West African Journal of Pharmacy (2025) 36 (1) 109 - 121

Unraveling the anxiolytic and antidepressant potential of *Chromolaena odorata* extract: a preclinical investigation

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https://doi.org/10.60787/wajp.vol36no1.382

ABSTRACT

Background: *Chromolaena odorata* Linn (L.) (Asteraceae) is a medicinal plant traditionally used to treat various ailments, including anxiety and depression. However, its neuropharmacological properties have not been extensively studied.

Objective: This study aimed to investigate the anxiolytic and antidepressant effects of *C. odorata* extract and explore its possible mechanism of action.

Methods: Acute oral toxicity testing was performed following OECD guidelines, and qualitative phytochemical analysis was carried out using standardized procedures. The anxiolytic effects of *C. odorata* extract were evaluated using the elevated plus maze (EPM) test, while its antidepressant activity was evaluated through the tail suspension test (TST). The extract was administered to mice at doses of 100, 200, and 400 mg/kg, with behavioral parameters carefully recorded over a five-minute observation period. At the fourteen day experimental period, the animals were euthanized using ethyl ether, and brain samples were collected to analyze serotonin and noradrenaline levels, and to evaluate *In-vivo* brain antioxidant activity.

Results: The result revealed that the LD_{50} of *C. odorata* extract was greater 2 g/kg. In the Elevated Plus Maze (EPM) test, the extract significantly increased the time spent ($P<0.01$; 400 mg/kg) and the frequency of exploratory behaviors ($P<0.05$; 400 mg/kg) in the open arms, while significantly reducing the time spent ($P<0.01$; 200-400 mg/kg) and the frequency of exploration ($P<0.05$, 0.01; 200-400 mg/kg) in the closed arms in comparison to the distilled water control group. In the Tail Suspension Test (TST), the extract significantly decreased the duration of immobility ($P<0.05$-0.001; 100-400 mg/kg) relative to the distilled water treated group. Furthermore, at 400 mg/kg, the extract significantly elevated serotonin ($P<0.01$) and noradrenaline ($P<0.0001$) levels compared to the negative control. The extract also demonstrated significant antioxidant activity, enhancing brain antioxidative biomarker levels.

Conclusion: This study highlights the anxiolytic and antidepressant potential of *C. odorata* extract. Its antidepressant effects may be attributed to increased serotonin and noradrenaline levels, along with the inhibition of oxidative radical generation.

Keywords: *Chromolaena odorata*; Medicinal plant; Anxiolytic; Antidepressant; Antioxidant; Serotonin

Exploration du potentiel anxiolytique et antidépresseur de l'extrait de *Chromolaena odorata*: une étude préclinique

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RÉSUMÉ

Contexte: Le *Chromolaena odorata* Linn (L.) (Asteraceae) est une plante médicinale traditionnellement utilisée pour traiter diverses affections, notamment l'anxiété et la dépression. Cependant, ses propriétés neuropharmacologiques n'ont pas fait l'objet d'études approfondies.

Objectif: Cette étude vise à examiner les effets anxiolytiques et antidépresseurs de l'extrait de *C. odorata* et à explorer son mécanisme d'action possible.

Méthodes: Des tests de toxicité orale aiguë ont été réalisés conformément aux lignes directrices de l'OCDE, et une analyse phytochimique qualitative a été effectuée selon des procédures normalisées. Les effets anxiolytiques de l'extrait de *C. odorata* ont été évalués à l'aide du test du labyrinthe en croix surélevé (EPM), tandis que son activité antidépressive a été évaluée à l'aide du test de suspension de la queue (TST). L'extrait a été administré à des souris à des doses de 100, 200 et 400 mg/kg, et les paramètres comportementaux ont été soigneusement enregistrés sur une période d'observation de cinq minutes. Au bout de la période expérimentale de quatorze jours, les animaux ont été euthanasiés à l'éther éthylique, et des échantillons de cerveau ont été prélevés pour analyser les taux de sérotonine et de noradrénaline, et pour évaluer l'activité in vivo antioxydante cérébrale.

Résultats: Les résultats ont révélé que la DL_{50} de l'extrait de *C. odorata* était supérieure à 2 g/kg. Dans le test du labyrinthe en croix surélevé (EPM), l' extrait a augmenté de manière significative le temps passé ($P<0,01$; 400 mg/kg) et la fréquence des comportements exploratoires ($P<0,05$; 400 mg/kg) dans les bras ouverts, tout en réduisant de manière significative le temps passé ($P<0,01$; 200-400 mg/kg) et la fréquence d'exploration ($P<0,05$, 0,01 ; 200-400 mg/kg) dans les bras fermés par rapport au groupe témoin à l'eau distillée. Dans le test de suspension de la queue (TST), l'extrait a réduit de manière significative la durée d'immobilité ($P<0,05$-0,001 ; 100-400 mg/kg) par rapport au groupe traité à l'eau distillée. En outre, à 400 mg/kg, l'extrait a augmenté de manière significative les taux de sérotonine ($p<0,01$) et de noradrénaline ($p<0,0001$) par rapport au témoin négatif. L'extrait a également démontré une activité antioxydante significative, augmentant les niveaux de biomarqueurs antioxydants cérébraux.

