the EOXA especially at a dose of 300 mg/kg b.wt is more effective than EOMM even at 300 mg/kg dose level in ameliorating depression in stressed rats.

Antidepressants that inhibit serotonin and/or norepinephrine reuptake lower immobility time and increase a rat's swinging activity in the forced swim and tail suspension studies, an activity that was not significantly altered in rats treated with EOXA and EOMM. Essential oils from plants such as Lavender, Rosemary and Lemon are well documented to reduce immobility while increasing curling behaviour (Isabel et al. 2019; Villareal et al. 2017; Hao et al. 2013). The reduction in immobility period can be considered as an antidepressant-effect index (Stéru et al., 1985). Besides, our findings suggested that fluoxetine therapy (10 mg/kg) reversed the reduced exploratory activity caused by the CUMS procedure in an open field study for 4 weeks. In the open field test, no increased exploratory activity was observed in animals treated with EOMM and EOXA at all doses, suggesting that the reduced FST immobility is not due to any psychomotor stimulant activity, thus confirming the antidepressant-like effect observed in FST.

The results of the hippocampal antioxidant defence state presented in Figs. 5 and 6 demonstrated that stress-induced by CUMS in rats was associated with a significant decrease in hippocampal SOD and CAT activity and depletion in levels of GSH and ascorbic acid as compared with unstressed rats. Also, the markers of lipid peroxidation (MDA) and protein oxidation (PC), as well as NO, were significantly elevated in the stressed group, which confirmed the susceptibility of brain tissues to oxidative stress. These results suggested that decreased antioxidant

