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Effects of *Costus afer* Extract in Mouse Models of Anxiety and Depression and Its Possible Mechanisms of Action

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ABSTRACT

Costus afer (Costaceae) is a perennial rhizomatous plant found in tropical Africa. It is used in traditional medicine to treat central nervous system disorders. The study investigated the anxiolytic- and antidepressant-like effects of the hydroethanol leaf extract of *Costus afer* and its possible mechanism(s) of action in mice. *C. afer* (25-200 mg/kg, p.o.), distilled water (10 mL/kg, p.o.), diazepam (1 and 3 mg/kg, p.o.) and imipramine (20 mg/kg, p.o.) were given 1 h before various tests, including hole-board, open field, elevated plus maze, light/dark exploration (anxiolytic-like activity), forced swim (FST) and tail suspension (TST) (antidepressant-like effect) tests. *C. afer* (50-200 mg/kg) increased number of head dips (hole-board test; $p < 0.05$), entries and dips in open arms (elevated plus maze test; $p < 0.05$-0.001), general square crossings (open field test; $p < 0.05$) and decreased time spent in the dark box (light/dark exploration test; $p < 0.05$). *C. afer*, with peak effect observed at 200 mg/kg, increased ($p < 0.01$) the latency of immobility and decreased ($p < 0.001$) the duration of immobility in both FST and TST. Sulpiride (dopamine D_2 receptor antagonist, 50 mg/kg), prazosin (α_1-adrenoceptor antagonist, 1 µg/kg) and metergoline (5-HT_2 receptor antagonist, 4 mg/kg) significantly ($p < 0.05$-0.01) blocked the anti-immobility effect of *C. afer* in FST. Findings showed that *C. afer* possess anxiolytic- and antidepressant-like activities, possibly mediated by α_1-adrenergic, dopamine D_2 and 5-HT_2 receptors.

Keywords *Costus afer*, Costaceae, Anxiolytic, Antidepressant, Mental disorders, Imipramine.

Introduction

Costus afer Ker Gawl (Costaceae) ("Bush sugarcane" or "Ginger lily") is a perennial rhizomatous and herbaceous plant found in tropical Africa, including Nigeria.[1] In Nigeria, it is called "Okpete / Okpoto" (Igbo), "Kakizawa" (Hausa), "Teteregun" (Yoruba) and "Mbritem" (Efik).[2] *C. afer* is commonly used for traditional therapeutic and other socio cultural purposes.[3] Preparations of *C. afer* are used for sleepiness, mental disorders and epileptic attack treatment, and to help dehydrating and weak patients.[3,4] Awouters *et al.*[5] also reported usefulness for treatment of cough, constipation, inflammation, arthritis, abdominal and chest pains, sepsis and edema, amongst other uses.

A number of studies have been conducted on the central nervous system effects of extracts of *C. afer*. Ezejiofor and Igweze[6] and Ezejiofor *et al.*[7] investigated the CNS depressant activity of the ethanol stem and leaf extracts of the plant in mice, respectively. The extract at doses of 300 and 800 mg/kg (ethanol stem extract) and 400 and 1200 mg/kg (ethanol leaf extract) increased the duration of phenobarbitone-induced hypnosis, suggesting CNS depressant property at these doses. In a study by Okoronkwo *et al.*[8] upon sub-acute administration (14 days), the ethanol leaf extract of *C. afer* was found to elicit anxiogenic effect at doses of 100 and 150 mg/kg.

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The combination of *C. afer* with two other plants has been reported to possess anti-anxiety effect with progressive changes in the temporal lobe integrity of the rat brain[9] and elicited improvement in short term memory and neuroprotective effect at low dose.[10] Other species of *Costus*, not *C. afer*, have been investigated and reported to possess antidepressant (*Costus speciosus*; leaf aqueous-ethanol extract)[11] and anti-Alzheimer activities (*Costus pictus*; leaf alcoholic extract).[12] To the best of our knowledge, the mechanism(s) of antidepressant activity of the hydroethanol leaf extract of *C. afer* has not been reported.