Conclusion: Cette étude met en évidence le potentiel anxiolytique et antidépresseur de l'extrait de *C. odorata.* Ses effets antidépresseurs pourraient être attribués à l'augmentation des taux de sérotonine et de noradrénaline, ainsi qu'à l'inhibition de la production de radicaux oxydatifs.

Mots-clés: *Chromolaena odorata* ; plante médicinale ; anxiolytique ; antidépresseur ; antioxydant ; sérotonine

INTRODUCTION

Co-morbid depression and anxiety represent an increasingly alarming global issue, impacting millions of individuals across the globe.[1] This co-morbidity complicates diagnosis, intervention, and overall health outcomes, thereby adversely affecting individuals' quality of life, productivity, and even somatic health.[2-3] Empirical studies indicate that approximately 50-60 % of individuals diagnosed with depression experience anxiety at the same time, and vice versa.[4] According to the World Health Organization (WHO), over 280 million individuals suffer from depression on a global scale, and about 264 million people struggle with anxiety.[5] The incidence of co-morbidity increases the severity of symptoms experienced. The dysregulation of biogenic amine neurotransmitters is pivotal in the pathophysiological mechanisms underlying both depression and anxiety.[6-7] Biogenic amines, including serotonin, dopamine and noradrenaline, are fundamental neurotransmitters that possess significant associations with mood regulation, emotional stability, and behavioral equilibrium; any disruption in their homeostasis may precipitate neuropsychiatric disorders such as depression and anxiety.[8-9] The overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), combined with the decline in the body's antioxidant defenses, has been increasingly recognized as a key factor in the development and progression of psychiatric disorders such as depression and anxiety.[10] Elevated levels of ROS and RNS can cause neural tissue damage, disrupting the neural circuits involved in mood and emotional regulation.[11] Pharmacological treatments, including antidepressant and anxiolytic agents, have shown significant efficacy in improving these conditions for many individuals.

Despite their widespread use, pharmacological treatments for anxiety and depression face several challenges, including concerns about efficacy, adverse effects, treatment adherence, individual variability in therapeutic response, and most notably, the financial burden of the medications.[12-13] Addressing these issues necessitates innovative treatment strategies, personalized approaches to medicine, improved management of side effects, and enhanced affordability of therapies to optimize patient outcomes and overcome the limitations of current interventions.

Chromolaena odorata Linn (L.) (Asteraceae), commonly referred to as Siam weed, Christmas bush, or devil weed, is a rapidly growing perennial shrub native to Central and South America, parts of Australia, and tropical and subtropical regions of Africa, Asia, and the Pacific.[14] This plant, which can reach heights of 2-3 meters under favourable conditions, features opposite leaves with a triangular to oval shape and serrated margins. It thrives in disturbed habitats, including roadsides, agricultural lands, and fallow fields. Within indigenous populations, *C. odorata* has long been utilized to treat various ailments, including malaria, wounds, diarrhoea, skin infections, toothaches, dysentery, abdominal pain, pharyngitis, seizures, hemorrhoids, as well as respiratory infections such as coughs and colds.[14] The antibacterial properties have been documented,[15-16] and studies such as those of Taiwo *et al*[17] have demonstrated its anti-inflammatory, antipyretic, and antispasmodic effects. Ethno-pharmacological research also highlights its diverse bioactivities, including antimicrobial, anti-inflammatory, anti-diarrhoeal, analgesic, anticancer, antidiabetic, antioxidant, wound healing, and haemostatic properties.[14] Preliminary reports from indigenous communities suggest that *C. odorata* may also have applications in managing neuropsychiatric disorders, such as anxiety and depression. However, its potential in this context remains unexplored in existing ethnopharmacological literature. Recognizing this gap, the present study aimed to investigate the anxiolytic and antidepressant-like effects of hydroethanol leaf extract of *C. odorata* using mouse models. The study also sought to elucidate the role of noradrenaline, serotonin, and reactive oxygen species in the plant's mechanism of action. The findings of this research may provide novel insights into addressing the therapeutic limitations of current antidepressant and anxiolytic medications.

MATERIALS AND METHODS

Plant material

The leaves of *Chromolaena odorata* were collected from Ijebu-Ode Town, Ogun State, Nigeria and identified and authenticated by Mr. Adeoti of the Department of Pharmacognosy, Faculty of Pharmacy, Olabisi Onabanjo University, Shagamu Campus.

Drugs and chemicals

Fluoxetine (Asset Pharmaceuticals, Lagos, Nigeria), Ethanol (Nosak Distilleries Limited, Lagos, Nigeria), Serotonin and Noradrenaline ELIZA kits (Elabscience, Wuhan, China), Antioxidant commercial ELIZA kits (Elabscience, Wuhan, China)

Extraction procedures

The extraction of *C. odorata* leaves followed the method described by Murtala *et al*.[18] A total of 550 grams of the

powdered, dried leaves was macerated in 1.5 liters of hydroethanol (1:1) for three days. The mixture was then decanted and filtered first through muslin cloth and subsequently through Whatman filter paper. This process, including decantation, and filtration protocols were repeated twice using the residual plant material. The combined filtrate was evaporated at 40^{o}C, to yield a dry, dark brown residue (8.2 %).