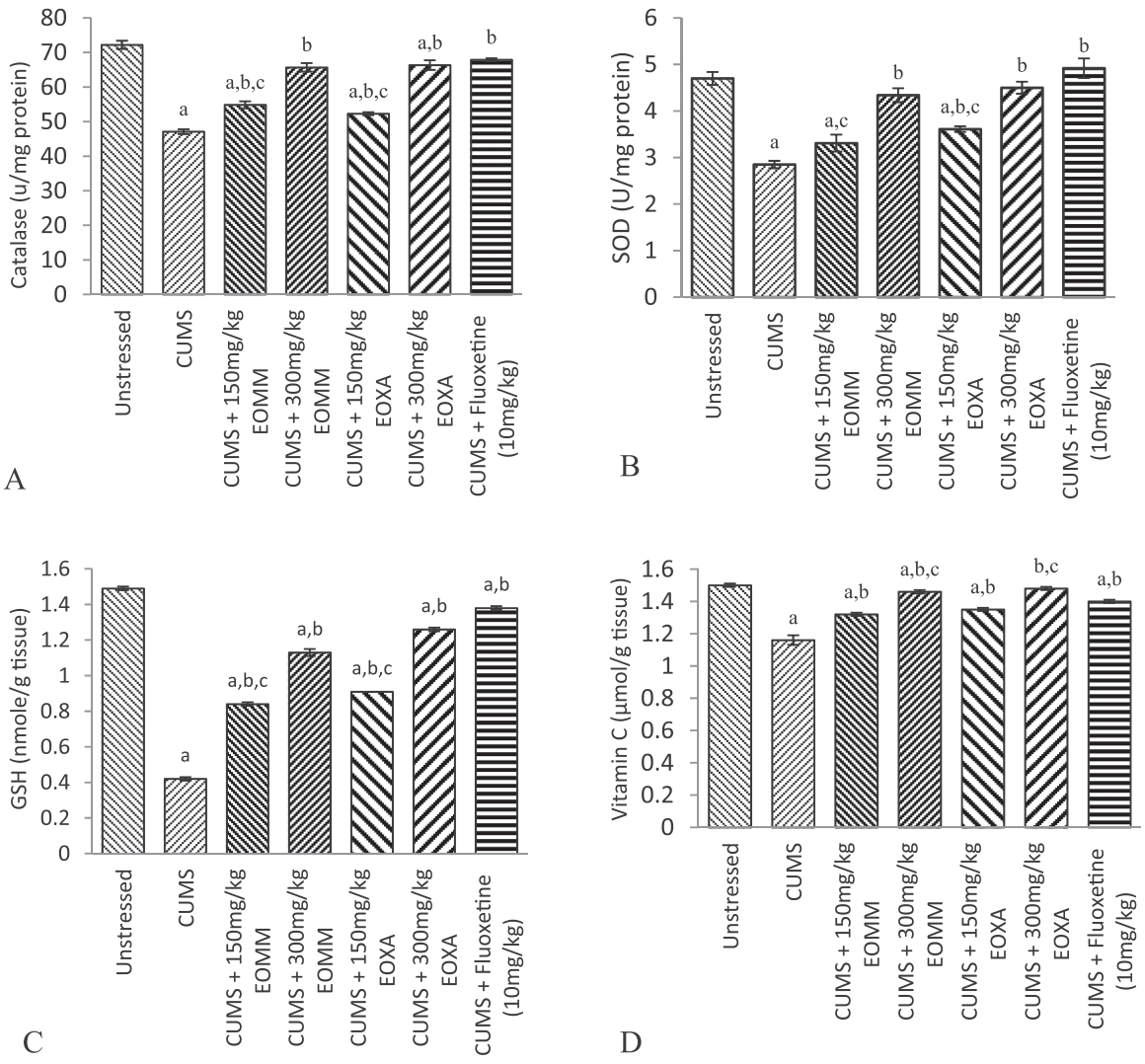

Fig. 4. Effect of different treatment on catalase level (A), Superoxide dismutase level (B), Glutathione level (C) and Vitamin C level (D) of the rat (one way ANOVA followed by an LSD test for multiple post hoc comparisons of means). a) $p < 0.01$ compared to an unstressed group, b) $p < 0.01$) compared to the stressed group. c) ($p < 0.01$) compared to fluoxetine group. CUMS: Chronic Unpredictable Mild Stress; EOMM: Essential oil of *M. myristica*; EOXA: Essential oil of *X. aethiopica*.

enzyme activities result in the accumulation of ROS and negatively correlated with the severity of depression. On the contrary, oral administration of both EOXA and EOMM at different doses of 150 mg/kg b.wt and 300 mg/kg b.wt for 5 weeks to rats with stress-induced by CUMS significantly restored the hippocampal redox balance state and attenuated CUMS-induced oxidative damage (Figs. 5 and 6). The best oxidative stress prevention effect was obtained with fluoxetine, followed with EOXA and then EOMM at the highest dose of 300 mg/kg b.wt in the hippocampal enzymatic/nonenzymatic antioxidant defence systems and oxidative stress markers. Lipid peroxidation occurs when the production of ROS is greater than the antioxidant potential (Zarrindast, 2012). Oxidative stress products provide key markers for the diagnosis and assessment of depressive status and the evaluation of antidepressant efficacy (Vaváková et al., 2015). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and nonenzymatic endogenous reduced glutathione GSH are responsible for detoxifying ROS in the brain tissue (Halliwell, 2006). The reduced concentration of ascorbic acid (Vitamin C) has also been observed in depressed patients, and its intravenous administration not only amplifies the effectiveness of antidepressants but also acts as an antidepressant itself (Gautam et al., 2012). Many nonenzymatic antioxidant deficiencies are associated with worsening symptoms of depression and anxiety (Gautam et al., 2012). Depression can cause shifts in the function of oxidative stress-related enzymes (Vaváková et al., 2015).

Several studies have shown that elevated CORT serum levels induced hippocampal ROS development, leading to memory function deficits and hippocampal dysfunction (Sato et al., 2010; You et al., 2009; Liu et al., 2012). In this study, compared to unstressed rats, rats treated with CUMS showed a significant increase in serum CORT level and MAO-A brain activity (Fig. 7). This effect was almost restored, reflecting the antidepressant-like capacity, by oral administration of both EOXA and EOMM and by fluoxetine relative to rats administered by CUMS. A growing body of proof has shown that MAO inhibition is effective in treating mice with depressive-like behaviour (Villarinho et al., 2012). According to the monoamine hypothesis, the concentrations of monoamines, such as serotonin, noradrenaline, and dopamine, in synaptic gaps are decreased in the depressive state. This research has shown that EOXA and EOMM can potentially lower the concentration of the stress hormone Corticosterone and stop reactive oxygen species formation by inhibiting MAO-A formation. MAOs are enzymes that metabolize monoamines and MAO-A levels are significantly increased throughout the brain in patients with depression (Meyer et al., 2006).

