Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Journal of Pharmacology and Toxicology

ISSN 1816-496X
DOI: 10.3923/jpt.2026.10.15

Research Article
Zingiber officinale Essential Oil as a Potential Therapeutic Alternative for Mental Health Management

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Abstract

Background and Objective: Insomnia-related stress is closely associated with elevated oxidative stress and increased production of reactive oxygen species (ROS), which can contribute to mental health disturbances. Natural antioxidants such as *Zingiber officinale* (ginger) essential oil may offer a therapeutic alternative for managing such conditions. The present study aimed to evaluate the efficacy of ginger (*Zingiber officinale*) essential oil in regulating ROS production in Wistar rats subjected to insomnia-induced stress. **Materials and Methods:** Ginger essential oil was extracted through hydrodistillation using a rotary evaporator and its bioactive compounds were identified using Gas Chromatography-Mass Spectrometry (GC-MS) in electron impact (EI) mode. Insomnia was induced in Wistar rats using two different models: (i) Continuous exposure to bright light with controlled heat for three days and (ii) Oral administration of coffee solution (20 mg/mL) at a dose of 50 mL/kg twice daily for three days. **Results:** The findings revealed that ginger essential oil exhibited significant antioxidant activity by reducing ROS levels in treated groups, with a reduction ranging from 11.98 to 53.72% compared to induced stress groups. **Conclusion:** *Zingiber officinale* essential oil demonstrates promising antioxidant potential and may serve as a credible alternative therapeutic approach for the management of insomnia-related health disorders.

Key words: Mental health, reactive oxygen species, *Zingiber officinale*, Ivory Coast

Citation: Alexis, B.G., B. Abou, A.D.S. David, B. Lydie and A.A. Hervé *et al*., 2026. *Zingiber officinale* essential oil as a potential therapeutic alternative for mental health management. J. Pharmacol. Toxicol., 21: 10-15.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

According to the World Health Organization[1], mental health is defined as "A state of well-being in which an individual can realize their own ability, cope with the normal stresses of life, work productively and efficiently and is able to contribute to their community". Mental health is viewed as a global public health issue and one of the United Nations' Sustainable Development Goals for 2030 (SDG 3). As such, mental health problems are public health issues. Stress and insomnia are considered to be two of the main risk factors for mental health disorders. Insomnia, or a lack of sleep, can be associated with oxidative stress as well[2,3]. According to Davinelli *et al.*[4], individuals with insomnia tend to exhibit increased levels of oxidative stress markers than those experiencing healthy sleep. The interaction between insomnia and stress create a negative feedback where the accumulation of reactive oxygen species (ROS) inhibits the physiological functions that regulate sleep[5,6] and similarly, a lack of sleep increases oxidative stress. Antidepressants, anxiolytics and hypnotics are often used to treat these disorders but may have negative side effects on physical health[7]. On account of these adverse effects, there is a growing interest in finding other therapeutic alternatives based on natural substances. Essential oils therefore, occupy a prominent place among these natural compounds[8,9].

This study evaluate the ability of ginger essential oils to regulate ROS production in Wistar rats subjected to insomnia-induced stress. This research study is intended to the treatment of mental health by using local natural resources available in our area.

MATERIALS AND METHODS

Study sites: Essential oil extraction and molecular characterisation were carried out at the Laboratory of Natural Substances (UFR SSMT). Animal experimentation was performed at the Laboratory of Animal Physiology and pharmacology for Natural Substances (Faculty of Biosciences). Biological parameters were measured at the Department of Medical and Fundamental Biochemistry at Institut Pasteur de Côte d'Ivoire (IPCI).

Materials

Plant material: *Zingiber officinale* rhizomes were collected in the Department of Tiassalé, Loh Djiboua Region, in the South-East of Côte d'Ivoire, from November to December 2024. The rhizomes were washed with tap water, then spread out in the laboratory at room temperature (25°C) protected from light for 5 days to remove residual water. Rhizomes were crushed using a laboratory mortar to obtained fragments. Then, from these fragments essential oils were extracted.

Experimental animals: Healthy adult male wistar rats of 4±0.5 months of age with a mean weight of 190±10 g were used. Animals were kept in cages with 12/12 hrs light/dark cycle and fed will standard food pellets and water was provided to them throughout the experiment. They were acclimatized three days prior to experiment. The experimental procedures and protocols used in this study were approved by the Ethics Committee of Félix Houphouët-Boigny University in accordance with Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes[10] and OECD guidelines for chemical testing[11].