Experimental animals

The study used six albino mice (3 male and 3 female), weighing between 25-30 g, obtained from the Animal Center of the Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu, Ogun State. The animals were housed under standard environmental conditions (23-25^{o}C; 12h/12h light-dark cycle) with unrestricted access to standard rodent pellet diet and water. A fourteen-day acclimatization period was allowed before the experiments began. Ethical clearance was secured from the Animal Care and Use Research Ethics Committee (ACUREC) of University of Lagos (CMUL/ACUREC/10/24/1618).

Qualitative phytochemical screening

The extract was screened using established analytical methods to identify the presence of various phytochemical constituents, including anthraquinones, saponins, flavonoids, cardiac glycosides, steroids, tannins, alkaloids, phlobatannins, and terpenoids.[19-20]

Acute toxicity test

Five groups of Swiss mice (five per group, 25 in total), were used to evaluate oral acute toxicity following the Organization for Economic Co-operation and Development (OECD) guidelines relevant to oral acute toxicity assessments.[21] The mice were fasted for 12 hours prior to the commencement of the experiment. *Chromolaena odorata* extract was administered per oral at a maximum dosage of 2000 mg/kg, while the control group received distilled water (10 mL/kg). Behavioral changes and signs of toxicity were closely monitored for the first two hours post-administration, with mortality recorded over 24 hours and for an additional fourteen days to assess delayed toxicity. The oral average lethal dose (LD_{50}) was then determined.[18,22]

Anxiolytic activity test

Elevated plus maze test

Distilled water (10 mL/kg), *C. odorata* (100, 200, and 400 mg/kg), and fluoxetine (20 mg/kg) were administered per oral to five groups of six mice each. One hour before treatment, each mouse was individually placed at the center of the maze, facing one of the closed arms. The total time spent in both the open and closed arms of the maze, along with the number of entries, was carefully recorded over a five-minute observation period.[24]

Antidepressant activity

Tail suspension test

Mice were administered *C. odorata* extract daily for 14 days, and 30 minutes after the treatment, the mice were immobilized for one hour to elicit restraint stress. The mice were randomly divided into five groups (six per group, totaling 30) and treated as follows: Group 1 received distilled water (10 mL/kg; Group 2 received fluoxetine (20 mg/kg); and Groups 3, 4, 5 and 6 were administered extract doses of 100, 200, and 400 mg/kg, respectively. On the fourteenth day, one hour after treatment, each mouse was suspended from a retort stand 50 cm above the ground, using adhesive tape approximately 1 cm from the tail tip. The latency to immobility and total immobility duration were recorded over a 5-minute observation period. A mouse was considered immobile if it showed no attempts to raise its head and maintained a downward head posture for more than 5 seconds.[25-26]

Brain tissue preparation for biochemical evaluations

On day 14, the mice were euthanized using ethyl ether, and the brain excised and preserved at -20 ^{o}C for quantification of amine neurotransmitters and antioxidant biomarkers. The brain tissues were homogenized in a phosphate buffer solution (PBS) at a weight-to-volume ratio of 1:9, using a glass homogenizer on ice. The resultant brain homogenates were then centrifuged at 5000 × g for 5 minutes at 4 ^{o}C to separate the supernatant, which was subsequently stored in the refrigerator at -80 ^{o}C.

Determination of noradrenaline and serotonin levels in the brain

Serotonin and noradrenaline level in the brain homogenates were assayed using ELISA kits (Elabscience, Wuhan, China) according to manufacturer's instruction.

Brain antioxidant assay

Evaluation of glutathione (GSH) content

Glutathione (GSH) levels in cerebral tissues were quantified using the method described by Kudo *et al.*[27]

Briefly, 0.4 mL of brain homogenate was mixed with an equal volume of 20 % trichloroacetic acid (TCA) and gently agitated. The mixture was then centrifuged at 4°C for 20 minutes at 5400 g. Next, 0.25 mL of the supernatant was combined with 2 mL of 0.6 mmol/L 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB), and the final volume was adjusted to 3 mL with a 0.2 mol/L phosphate buffer (pH 8.0). Absorbance was measured at 412 nm, using a spectrophotometer, with a blank reagent (2 mL of 0.6 mmol/L DTNB and 1 mL of phosphate buffer) as the reference. The concentration of reduced GSH was expressed as nanomoles per milligram of protein (nmol/mg protein).