Anxiety and depression oftentimes are co-morbid disorders. Depression, a low level energy condition, evolves emotions such as worried thoughts, despondency and feeling overwhelmed, while anxiety which is of high energy state produces phobias and panic situations.[13] Up to 85% of depressive patients have also been diagnosed with anxiety disorders and an intriguing nexus has also been reported between anxiety, depression and insomnia.[13,14]

Depression, a leading cause of disability, affects over 300 million of world population without any regard for age.[15] Depression incidence is higher in developed countries (15%) relative to developing countries (11%).[16] Antidepressant drugs are most commonly used for both anxiety and depression because psycho-therapeutic agents often provide relief for patients suffering from both conditions. However, because of adverse effects and concerns about effectiveness, folkloric products now represent a credible alternative to addressing the menace of these disorders.

The study investigated the anxiolytic and antidepressant-like effects of the hydroethanol leaf extract of *Costus afer* and its possible mechanism(s) of action in mice.

Materials and Methods

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Plant material
The leaves of *C. afer* were collected from Ikenne Town, Ogun State, Nigeria in the month of July 2019. The identification/authentication of the plant (LUH8018) was done in the Department of Botany, University of Lagos, Nigeria.

Extraction
The extraction of the plant material (*C. afer* leaves) was done as previously reported by the authors.[17] 510 g of the powdered dried leaves were macerated in 1.5 L of hydroethanol (1:1) for 3 days. The combined filtrate from the exhaustive extraction process was evaporated to dryness at 40^{o}C to give a dark brown solid.

Experimental animals
Mice (20-25 g; either sex) sourced from the institutional Laboratory Animal Centre (CMUL - College of Medicine of the University of Lagos, Nigeria) were kept in appropriate compartments with free access to rodent feed and water. Two weeks window of acclimatization was observed before the start of the experimental sessions. Ethical approval was obtained from the institutional ethics committee (CMUL/HREC/12/17/330). Male:female mice ratio was 3:2 (n=5) in the models used in this study, except for the social interaction test (male mice only used) and the determination of mechanism(s) of action (equal representation of male and female mice). Animals were randomised into the different groups.
Treatment of animals and assessment of neurobehavioural parameters were done by different individuals to eliminate bias.

Acute toxicity test
C. afer was administered to a group of mice (n=5) fasted for 12 h at 2 g/kg p.o. Extract doses of 25, 50, 100, 200 and 400 mg/kg were given i.p. to 5 groups (n=5) of mice. The control groups were treated with distilled water 10 mL/kg p.o. and i.p. Animals were observed for 120 min post-treatment for behavioural manifestations. Mortality within 24 h was noted. Surviving animals were observed for signs of delayed toxicity for 7 days. The i.p. median lethal dose (LD_{50}) was obtained by the Behrens-Karber method and log-probit analysis.[18]

Gas chromatography-mass spectrometry (GC-MS) analysis
Although this study centred mainly on neuropharmacological activity evaluation of the hydroethanol leaf extract of *C. afer*, GC-MS analysis was done for fingerprinting prior to further research using a bioactivity guided fractionation approach to identify specific phytomolecules responsible for the reported biological activities in this study.
GC-MS analysis was done as reported by Anyasor *et al.*[19] using Agilent Technologies (Santa Clara, CA, USA) GC systems (GC-7890A/MS-5975C model; MSD - 5975C, injector - 7683B series). Specifications used were initial temperature (held for 2 min.) = 100^{o}C and final temperature (at rate of 10^{o}C/min) = 270^{o}C. The volume of the extract injected was 1 µL of 0.2 g/mL. The heater temperature was 250^{o}C while pressure was 3.2652 psi with splitless injection mode type. The system was equipped with HP-5MS column (30 m × 320 µm × 0.25 µm) and helium carrier gas (99.9999% purity). The set flow rate was 1.4963 mL/min with average velocity of 45.618 cm/s. Identification of compounds present in the extract was done by comparison of retention times and mass spectra of the authentic samples obtained by GC with the mass spectra from the National Institute of Standards and Technology (NIST), United States database.