M. myristica and *X. aethiopica* are two spices which are very rich in antioxidants phytochemicals. The present study revealed that the essential oils (EOXA and EOMM) from these spices was also good antidepressants. Further analysis of our data has shown that the EOXA,

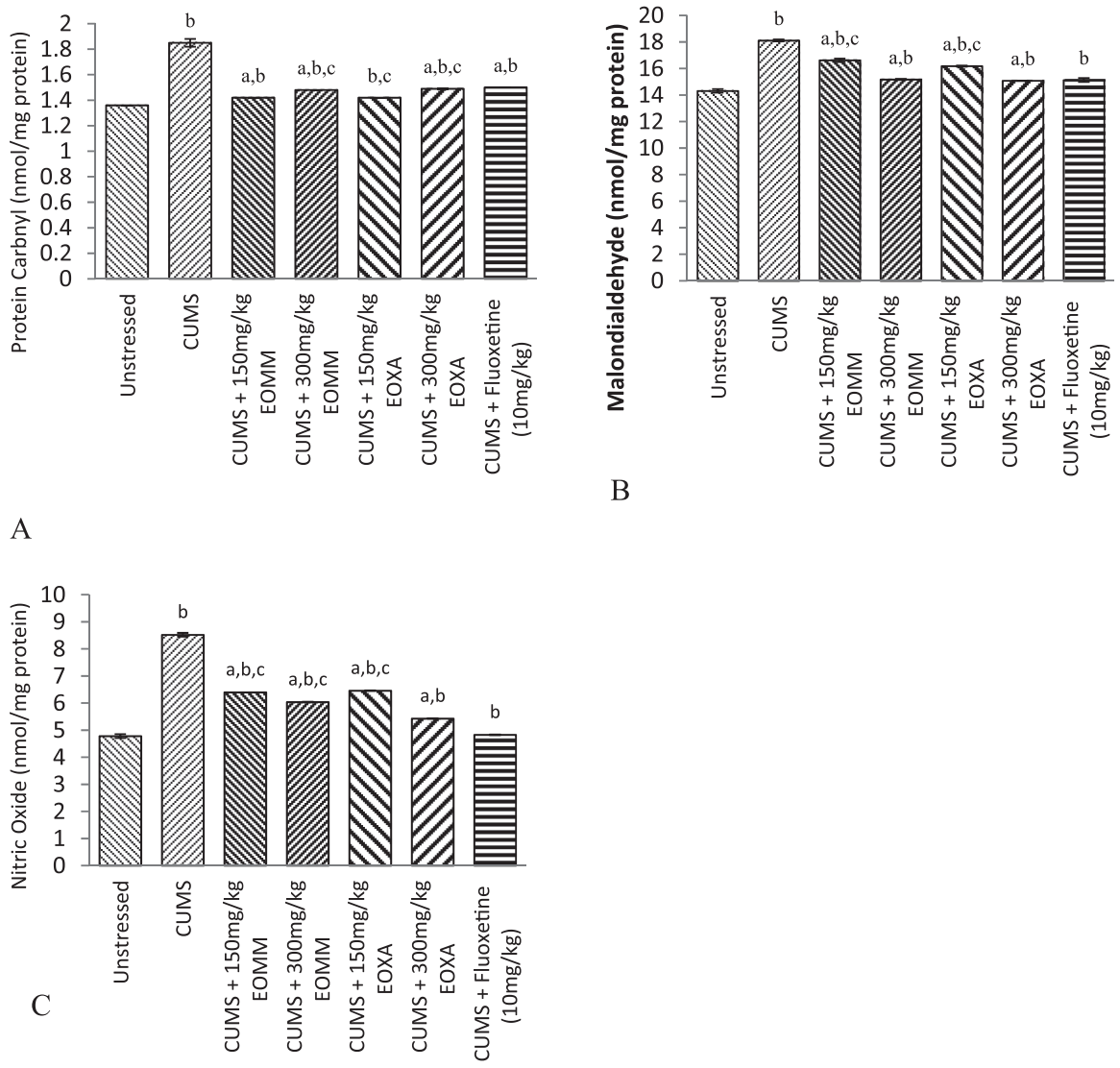

Fig. 5. Effect of different treatment on Protein carbonyl content (A), Malondialdehyde level (B) and Nitric Oxide level (C) of the rat (one way ANOVA followed by an LSD test for multiple post hoc comparisons of means). a) $p < 0.01$ compared to an unstressed group, b) $p < 0.01$) compared to the stressed group. c) ($p < 0.01$) compared to fluoxetine group. CUMS: Chronic Unpredictable Mild Stress; EOMM: Essential oil of *M. myristica*; EOXA: Essential oil of *X. aethiopica*.