Technical equipment: The technical material included a rotary evaporator (Rotavapor), a Perkin Elmer Autosystem XL chromatograph equipped with an automatic injector and an apolar column (Rtx-1) (60 cm long, 0.22 mm thick film: 0.25 pm), coupled with a Perkin Elmer mass detector, TurboMass (GC-MS) and an ELISA chain.

Reagents: The reagents used were anhydrous sodium sulfate and an ELISA (Enzyme-Linked Immunosorbent Assay) kit for measuring ROS and ether levels.

Extraction of essential oils: Essential oils were extracted through hydrodistillation, by adding 100 g of plant material to 1 L of distilled water and then boiled under atmospheric pressure. The boiling water caused the plant cells to burst, releasing the molecule odors, which form a mixture of azeotropic vapors. The aromatic molecules were obtained by decantation after condensation of this mixture on a cold surface. In order to remove any water that may have been retained in the organic phase, the oils are filtered to collect the water-free organic phase after a dehydration phase carried out using anhydrous sodium sulfate. The oils were stored in suitable containers, protected from light and at temperatures not exceeding 25°C. A sample of each extracted essential oil was taken for chemical and physicochemical analysis according to the method of Kennas and Boussalah[12].

Molecular characterization of essential oils: The coupling of GC with mass spectrometry (GC-MS) in electron impact (EI) mode is the reference analysis technique for essential oils.

After separation by GC, constituents of the mixture are bombarded by a high-energy beam of electrons of around 70 eV. After ionization, the molecules are fragmented through collisions with the electrons, resulting in positive ionic fragments that produce a characteristic mass spectrum for each constituent. Hence, each chromatographic peak produces a corresponding mass spectrum. The compounds are identified by comparing their mass spectra with those of reference compounds in computerized spectrum libraries, which are either commercially available or developed in laboratories[13]. The chemical composition determined for an essential oil defines its chemotype.

Inducing insomnia in rats: Two methods were used to induce insomnia in rats. In the first method, the animals were exposed to a continuous bright light with controlled temperature (LC) for three days using an incandescent light bulb. The second method involved administration of 50 mL/kg from a coffee solution (20 mg/mL) by oral route (via gavage) twice daily for three days.

For both methods, the rats were divided into six groups of three rats per group according to the following experimental design:

- **Group 1:** Negative control (NC) rats in this group were not subjected to any insomnia induction protocol and did not receive any essential oils. They served as controls for environmental conditions
- **Group 2:** Positive control this group included rats with insomnia, induced by continuous exposure to incandescent light with controlled temperature (PCLH) or coffee gavage (PCC) for three days, without any essential oil treatment
- **Group 3:** Rats with insomnia treated with ginger essential oil (PCLH+G or PCC+G). In this group, the animals were treated with ginger essential oil for three days while being exposed to insomnia induction in both methods
- **Group 4:** Rats with insomnia treated with the reference essential oil (PCLH+R or PCC+R), the rats were treated with the reference essential oil for 3 days while being exposed to insomnia in both methods
- **Groups 5 and 6 :** Rats with no insomnia treated with essential oils (G+S or R+S), the rats in these groups were not exposed to any insomnia induction protocol, but were treated with either ginger essential oil (Group 5) or the reference essential oil (Group 6)

Measuring ROS using ELISA: This method is based on the formation of a sandwich complex. Serum containing ROS (the antigen) is added to wells of a microplate assay on which antibodies against ROS are fixed. The microplate is then incubated to allow binding to the antibodies. Next, a secondary antibody conjugated to horseradish peroxidase (HRP), which is specific to reactive oxygen species, is added to bind to the primary antibody coupled to the antigen, thus forming a sandwich complex. Free components are removed by washing and a TMB (3,3',5,5'-tetramethylbenzidine) substrate solution is added to each well. Only the wells containing ROS and the ROS-specific HRP-conjugated antibody will turn blue before turning yellow when the stop solution is added. The optical density (OD) is then measured by spectrophotometry at a wavelength of 450 nm. The OD value is proportional to the concentration of ROS[14].

Statistical analysis: An Analysis of Variance (ANOVA) was performed to identify overall differences between the different experimental conditions, followed by Fisher LSD *post-hoc* tests to identify specific pairs of groups with significant differences. The significance threshold was set at $p<0.05$.