Evaluation of malondialdehyde (MDA) levels

To assess malondialdehyde (MDA) levels, 0.4 mL of brain tissue was mixed with 1.6 mL of Tris-potassium chloride (Tris-KCl) buffer, followed by the addition of 0.5 mL of a 30% trichloroacetic acid (TCA). Subsequently, 0.5 mL of a 0.75 % thiobarbituric acid (TBA) was added, and the mixture was incubated in a water bath at 80 °C for 45 minutes. After incubation, the mixture was cooled on ice and centrifuged at 1200 g for 15 minutes. The clear supernatant was then collected, and its absorbance was measured at 532 nm against distilled water blank. The MDA concentration was calculated using a molar extinction coefficient of 1.56 × 105 mol/L/cm, and expressed as nanomoles of MDA per milligram of protein (nmol MDA mg–1 protein).[28]

Determination of catalase levels

Catalase (CAT) activity was determined using the method described by Popov *et al*.[29] The assay was based on the decomposition kinetics of hydrogen peroxide (H_2O_2) at 240 nm. The reaction mixture consisted of 3 mL of phosphate-buffered saline and 10.5 mL of brain tissue homogenate supernatant. Absorbance at 240 nm was recorded using a spectrophotometer, and CAT activity was expressed in kilograms of protein.

Determination of superoxide dismutase levels (SOD)

Superoxide dismutase (SOD) activity in brain tissue homogenates was assessed following the method described by Popov *et al*.[29] The reaction mixture contained 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.3 mM xanthine, 0.6 mM nitro blue tetrazolium (NBT), and 50 µL of xanthine oxidase, all dissolved in 10 mM sodium phosphate buffer at pH 7.4. The mixture was maintained in an ice bath before adding 20 µL of brain supernatant to the wells containing the reaction mixture. The reaction mixture was incubated at 37 °C for 15 minutes, and absorbance was measured at 560 nm using a spectrophotometer.

Statistical analysis

The results of this study are expressed as mean ± S.E.M. Data were analyzed using one-way ANOVA, followed by Dunnett's post-hoc test, using GraphPad Prism 6 Software (GraphPad Software Inc., CA, USA). Statistical significance was set at $P < 0.05$.

RESULTS

Acute toxicity

Oral administration of *C. odorata* extract at doses of up to 2 g/kg showed no signs of behavioral toxicity such as lethargy, tachycardia, hypokinesia, restlessness, lacrimation, anorexia, or mortality within 24 hours to 14 days in mice was observed. The oral LD_{50} of *C odorata* was estimated to be greater than 2 g/kg.

Preliminary phytochemical screenings

Qualitative phytochemical screening of *C. odorata* revealed the presence of anthraquinones, saponins, flavonoids, cardiac glycosides, steroids, tannins, and alkaloids.

Elevated plus maze test

One-way ANOVA showed that *C. odorata* at 100 and 400 mg/kg significantly increased ($p < 0.05$, $p < 0.01$, respectively) the time spent in the open arms of the maze compared to the distilled water-treated group (Table 1). Similarly, fluoxetine, the standard drug, significantly increased ($p < 0.05$) open-arm exploration relative to the negative control group (Table 1). Regarding the time spent in the closed arms, *C. odorata* at 400 mg/kg and fluoxetine (20 mg/kg) significantly reduced ($p < 0.05$) the duration spent in the closed arms compared to the negative control group (Table 1). In terms of open-arm entries, *C. odorata* extract at 200-400 mg/kg ($P < 0.01$) and fluoxetine ($P < 0.05$; 20 mg/kg) significantly increased open-arm exploration compared to the control group (Table 1). Conversely, the extract at 200-400 mg/kg ($p < 0.05$, $p < 0.01$) significantly reduced closed-arm entries relative to the negative control group (Table 1). Fluoxetine significantly decreased closed-arm explorations compared to the negative control group (Table 1).

Table 1: Effect of hydroethanol leaf extract of *C. odorata* in the elevated plus maze test in mice

Treatment	Dose (mg/kg)	Time spent in open arms (sec)	Time spent in close arms (sec)	No of entry into open	No of entry into close open
D/water	**10 mL/kg**	1.36±0.11	2.83±0.12	1.33±0.33	6.00±0.57
C. odorata	**100**	3.55±0.58[a]	1.81±0.69	3.33±0.33	3.33±0.66
C .Odorata	**200**	3.23±0.72	2.13±0.94	5.33±1.20[b]	1.66±0.66[b]
C. odorata	**400**	4.38±0.15[b]	0.60±0.15[a]	5.33±0.33[b]	2.33±0.88[a]
Fluoxetine	**20**	3.71±0.68[a]	0.43±0.17[a]	4.66±0.88[a]	2.00±0.57[b]

(n=6). [a]*P* < 0.05, [b]*P* < 0.01 vs. Distilled water (One way ANOVA followed by Dunnett's post-hoc test)

Tail suspension test (TST)

C. odorata extract (100 mg/kg) significantly increased the latency to immobility (p<0.05) compared to the negative control group (Table 2). Regarding the immobility duration, *C. odorata* (100-400 mg/kg) produced a significant reduction (p < 0.05-0.001) compared to the negative control cohort (Table 2). Similarly, fluoxetine significantly prolonged the latency to immobility (p < 0.001) and significantly reduced immobility duration (p < 0.0001) relative to the negative control group (Table 2).