especially at a dose of 300 mg/kg b.wt, is more effective than EOMM even at 300 mg/kg dose level in ameliorating depression in stressed rats. Studies have shown that the essentials oil from *M. myristica* and *X. aethiopica* essentials are dominated by monoterpenes (Bakarnga-Via et al., 2014). Monoterpenes have been reported to possess antidepressant activities (Salakhutdinov et al., 2017). We, therefore, hypothesized that the antidepressant property of EOXA and EOMM might be due to its rich monoterpenes content. In summary, the study has revealed the benefits of EOXA and EOMM administration to reverse the development of depression. The result also suggests that EOXA and EOMM may have regulated the antidepressant pathways by the restoration of changes in oxidative stress and a decrease in serum Corticosterone and MAO-A levels in the brain. These findings are consistent with evidence recorded on the multifaceted action of essential oils via modulation effect on both serotonergic and dopaminergic systems in the brain, especially in the striatum and hippocampus (Sănchez-vidaña et al., 2019), as well as promoting neurogenesis and improving dendritic branching in stressed rats (Chemg-wei et al., 2013).

4.1. Conclusion

In conclusion, the study found that depression-like states are relieved by both EOXA and EOMM by reducing oxidative stress with a decrease in serum CORT and brain MAO-A levels. This research, in line with our hypothesis, supported the link between oxidative stress and inflammatory responses in depression and provides in vivo evidence of the effectiveness of essential oils *M. myristica* and *X. aethiopica* in reducing stress and anxiety-like behaviour and biochemical changes in CUMS induced rats. Consequently, *M. myristica* and *X. aethiopica* essential oils can be effective, low-cost and an alternative therapeutic agent for the treatment of stress-related disorders including depression. Nonetheless, more research is needed to explain these findings in humans, which may help a novel therapeutic approach to slow down depression symptoms.

Funding

This project did not receive any sort of funding from granting bodies in the public, commercial, or not-for-profit sectors.

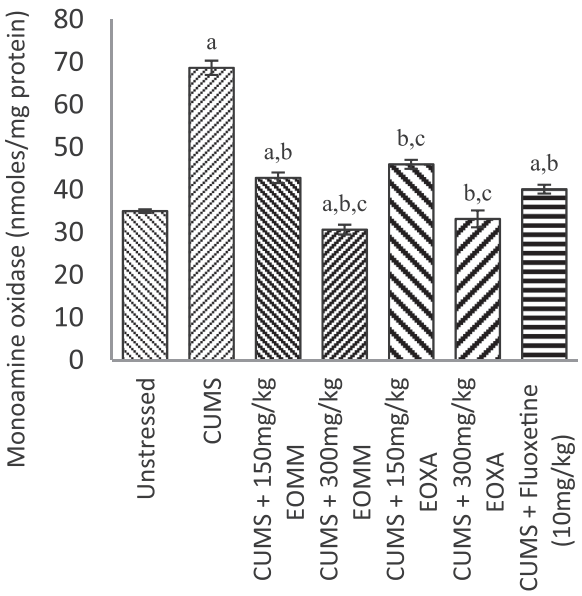

Fig. 6. Effect of different treatment on Monoamine oxidase level of the rat (one way ANOVA followed by an LSD test for multiple post hoc comparisons of means). a) $p < 0.01$ compared to an unstressed group, b) $p < 0.01$) compared to the stressed group. c) ($p < 0.01$) compared to fluoxetine group. CUMS: Chronic Unpredictable Mild Stress; EOMM: Essential oil of *M. myristica*; EOXA: Essential oil of *X. aethiopica*.

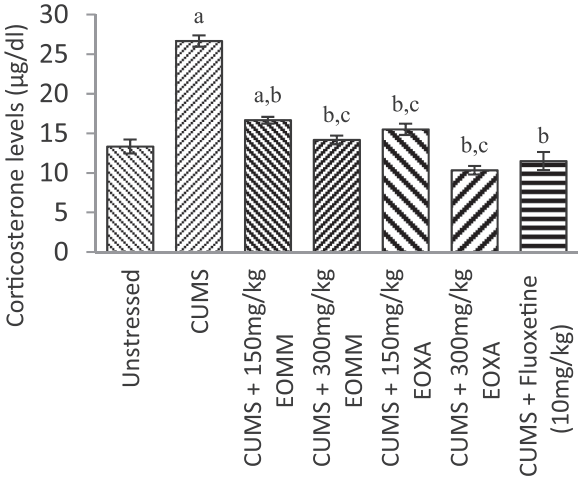

Fig. 7. Effect of different treatment on the Corticosterone level of the rat (one way ANOVA followed by an LSD test for multiple post hoc comparisons of means). a) $p < 0.01$ compared to an unstressed group, b) $p < 0.01$) compared to the stressed group. c) ($p < 0.01$) compared to fluoxetine group. CUMS: Chronic Unpredictable Mild Stress; EOMM: Essential oil of *M. myristica*; EOXA: Essential oil of *X. aethiopica*.