RESULTS AND DISCUSSION

Results

Extraction yield and characterization of molecular constituents: According to the experimental plan, a drying time of 5 days and an extraction time of 6 hrs yielded an optimum yield of 1.6%. The GC-MS analysis of the essential oils extracted from ginger identified 67 constituents, representing 96.1% of the total chemical composition of the sample. The main families of chemical compounds represented were oxygenated monoterpenes (58.1%), followed by hydrocarbon sesquiterpenes (32.9%) and hydrocarbon monoterpenes (22.9%). Oxygenated sesquiterpenes were represented in lower concentrations (8.7%). The chemical compositions of these essential oils showed a predominance of five compounds: Geranial (17.0%), 1,8-cineole (14.4%), camphene (14.0%), p-zingiberene (15.1%) and neral (10.5%). In addition to these major compounds, some constituents such as 1-sesquiphellandrene (6.1%), borneol (4.7%), (2E,6E)-alpha-farnesene (4.2%), alpha-pinene (3.5%), geraniol (3.2%) and ß-bisabolene (2.8%) were also identified in the sample.

Table 1: Regulation of ROS production compared to controls

Model	Tested oil	Mean of ROS±SD (pg/mL)	Regulation of ROS production
Healthy rats			
	None (Negative control)	70.87±5.76	
	Ginger oil	86.68±1.98	+22.3%
	Reference oil	97.24±12.89	+37.2%
Stressed rats			
Bright light+heat	Positive control (PCLH)	104.77±7.76	
	PCLH+Ginger oil	92.21±3.76	-12%
	PCLH+Reference oil	107.28±0	+2.4%
Stressed rats			
Coffee	Positive control (PCC)	245.42±50.75	
	PCC+Ginger oil	113.56±13.37	-53.7%
	PCC+Reference oil	128.00±29.26	-47.8%

ROS: Reactive oxygen species, SD: Standard deviation, PCLH: Positive control (bright light+heat) and PCC: Positive control (coffee)

Physical signs of stress: Rats exposed to stress induced by exposure to insomnia showed physical signs such as increased nervousness, excitement and loss of appetite.

Production of reactive oxygen species: The experimental results showed that the production of reactive oxygen species (ROS) increased significantly in rats exposed to insomnia compared to control group ($p<0.05$). The ROS concentrations were 104.77±7.76 pg/mL in rats exposed to bright light combined with heat and 245.41±50.75 pg/mL in those given caffeine by gavage, compared to 70.87±5.76 pg/mL in healthy controls. A comparative analysis of the two insomnia induction models showed a statistically significant difference between caffeine and the combination of bright light and heat. The caffeine model induced more pronounced oxidative stress with a mean ROS of 245.41 pg/mL, which is 2.3 times higher than that observed with the combination of bright light and heat.

The results also showed variability in the response of essential oils to stress according to the model. Indeed, in the context of moderate oxidative stress induced by exposure to light combined with heat (Table 1), ginger essential oil (PCLH+G) exhibited a significant 12% decrease in ROS concentrations compared to the stressed control group (PCLH). In contrast, the reference essential oil (PCLH+R) caused a slight decrease in ROS concentrations of about 2%. Fisher's LSD test, *post hoc* comparisons showed that treatment with ginger essential oils induced a significant decrease in ROS compared to pathological controls (PCLH) ($p<0.05$), while true lavender oil (reference) showed no significant decrease ($p>0.05$). Furthermore, a comparison of the effects of the two essential oils revealed that ginger was more effective in terms of antioxidant protection ($p<0.05$). This response is much more pronounced in the severe stress model caused by coffee administration (Table 1). The results revealed a strong antioxidant effect of the essential oils, with mean reductions of 53.7% and 47.8% in ROS for ginger oil (PCC+G) and reference oil (PCC+R), respectively, compared to the stressed control group (PCC). Fisher's *post-hoc* tests clearly indicate that both essential oils had a significant effect compared to the control group (PCC, $p<0.001$) and the effect of ginger essential oils was significantly greater than that of the reference oil ($p<0.05$). A comparative analysis between the regulatory effect of essential oils and healthy control rats showed no statistically significant difference ($p>0.05$).