Table 2: Effects of hydroethanolic leaf extract of *Chromolaena odorata* in the tail suspension test in mice

Treatment	Dose (mg/kg)	Onset of immobility (min)	Duration of immobility (min)
Distilled water	10 mL /kg	0.35± 0.02	3.51±0.17
C. Odorata	100	0.77 ± 0.06[a]	2.66± 0.15[a]
C .Odorata	200	0.31± 0.03	2.02±0.38[c]
C. Odorata	400	0.36± 0.11	2.29±0.09[b]
Fluoxetine	20	1.08± 0.13[c]	0.73±0.08[d]

(n=6). [a]*P*<0.05, [b]*P*<0.01, [c]*P*<0.001, [d]*P*<0.0001 vs. Distilled water (One way ANOVA followed by Dunnett's post-hoc test)

Effect of *C. odorata* on Noradrenaline and Serotonin in Brain homogenates

Regarding the influence of the extract on noradrenaline levels, *C. odorata* at 400 mg/kg significantly increased (p < 0.0001) brain noradrenaline concentration compared to the group treated with distilled water (Figure 1). In terms of serotonin levels, *C. odorata* at 400 mg/kg significantly elevated (P < 0.01) in serotonin levels relative to the negative control cohort. Fluoxetine (20 mg/kg) also produced a significant increase (P < 0.01) in serotonin levels compared to the negative control (Figure 2).

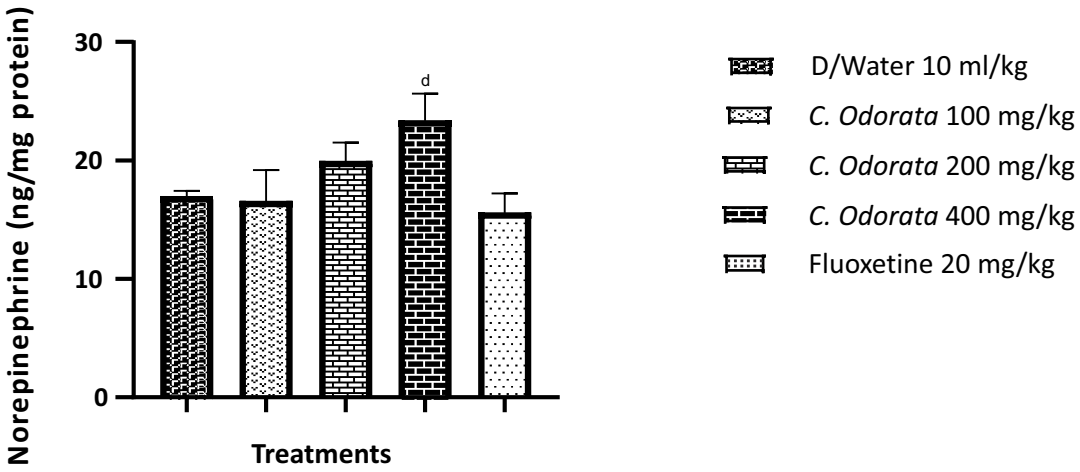

Figure 1: Evaluation of noradrenaline function in the brain on the elucidation of possible mechanism(s) of antidepressant-like effect of *C. odorata* using Tail Suspension Test. (n=6). ^{d}P<0.0001 vs. Distilled water (One way ANOVA followed by Dunnett's post-hoc tests).

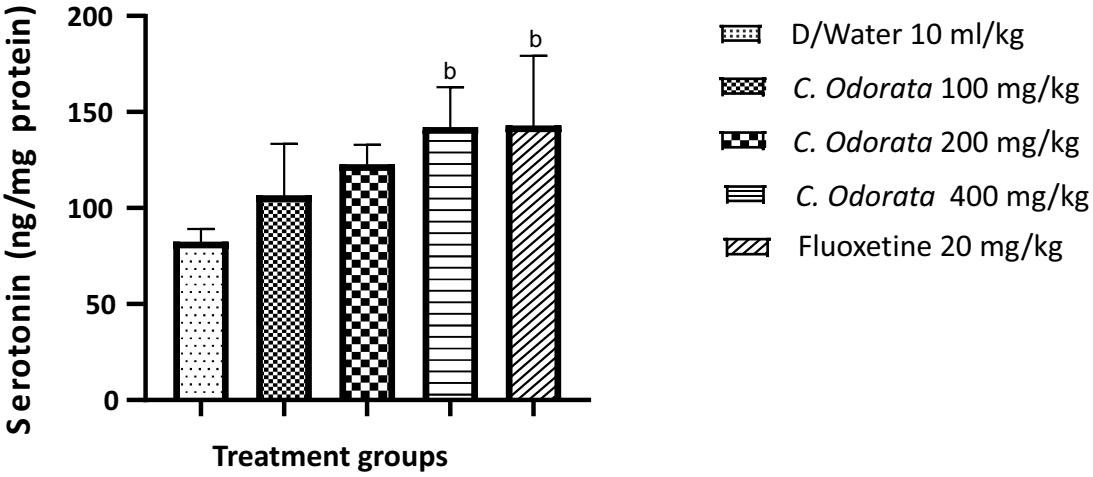

Figure 2: Evaluation of serotonin function in the brain on the elucidation of possible mechanism(s) of antidepressant-like effect of *C. odorata* using TST. (n=6). $^{b}P < 0.01$ vs. Distilled water (One way ANOVA followed by Dunnett's post-hoc tests)

Brain Antioxidant Determinations

C. odorata (100-400 mg/kg) produced a significant decrease in malondialdehyde (MDA) levels ($p < 0.01$, $p < 0.001$) compared to the distilled water-treated group. Glutathione (GSH) levels increased significantly at 400 mg/kg ($p < 0.05$). Catalase activity showed a significant elevation at 200-400 mg/kg ($p < 0.05$, 0.01), while superoxide dismutase (SOD) levels significantly increased at 200 mg/kg ($p < 0.05$).