Compliance with Ethical Standards

The animal experimentation committee of Imo state University, Department of Biochemistry approved the protocol for all animal experiments carried out in this study. Processes involving experimental animals were carried out following the National Institute of Health Care Guide for the Care and Use of Laboratory Animals (NIH publication #85 – 23, revised in 1985). We made all efforts to minimalize the animal's pain and to reduce the number of animals used in the experiment.

CRediT authorship contribution statement

Ekeanyanwu, Raphael Chukwuma: Conceptualization, Methodology, Validation, Investigation, Writing - Review & Editing, Supervision, Project administration; **Nkwocha, Chinelo Chinenye**; **Ekeanyanwu, Chidinma Lynda**: Writing - Original Draft, Project administration, Visualization, Resources.

Acknowledgement

The authors are thankful to the management of Imo State University for providing Intuitional and Infrastructural support.

Contributions

The authors contributed equally to this research.

Conflicts of Interest

The authors declare no conflict of interest.

References

Akinwunmi, K.F., Oyedepo, O.O., 2015. *In vitro* anti-inflammatory evaluation of African nutmeg (*Monodora myristica*) seeds. Euro J Med. Plants 8, 167–174. https://doi.org/10.9734/EJMP/2015/17853.

APA, 2013. Diagnostic and statistical manual of mental disorders. 5th ed. Washington, DC. DOI:⟨10.1176/appi.books.9780890425596⟩.

Asekun, O.T., Adeniyi, B.A., 2004. Antimicrobial and cytotoxic activities of the fruit essential oil of *Xylopia aethiopica* from Nigeria. Fitoterapia 75 (3/4), 368–370. https://doi.org/10.1016/j.fitote.2003.12.020.

Bakarnga-Via, I., Hzounda, J.B., Fokou, P.V., Tchokouaha, L.R., Gary-Bobo, M., Gallud, A., Garcia, M., Walbadet, L., Secka, Y., Dongmo, P.M., Boyom, F.F., Menut, C., 2014. Composition and cytotoxic activity of essential oils from *Xylopia aethiopica* (Dunal) A. Rich, *Xylopia parviflora* (A. Rich) Benth) and *Monodora myristica* (Gaertn) growing in Chad and Cameroon. BMC Complement. Altern. Med. 14 (14), 125. https://doi.org/10.1186/1472-6882-14-125.

Bhardwaj, P., Kantharia, N.D., Yadav, P., Panwar, A., 2009. Effect of fluoxetine on some cognitive functions of patients of depression. Indian J. Psychol. Med. 31 (1), 24–29. https://doi.org/10.4103/0253-7176.53311.

Biney, R.P., Benneh, C.K., Ameyaw, E.O., Boakye-Gyasi, E., Woode, E., 2016. *Xylopia aethiopica* fruit extract exhibits an antidepressant-like effect via interaction with serotonergic neurotransmission in mice. J. Ethnopharmacol. 184, 49–57. https://doi.org/10.1016/J.JEP.2016.02.023.

Can, A., Duo, D.T., Terrillion, C.E., Piantadosi, S.C., Bhat, S., Gould, T.D., 2011. The tail suspension test. J. Vis. Exp. 59, 3759. https://doi.org/10.3791/3769.

Chemg-wei, H., Wen-sung, L., Chi-tang, H., Lee-Yan, S., 2013. Antidepressant-like effect of lemon essential oil is through a modulation in the levels of norepinephrine, dopamine and serotonin in mice: use of the tail suspension test. J. Funct. Foods 5, 370–379.

Ekeanyanwu, R.C., Mgbedo, N.E., Njoku, A.T., 2020. Safety assessment of essential oils from *Xylopia aethiopica* (Dunal) fruit from Nigeria. Biomed. Res. Ther. 7 (7), 3865–3876. https://doi.org/10.15419/BMRAT.V7I7.616.

Ekeanyanwu, R.C., Njoku, O.U., 2015. Flavonoid-rich fraction of the *Monodora tenuifolia* seed extract attenuates behavioural alterations and oxidative damage in forced-swim stressed rats. Chin. J. Nat. Med. 13 (3), 183–191. https://doi.org/10.1016/s1875-5364(15)30003-0.

Gautam, M., Agrawal, M., Gautam, M., Sharma, P., Gautam, A., Gautam, S., 2012. Role of antioxidants in generalised anxiety disorder and depression. Indian J. Psychiatry 54 (3), 244–247. https://doi.org/10.4103/0019-5545.102424.