Discussion: The main objective of this study was to assess the antioxidant effect of ginger essential oil (*Zingiber officinale*) on experimentally induced oxidative stress in Wistar rats through direct measurement of reactive oxygen species (ROS). To conduct this study, two distinct models of insomnia induction, responsible for oxidative stress, were used. These involved prolonged exposure of rats to bright light combined with heat (PCLH) or gavage of rats with caffeine (PCC). Analysis of results showed a significant increase in plasma of ROS levels in the pathological control group, 271.4 pg/mL for the caffeine model (PCC) and 104.77 pg/mL for the bright light and heat model, compared to 70.9 pg/mL in the negative control group (NC). The significant increase in oxidative stress levels (47.8% for the PCLH model and 246.3% for the PCC model) demonstrates the effectiveness of the experimental models used to induce insomnia. A comparison of the two methods to induce oxidative stress (light+heat and coffee) shows major differences in the way they trigger reactive oxygen species (ROS), depending on the type of stress applied. Exposure to light combined with heat caused a moderate increase in ROS, with an estimated mean concentration of 104.77 pg/mL. However, a much more pronounced response was observed following coffee ingestion, with ROS levels reaching 245.41 pg/mL. This concentration, which is twice as high as that observed with light combined with heat, suggest a distinct and potentially more intense oxidative attack with

caffeine. Similar results were obtained in a study conducted by Guo *et al.*[15] on animals exploring the impact of different stresses on oxidative balance. Their work showed that exposure to environmental factors modulating oxidative stress, such as UVB radiation, similar to our light combined with heat model, led to a moderate increase in ROS. In addition, this increase was accompanied by the activation of enzymatic antioxidant defenses such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). The results also confirmed ginger's strong antioxidant potential in both insomnia induction models, with a decrease in ROS concentrations ranging from 11.98 to 53.72%. This decrease in ROS is consistent with the work of Prasad *et al.*[14], who reported a 45% reduction in lipid peroxides (TBARS) after administering ginger extract to rats exposed to malathion. Although their methodology is based on indirect measurement, it revealed antioxidant activity similar to that obtained in this study using direct ELISA measurement of ROS. This 54% reduction in ROS suggests a significant antioxidant effect, attributed to the chemical composition (chemotype) of ginger essential oils containing a high concentration of biomolecules such as geranial, 1,8-cineole and gingerol, well-known for their therapeutic properties[8]. A comparative analysis of the antioxidant effects of ginger and true lavender essential oils (reference essential oil) showed antioxidant activity that was significantly higher than that of the reference essential oil, regardless of the level of oxidative stress. Ginger essences significantly reduced ROS, with a difference of 9.89% for moderate stress and 5.88% for severe stress. This effect of ginger essential oils, which brought ROS concentrations close to those of the control rats, suggests a more stable modulation of oxidative metabolism. This observation could be explained by the fact that ginger essential oils were in their raw state, unlike the true lavender oils, which are a commercial formulation. Our results are in accordance with those of Ahmed *et al.*[16], Bellik *et al.*[17] and Bilto *et al.*[9], who also demonstrated the antioxidant activity of ginger extracts in *in vivo* experimental models. During their experiment, it was shown that in rats, the administration of ginger extract led to a significant increase of the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), compared to a control group treated with a neutral oil. The gains observed in their study ranged from 10 to 20% depending on the markers, which corresponds relatively to the difference in efficacy observed in our experimental study between ginger essential oil and true lavender essential oil. Thus, despite different protocols and biological models, the consistency between the two studies reinforces the robustness of the hypothesis that ginger essential oil exhibits effective antioxidant activity under a moderate or severe oxidative stress.

CONCLUSION

This study revealed that ginger essential oils have significant antioxidant properties as demonstrated by their ability to lower serum levels of reactive oxygen species (ROS) in rats undergoing oxidative stress induced by prolonged experimental insomnia. That strong antioxidant activity of ginger essential oils stems from the presence of bioactive constituents such as 1,8-cineole, geranial and gingerol, that exhibit potent antioxidant properties. these results could justify the use of ginger essential oils as powerful natural antioxidants in the fight against diseases that cause oxidative stress. Ginger essential oils could therefore represent a credible therapeutic alternative in the medical treatment of mental health disorders. However, formulation and short, medium and long-term toxicity studies need to be done before any therapeutic application.

SIGNIFICANCE STATEMENT

This study demonstrates that *Zingiber officinale* essential oil effectively reduces reactive oxygen species (ROS) in experimental models of insomnia-induced stress, highlighting its antioxidant and potential neuroprotective properties. These findings support the development of ginger essential oil as a natural therapeutic candidate for managing oxidative stress associated with sleep disturbances and mental health disorders. Further preclinical and clinical studies are warranted to validate its efficacy and safety in humans.

ACKNOWLEDGMENT

We would like to express our gratitude to the administrators of the institutions that facilitated this work. These were the Institut Pasteur of Côte d'Ivoire for providing the technical platform and Félix Houphouët Boigny University for allowing the animal experiments to be conducted.

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