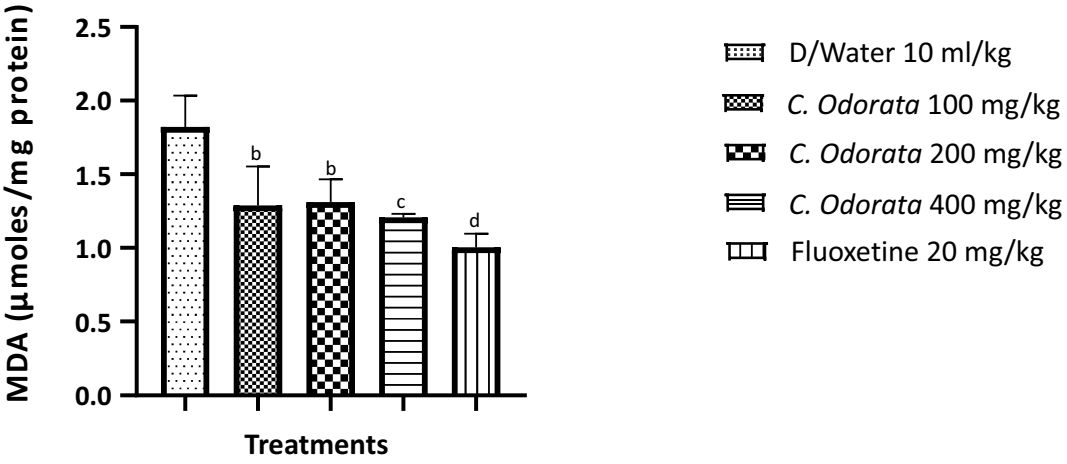

Figure 3: Evaluation of MDA level in the brain on the elucidation of possible mechanism(s) of antidepressant-like effect of *C. odorata* using Tail Suspension Test. (n=6). $^{b}P < 0.01$, $^{c}P < 0.001$, $^{d}P < 0.0001$ vs. Distilled water (One way ANOVA followed by Dunnett's post-hoc tests).

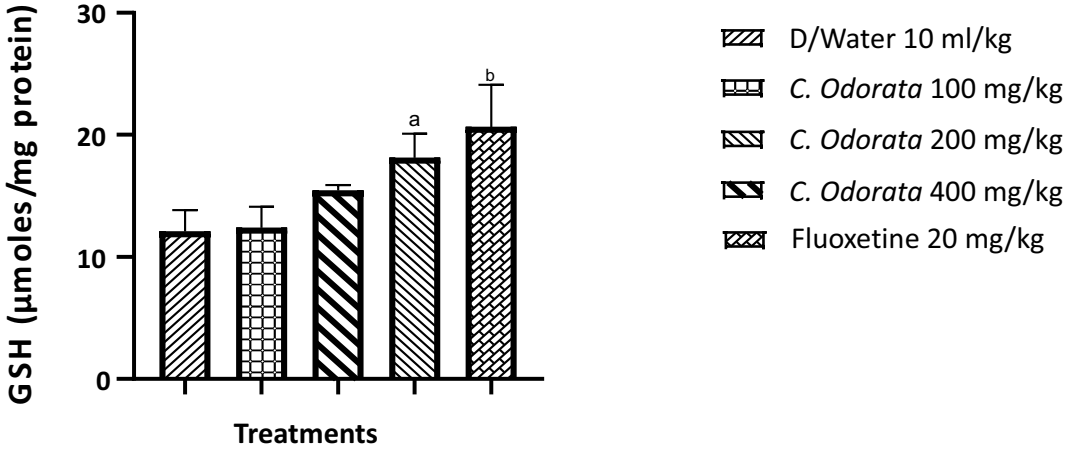

Figure 4: Evaluation of GSH level in the brain on the elucidation of possible mechanism(s) of antidepressant-like effect of *C. odorata* using Tail Suspension Test. (n=6). $^{a}P < 0.05$, $^{b}P < 0.01$ vs. Distilled water (One way ANOVA followed by Dunnett's post-hoc tests).