Gogas, K.R., Lechner, S.M., Markison, S., William, J.P., McCarthy, N., Grigoriadis, D.E., Foster, A.C., 2007. Anxiety. Comprehensive Med Chem II, pp. 85–115.

Gumnick, J.F., Nemeroff, C.B., 2000. Problems with currently available antidepressants. J. Clin. Psychiatry 61 (Supplement 10), 5–15. ⟨https://pubmed.ncbi.nlm.nih.gov/10910012/⟩.

Gureje, O., Uwakwe, R., Oladeji, B., Makanjuola, V.O., Esan, O., 2010. Depression in adult Nigerians; results from the Nigerian Survey of Mental Health and Well being. J. Affect. Disord. 120 (1–3), 158–164. https://doi.org/10.1016/j.jad.2009.04.030.

Halliwell, B., 2006. Oxidative stress and neurodegeneration: where are we now? J. Neurochem. 97 (6), 1634–1658. https://doi.org/10.1111/j.1471-4159.2006.03907.x.

Hao, C., Lui, W., Ho, K., Sheen, L., 2013. Antidepressant-like effect of lemon essential oil is through a modulation in the levels of norepinephrine, dopamine and serotonin in mice; use of the tail suspension test. J. Funct. Foods 5, 370–379. https://doi.org/10.1016/j.jff.2012.11.008.

Hassan, A.M., Almagboul, A.Z., Abdelgadir, H., 2014. Antimicrobial activity of the fruits essential oil of *Xylopia aethiopica* (Dunal) A. Rich. Adv. Med. Plant Res. 2 (4), 58–61. ⟨http://www.netjournals.org/pdf/AMPR/2014/4/14-027.pdf⟩.

Isabel, S.D., Kai-Ting, P.K., Kai-Hang, F.T., Ka-wing, C.J., Kwok-wai, L.W., Pui-Kin, S., Wui-man, L.B., Wing-Hong, T.H., 2019. Lavender essential oil ameliorates depression-like behaviour and increases neurogenesis and dendritic complexity in rats. Neuroscience 701, 180–192. https://doi.org/10.1016/j.neulet.2019.02.042.

Ishola, I.O., Ikumawoyi, V.O., Afolayan, G.O., Olorife, O.J., 2016. Antinociceptive and anti-inflammatory properties of hydroethanolic seed extract of *Monodora myristica* (Annonaceae) in rodents. West Afr. J. Pharm. 27, 22–32. ⟨http://www.wapharm.org/wajp/admin/12389900798187/antinociceptive_and_anti-inflammatory_properties_of_hydroethanolic.pdf⟩.

Karioti, A., Hadjipavlou-Litina, D., Mensah, M.L.K., Fleischer, T.C., Skaltsa, H., 2004. Composition and antioxidant activity of the essential oils of *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) leaves stem bark and fresh and dried fruits growing in Ghana. J. Agric. Food Chem. 52 (26), 8094–8098. https://doi.org/10.1021/jf040150j.

Konan, N., Kouame, B.A., Mamyrbekova-Bekro, J.A., Nemlin, J., Yves-Alain, B., 2009. Chemical composition and antioxidant activities of essential oil of *Xylopia aethiopica* (Dunal)A rich. Eur. J. Sci. Res. 37 (2), 311–318.

Koudou, J., Etou Ossibi, A.W., Akikokou, M., Bessiere, J.M., 2007. Chemical composition and hypotensive effects of essential oils of *Monodora myristica* Gaertn. J. Biol. Sci. 7 (6), 937–942. https://doi.org/10.3923/jbs.2007.937.942.

Lehrner, J., Eckersberger, C., Walla, P., Pötsch, G., Deecke, L., 2000. Ambient odour of orange in a dental office reduces anxiety and improves mood in female patients. Physiol. Behav. 71 (1–2), 83–86. https://doi.org/10.1016/s0031-9384(00)00308-5.

Lim, G.Y., Tam, W.W., Lu, Y., Ho, C.S., Zhang, M.W., Ho, R.C., 2018. Prevalence of depression in the community from 30 countries between 1994 and 2014. Sci. Rep. 8, 2861. https://doi.org/10.1038/s41598-018-21243-x.

Liu, Y., Guan, W., Ren, G., Yang, Z., 2012. The possible mechanism of silver nanoparticle impact on hippocampal synaptic plasticity and spatial cognition in rats. Toxicol. Lett. 209 (3), 227–231. https://doi.org/10.1016/j.toxlet.2012.01.001.