Neuropharmacological activities evaluation
Hole Board Test
One hour after oral administration of distilled water (10 mL/kg), *C. afer* (25, 50, 100 and 200 mg/kg) and diazepam (Swipha Pharmaceuticals, Lagos, Nigeria; 1 and 3 mg/kg) to 7 groups of 5 mice each, every mouse was dropped at one corner of the hole-board (wooden board - 40 cm × 40 cm - with four equidistant holes - 1 cm diameter × 2 cm depth) and observation was done for 5 min. The number and duration of head dips, and sectional crossings were recorded for each mouse.[20]

Elevated plus maze test
One hour after treatment as stated in the hole board test, each mouse was placed at the centre of the elevated plus maze (consists of 2 open arms and 2 closed arms - 50 × 10 × 40 cm each - elevated to a height of 50 cm) and observed for 5 min.[21] The cumulative times spent in the open and closed arms of the maze by each mouse, the numbers of entries into the arms and head dips in the open arm were recorded.

Open field test
Sixty minutes post-oral treatment as described in the hole board test, each mouse was gently placed at the centre of the open field (wooden box with dimensions of 50 cm × 50 cm × 25 cm with plain floor divided into 8 cm × 8 cm with 16 squares on it). Squares at the middle were designated as centre squares while others adjacent to the walls were tagged periphery squares. The number of general and centre squares crossings (all four paws), rearings and assisted rearings within 5 min were recorded.[22]

Light/Dark exploration test
Sixty minutes after pre-treatment as mentioned in the hole board test, each mouse was placed individually in the lit part of the box (50 cm × 25 cm × 25 cm segmented into 2 compartments - light and dark). Latency of entry into the dark box, number of entries into the light and dark compartments, total time spent in the light compartment, and number of rearings and assisted rearings were recorded within 5 min.[23]

Forced swim test
Mice were individually forced to swim in transparent cylindrical container (10 cm diameter and 25 cm height) containing water up to the 19 cm mark. Mice were randomly selected into 6 groups of 5 animals each and respectively treated as follows: distilled water (10 mL/kg, p.o.; Group 1); imipramine (Remedica Ltd., Lagos, Nigeria; 20 mg/kg, p.o.; Group 2); and extract (25, 50, 100 and 200 mg/kg, p.o.; Groups 3-6 respectively). After 60 min, each mouse was subjected to the forced swim test for 5 min. The latency and cumulative duration of immobility were recorded.[24]

Tail suspension test
Mice were treated as mentioned in the forced swim test. Sixty minutes post-treatment, each mouse was suspended by the tail in turn on a retort stand 50 cm above the floor with the help of adhesive tape. The latency and cumulative duration of immobility within 5 min were recorded.[25]

Elucidation of possible mechanism(s) of antidepressant-like effect
Four different catecholamine antagonists were respectively given i.p. to separate groups of mice 15 min before extract (200 mg/kg, p.o.) administration. The antagonists include sulpiride (dopamine D_2 receptor antagonist, 50 mg/kg), prazosin (α_1-adrenoceptor antagonist, 1 µg/kg), metergoline (5-HT_2 receptor antagonist, 4 mg/kg) and yohimbine (α_2-adrenoceptor antagonist, 1 mg/kg). One hour post extract administration, mice were subjected to the FST.[21]

Statistical analysis
Values were expressed as Mean ± S.E.M. The data were analysed using one-way Analysis of Variance (ANOVA) followed by Dunnett's and Tukey's multiple comparison tests using GraphPad Prism 5 Software (GraphPad Software Inc., CA, USA). Results were considered significant at $p < 0.05$.