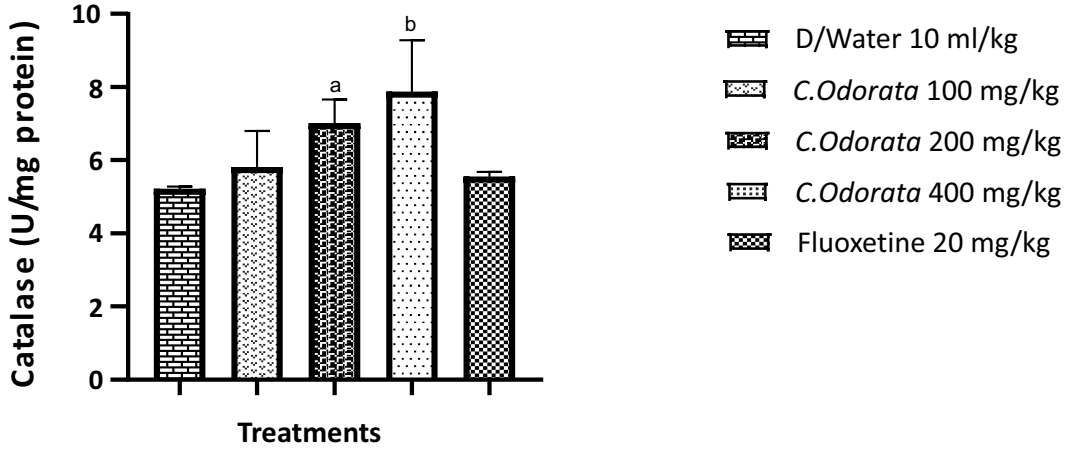

Figure 5: Evaluation of Catalase level in the brain on the elucidation of possible mechanism(s) of antidepressant-like effect of *C. odorata* using Tail Suspension Test. (n=6). $^{a}P < 0.05$, $^{b}P < 0.01$ vs. Distilled water (One way ANOVA followed by Dunnett's post-hoc tests).

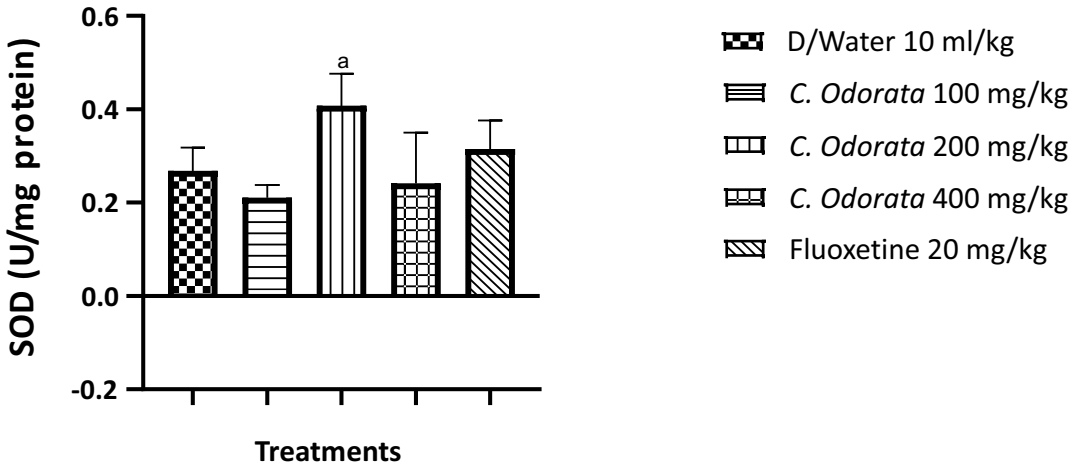

Figure 6: Evaluation of SOD level in the brain on the elucidation of possible mechanism(s) of antidepressant-like effect of *C. odorata* using Tail Suspension Test. (n=6). $^{a}P < 0.05$ vs. Distilled water (One way ANOVA followed by Dunnett's post-hoc tests)

DISCUSSION

The present study provides evidence supporting the antidepressant and anxiolytic potential of *C. odorata* extract, demonstrated by its significant reduction in immobility time in the tail suspension test and decreased anxiety-like behavior in the elevated plus maze test. These findings suggest that *C. odorata* extract could serve as a promising therapeutic option for managing depression and anxiety disorders.

The study used the elevated plus maze test to assess anxiolytic effects, while the tail suspension test was used to evaluate its antidepressant activity. In addition, the possible mechanism of antidepressant activity of the extract was elucidated.

The duration of exploration and frequency of entries into the open and closed arms are key parameters in the elevated plus maze (EPM) test, a widely used model for assessing anxiety-like behavior in rodents.[30] Existing literature suggest that mice with increased anxiety tend to avoid the open arms, reflecting a fear of exposure and potential threats.[31-32] This avoidance pattern aligns with both physiological and subjective measures of anxiety, thereby validating its use as a reliable indicator in animal models.[32-33]

In this study, *C. odorata* significantly increased the time spent and frequency of exploration in open arms, while reducing both parameters in the closed arms. This shift in behaviour suggests an anxiolytic effect of the extract, as increased open-arm exploration and reduced closed-arm preference are indicative of reduced anxiety levels.