Lucki, I., 1997. The forced swimming test as a model for core and component behavioural effects of antidepressant drugs. Behav. Pharmacol. 8, 523–532. https://doi.org/10.1097/00008877-199711000-00010.

Mann, J.J., 2005. The medical management of depression. N. Engl. J. Med. 353, 1819–1834. https://doi.org/10.1056/NEJMra050730.

Meyer, J.H., Ginovart, N., Boovariwala, A., Sagarati, S., Hussey, D., Garcia, A., Young, T., Praschak-Rieder, N., Wilson, A.A., Houle, S., 2006. Elevated Monoamine oxidase A levels in the brain: an explanation for the monoamine imbalance of major depression. Arch. Gen. Psychiatry 63 (11), 1209–1216. https://doi.org/10.1001/archpsyc.63.11.1209.

Moukette, B.M., Piene, C.A., Njimou, J.R., Biapa, C.P.N., Mario, B., Ngogang, B., 2015. In vitro antioxidant properties, free radicals scavenging activities of extracts and polyphenol composition of a non-timber forest product used as a spice; *Monodora myristica*. Biol. Res. 48, 1–17. https://doi.org/10.1186/s40659-015-0003-1.

Nemeroff, C.B., Owens, M.J., 2002. Treatment of mood disorders. Nat. Neurosci. 5 (Supplements), 1068–1070. https://doi.org/10.1038/nn943.

Njoku, U.O., Akah, P.A., Okonkwo, C.C., 2012. Antioxidant activity of seed extracts of *Monodora tenuifolia* (ANNONACEAE). IJBAS-IJEN 12, 2.

Noudjou, F., Kouninki, H., 2007. Composition of *Xylopia aethiopica* (Dunal) A Rich. Essential oils from Cameroon and identification of a minor diterpene: ent-13-epi manoyl oxide. Biotechnol. Agron. Soc. Environ. 11 (3), 193–199. ⟨https://popups.uliege.be/1780-4507/index.php?id=830⟩.

Okoh, L., Ajayi, A.M., Ben-Azu, B., Akinluyi, E.T., Emokpae, O., Umukoro, S., 2020. D-Ribose-L-cysteine exhibits adaptogenic-like activity through inhibition of oxido-inflammatory responses and increased neuronal caspase-3 activity in mice exposed to unpredictable chronic mild stress. Mol. Biol. Rep. 47 (10), 7709–7722. https://doi.org/10.1007/s11033-020-05845-1.

Okwu, D.E., 2001. Evaluation of the chemical composition of indigenous spices and flavouring agents. Global J. Pure Appl. Sci. 7 (3), 455–459. https://doi.org/10.4314/gjpas.v7i3.16293.

Oliveira, J.D., Alves, D.K.M., Miranda, M.L.D., Alves, J.M., Xavier, M.N., Cazal, C.M., Alves, C.C.F., 2017. Chemical composition of essential oil extracted from leaves of *Campomanesia adamantium* subjected to different hydrodistillation times. Ciênc. Rural 47 (1), 1–7. https://doi.org/10.1590/0103-8478cr20151131.

Onyenekwe, P.C., Ogbadu, G., Deslaurier, H., Gagnon, M., Collin, G.J., 1993. Volatile constituents of the essential oil of *Monodora myristica* (Gaertn) Dunal. J. Sci. Food Agric. 61, 379–381. https://doi.org/10.1002/jsfa.2740610317.

Salakhutdinov, N.F., Volcho, K.P., Yarovaya, O.I., 2017. Monoterpenes as a renewable source of biologically active compounds. Pure Appl. Chem. 89 (8), 1105–1117. https://doi.org/10.1515/pac-2017-0109.

Sānchez-vidaña, D.I., Po, K.I., Fund, T.K., Chow, J.K., Lau, W.K., So, P., Lau, B.W., Tsang, H.W., 2019. Lavender essential oil ameliorates depression-like behaviour and increases neurogenesis and dendritic complexity in rats. Neurosci. Lett. 701, 180–192. https://doi.org/10.1016/j.neulet.2019.02.042.

Sato, H., Takahashi, T., Sumitani, K., Takatsu, H., Urano, S., 2010. Glucocorticoid generates ROS to induce oxidative injury in the Hippocampus, leading to impairment of cognitive functions of rats. J. Clin. Biochem. Nutr. 47 (3), 224–232. https://doi.org/10.3164/jcbn.10-58.