Results and Discussion

In the management of CNS disorders, there is need for paradigm shift to accommodate effective complimentary medicines which are likely to be more accessible, affordable and safe. Accordingly, the anxiolytic- and antidepressant-like activities of the hydroethanol leaf extract of *C. afer* were evaluated based on use in traditional medicine.
Anxiety, agitation, restlessness and insomnia are common and expensive psychiatric problems which are essentially managed with conventional anxiolytics and hypnotics.[26] Herbal therapies which are easily accessible represent credible options to treating these conditions. In the hole board test, *C. afer* (50-200 mg/kg) significantly

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increased ($p < 0.05$) the number of head dips compared to control. Both 1 and 3 mg/kg of diazepam did not significantly change ($p > 0.05$) the number of head dips relative to control. *C. afer* and diazepam (1 and 3 mg/kg) elicited non-significant increase ($p > 0.05$) in the duration of head dips compared with the control. As regards the number of sectional crossings, there was significant increase ($p < 0.001$) in value at 50 mg/kg of the extract relative to control (Table 1). Animals manifest anxiolytic state by eliciting increased head dips and locomotive activity in the hole board procedure.[27,28] *C. afer* at 50-200 mg/kg significantly ($p < 0.05$) increased the number of head dips indicating possible anxiolytic activity. The extract at the dose of 50 mg/kg significantly increased the number of sectional crossings, indicating enhanced locomotion. Based on the findings in this model, *C. afer* possess anxiolytic-like activity, without stimulant action, especially at 100 and 200 mg/kg (higher doses).

In the open field test, *C. afer* at the dose of 100 mg/kg significantly increased ($p < 0.05$) the number of general square crossings. At 50 mg/kg of the extract, there was significant increase ($p < 0.001$) in the number of rearings. Also, there was significant increase ($p < 0.01$, 0.001) in the number of assisted rearings at 50, 100 and 200 mg/kg of the extract (Table 2). Barua *et al.*[20] associated anxiolytic effect of a plant extract with increase in the number of rearings, assisted rearings and centre square crossings in the open field test. Animals show anxiety and fear by expressing decrease in the exploration of the general square crossings, centre square crossings, rearings, assisted rearings, and increased urination and defecation when introduced into a strange environment.[29] *C. afer* (50, 100 and 200 mg/kg) significantly increased the number of rearings, assisted rearings, and non-significantly increased the number of centre square crossings. These effects suggest potential anxiolytic-like activity of the extract.

In respect of the elevated plus maze test, the extract at 100 and 200 mg/kg produced non-significant increase ($p > 0.05$) in time spent in the open arms compared to control. At 100 and 200 mg/kg, the extract produced significant increase ($p < 0.05$, 0.01) in the number of entries into the open arms compared to control. In respect of number of head dips in the open arms, *C. afer* at 200 mg/kg elicited significant increase ($p < 0.001$) relative to control. Diazepam (1 mg/kg) produced significant increase ($p < 0.05$) in the number of head dips compared with control. With regards to number of entries and time spent in the closed arms, the extract at all doses non-significantly reduced ($p > 0.05$) values relative to the control. Diazepam (1 mg/kg) produced significant ($p < 0.01$) decrease in time spent in the closed arms compared to the control (Table 3). The EPMT represents the most important anxiolytic procedure in the quest for new benzodiazepine-like anxiolytic drugs.[30] Santos *et al.*[31] mentioned that rodents spend more time in the closed arms of the EPM. Aversion to the open arm indicates fear and anxiety while greater exploration of the open arms is suggestive of anxiolytic activity. In this study, the extract at 100 and 200 mg/kg non-significantly increased the time spent in open arms. *C. afer* at 100 and 200 mg/kg significantly increased the number of entry into the open arms, while at 200 mg/kg there was a significant increase in the number of head dips in the open arms. The aggregates of increased number of entries and head dips in the open arms suggest anxiolytic-like activity, especially at the higher doses.