Similarly, behavioral tests such as the forced swim test (FST) and tail suspension test (TST) serve as established predictors of depressive states in rodent models.[34] Increased immobility in these tests is associated with depressive-like behaviours, including reduced motivation and behavioral despair.[35] Conversely, a decrease in immobility duration suggests an improvement in mood or coping mechanisms.[36] In the present study, *C. odorata* administration at all tested doses significantly reduced the immobility of duration, indicating its potential antidepressant effects.

Deficiencies in the noradrenaline (NA) and serotonin (5-HT) neurotransmitter systems contribute to the exacerbation of depressive symptoms, given their critical roles in mood modulation, cognitive function, and stress response.[37] Research indicates that reduced activation of serotonergic and noradrenergic pathways is implicated in the pathophysiology of depression, leading to impaired mood regulation and increased anxiety.[38] In this study, administration of *C. odorata* at 400 mg/kg significantly increased noradrenaline and serotonin levels. In rodent models of depression, elevated concentrations of these neurotransmitters are commonly associated with reduced immobility in behavioural tests such as forced swim test (FST) and tail suspension test (TST).[39-40] Futhermore, many studies have demonstrated the involvement of nearly all serotonin receptor subtypes in mediating antidepressant and anxiolytic effects.[41] Consistent with these findings, the ability of *C. odorata* to enhance noradrenaline and serotonin levels suggest its potential as an antidepressant agent.

Oxidative biomarkers serve as measurable indicators of oxidative damage and antioxidant defense mechanisms, often showing alterations in individuals with depression.[42-43] Oxidative stress exacerbates inflammatory processes, disrupts neurotransmitter balance and contributes to neurotoxicity.[44] Additionally, oxidative damage to monoamine systems, including serotonin, dopamine, and noradrenaline can impair mood regulation.[45] However, antioxidants play a crucial role in preserving neuronal integrity and synaptic function, both of which are often compromised in depressive disorders.[46-47] In this study, *C. odorata* significantly reduced malondialdehyde (MDA) levels while increasing in glutathione (GSH), catalase, and superoxide dismutase (SOD) levels in the brain. The enhancement of antioxidant defenses suggests a neuroprotective effect, further supporting the extract's antidepressant potential.

Oral administration of *C. odorata* extract at doses reaching up to 2 g/kg showed no observable evidence of behavioral toxicity, including lethargy, tachycardia, hypokinesia, agitation, lacrimation, and anorexia, nor was there any mortality recorded during the 24-hour to 14-day observation period. The estimated oral LD_{50} of *C. odorata* in this present study was determined to be greater than 2 g/kg, which is consistent with those of Juan et al48 who reported signs of toxicity only at doses between 2-5 g/kg and estimated the LD_{50} to exceed 5 g/kg.

Qualitative phytochemical analysis of *C. odorata* confirmed the presence of bioactive compounds such as anthraquinones, saponins, flavonoids, cardiac glycosides, steroids, tannins, and alkaloids. These results are similar to the findings of Fagbohun *et al*.[49] and Paulose *et al*.[50] who identified similar phyto-constituents in *C. odorata* extracts. Existing literature suggests that tannins and phenolic compounds can mitigate oxidative stress and inflammation in neural tissues, potentially reducing the risk of neuropsychiatric disorders such as anxiety and depression, as well as neurodegenerative conditions like Alzheimer's and Parkinson's disease.[51-52] In addition, flavonoids have been shown to exert neuroprotective effects by counteracting oxidative stress, inflammation, and excitotoxicity, while also enhancing cognitive function through improved cerebral blood flow and neurotransmitter modulation.[53-54] Recent studies by Ali *et al*.[55] and Liew *et al*.[56] further highlighted the neuroprotective effects of alkaloids, demonstrating their ability to regulate neurotransmitter levels, reduce oxidative stress, and suppress neuroinflammation. Similarly, neurosteroids have been recognized for their potential therapeutic roles in managing neurodegenerative and mood-related disorders.[57] Murtala *et al*.[58] attributed the antidepressant and anxiolytic properties of Datura stramonium extract to the presence of phytoconstituents, including flavonoids, steroids, saponins, and alkaloids, as evidenced in their individual investigations. Given the phytoconstituents identified in *C. odorata*, its established anxiolytic and antidepressant effects, may be attributed to the presence of tannins, phenols, flavonoids, and alkaloids.

CONCLUSION

This study provides compelling evidence that *C. odorata* extract possesses anxiolytic and antidepressant properties. Its antidepressant effects appear to be mediated, at least in part, by the up-regulation of serotonin and noradrenaline signaling, two key neurotransmitter systems involved in depression. Additionally, the extract's ability to mitigate oxidative species suggests a potential neuroprotective mechanism that may contribute to its antidepressant action. These findings support the traditional use of *C. odorata* in folk medicine for managing anxiety and depression-related disorders.

ACKNOWLEDGMENT

The authors convey their sincere appreciation to Dr. Abayomi Ajayi of the University of Ibadan, Nigeria, for his invaluable contributions. The authors further extend their gratitude to Mr. Osipitan Luqman and Mr. Kasumu Busayo from the Department of Pharmacology and Therapeutics, Olabisi Onabanjo University, Nigeria, for their diligent and commendable efforts.

We affirm that there are no conflicting interests.

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