Shukkoor, M.S.A., Baharuldin, M.T.H.B., Jais, A.M.M., Moklas, M.A.M., Fakurazi, S., 2017. Antidepressant-like effect of lipid extract ofchanna striatusin postpartum model of depression in rats. Evid.-Based Complement. Altern. Med. 2017, 2986090 https://doi.org/10.1155/2017/1469209.

De Sousa, D.P., Silva, R.H.N., de Silva, E.F., Gavioli, E.C., 2017. Essential oils and their constituents: an alternative source for novel antidepressant. Molecules 22, 1290. https://doi.org/10.3390/molecules22081290.

Spijker, S., 2011. Dissection of rodent brain regions. Neuroproteomics 57, 13–26. https://doi.org/10.1007/978-1-61779-111-6_2.

Stéru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test; a new method for screening antidepressant in mice. Psychopharmacology 85, 367–370. https://doi.org/10.1007/BF00428203.

Tegang, A.S., Buemo, T.M.N., Dongmo, P.M.J., Ngoune, L.T., 2018. Essential oil of *Xylopia aethiopica* from Cameroun: chemical composition, antiradical and in vitro antifungal activity against some mycotoxigenic fungi. J. King Saud Univ.-Sci. 30 (4), 466–471. https://doi.org/10.1016/j.jksus.2017.09.011.

Thapa, S.B., Martinez, P., Clausen, T., 2015. Depression and its correlates in South Africa and Ghana among people aged 50 and above; findings from the WHO study on global AGEing and adult health. J. Psychiatry 17 (6), 1000167. https://doi.org/10.4172/2F1994-8220.1000167.

Vaváková, M., Ďuračková, Z., Trebatická, J., 2015. Markers of oxidative stress and neuroprogression in depression disorder. Oxid. Med. Cell. Longev. 2015, 898393 https://doi.org/10.1155/2015/898393, 12 pages.

Villareal, M.O., Ikeya, A., Sasaki, K., Ben Arfa, A., Neffati, M., Isoda, H., 2017. Anti-stress and neuronal cell differentiation induction effects of *Rosmarinus officinalis* L. essential oil. BMC Complement. Altern. Med. 17, 549. https://doi.org/10.1186/s12906-017-2060-1.

Villarinho, J.G., Fachinetto, R., de Vargas Pinheiro, F., da Silva Sant'Anna, G., Machado, P., Dombrowski, P.A., da Cunha, C., de Almeida Cabrini, D., Pinto Martins, M.A., Gauze Bonacorso, H., Zanatta, N., Antonello Rubin, M., Ferreira, J., 2012. Antidepressant-like effect of the novel MAO inhibitor 2-(3,4-dimethoxy-phenyl)-4,5-dihydro-1H-imidazole (2-DMPI) in mice. Prog. Neuropsychopharmacol. Biol. Psychiatry Behav. Brain Res. 39 (1), 31–39. https://doi.org/10.1016/j.pnpbp.2012.04.007.

Wang, M., Song, J., Xiao, W., Yang, L., Yuan, J., Wang, W., Yu, Z., Xie, M., 2012. Changes in lipid-sensitive two-pore domain potassium channel TREK-1 expression and its involvement in astrogliosis following cerebral ischemia in rats. J. Mol. Neurosci. 46, 384–392. https://doi.org/10.1007/s12031-011-9598-z.

WHO, 2008. The WHO STEPwise Approach to Chronic Disease Risk Factor Surveillance (STEPS). WHO STEPS Instrument,, Geneva, Switzerland. ⟨https://www.who.int/ncds/surveillance/steps/STEPS_Instrument_v2.1.pdf⟩.

You, J.M., Yun, S.J., Nam, K.N., Kang, C., Won, R., Lee, E.H., 2009. Mechanism of glucocorticoid-induced oxidative stress in rat hippocampal slice cultures. Can. J. Physiol. Pharmacol. 87 (6), 440–447. https://doi.org/10.1139/y09-027.

Zarrindast, M.R., 2012. Acute administration of Zn, Mg, and thiamine improves postpartum depression conditions in mice. Arch. Iran. Med. 15 (5), 306 doi:012155/aim.0012.

Zhao, J., Niu, C., Wang, J., Yang, H., Du, Y., Wei, L., Li, C., 2018. The depressive-like behaviours of chronic unpredictable mild stress-treated mice ameliorated by Tibetan medicine Zuotai: involvement in the hypothalamic-pituitary-adrenal (HPA) axis pathway. Neuropsychiatr. Dis. Treat. 14, 129–141. https://doi.org/10.2147/NDT.S151107.