Concerning the light/dark exploration test, *C. afer* (200 mg/kg) and diazepam (1 mg/kg) produced non-significant increase ($p > 0.05$) in the latency of entry into the dark box compared to the control. With regards to time spent in the light box, *C. afer* (25-200 mg/kg) produced non-significant increase ($p > 0.05$) relative to the control. Diazepam (1 mg/kg) produced significant increase ($p < 0.01$) in the time spent in the light box. *C. afer* (100 mg/kg) produced significant decrease ($p < 0.05$) in time spent in the dark box compared to the control. Diazepam 1 mg/kg elicited significant reduction ($p < 0.0001$) in time spent in the dark box relative to the control. *C. afer* (25-200 mg/kg) produced significant ($p < 0.0001$, 0.001) increase in the number of assisted rearings compared to the control. As regards the number of transitions, there was significant increase ($p < 0.0001$) at extract doses of 25-200 mg/kg relative to the control (Table 4). The time spent in each compartment of the light/dark box is a reflection of aversion.[32] Anxiety is considered to be low if the length of time spent in the lit compartment is high. The extract at 100 mg/kg significantly decreased the time spent in the dark box. The extract at 25-200 mg/kg significantly increased the number of assisted rearings in the lit compartment and transitions between the light and dark compartments, suggesting anxiolytic-like activity.

Table 1: Effect of hydroethanol leaf extract of *C. afer* in the hole board test in mice

Treatment	Dose (mg/kg)	No. of head dips	Duration of head dips (sec.)	No. of sectional crossings
Distilled water	10 (mL/kg)	8.80 ± 2.33	0.36 ± 0.12	6.40 ± 2.06
Diazepam	1	6.60 ± 1.63	0.92 ± 0.31	1.60 ± 0.24
Diazepam	3	3.00 ± 1.00	1.43 ± 0.59	2.00 ± 0.70
C. afer	25	14.40 ± 1.36	0.78 ± 0.31	$8.60 \pm 1.12^{\beta}$
C. afer	50	$20.40 \pm 3.55^{a,\beta}$	1.11 ± 0.21	$15.00 \pm 1.58^{c,\delta}$
C. afer	100	$19.80 \pm 0.73^{a,\beta}$	0.77 ± 0.21	$10.60 \pm 0.81^{\gamma}$
C. afer	200	$19.80 \pm 3.07^{a,\beta}$	0.84 ± 0.27	$11.20 \pm 1.46^{\gamma}$

Values are Mean ± S.E.M. (n = 5). $^{a}p < 0.05$, $^{c}p < 0.001$ vs. distilled water; $^{\beta}p < 0.01$, $^{\gamma}p < 0.001$, $^{\delta}p < 0.0001$ vs. diazepam 1 mg/kg (one-way ANOVA followed by Tukey's multiple comparison test).

Table 2: Effect of hydroethanol leaf extract of *C. afer* in the open field test in mice

Treatment	Dose (mg/kg)	No. of general square crossings	No. of centre square crossings	No. of rearings	No. of assisted rearings
Distilled water	10 (mL/kg)	56.60 ± 7.97	4.80 ± 1.31	3.40 ± 0.92	8.80 ± 1.71
Diazepam	1	90.00 ± 7.21	0.80 ± 0.37	0.40 ± 0.24	3.80 ± 1.82
Diazepam	3	44.00 ± 20.52	0.40 ± 0.24	0.60 ± 0.24	2.00 ± 0.31
C. afer	25	60.20 ± 2.99	5.20 ± 2.01	$5.60 \pm 1.50^{\beta}$	$16.00 \pm 2.25^{\beta}$
C. afer	50	89.20 ± 11.73	5.20 ± 1.24	$9.80 \pm 1.15^{c,\delta}$	$20.80 \pm 2.97^{b,\delta}$
C. afer	100	$108.6 \pm 6.47^{a,\beta}$	$10.00 \pm 2.16^{\gamma}$	$6.00 \pm 1.00^{\beta}$	$23.80 \pm 2.63^{c,\delta}$
C. afer	200	88.80 ± 6.73	$7.00 \pm 0.70^{\alpha}$	4.40 ± 0.50	$23.20 \pm 2.08^{c,\delta}$

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Values are Mean ± S.E.M. (n=5). [a]p < 0.05, [b]p < 0.01, [c]p < 0.001 vs. distilled water; [α]p < 0.05, [β]p < 0.01, [γ]p < 0.001, [δ]p < 0.0001 vs. diazepam 1 mg/kg (one-way ANOVA followed by Tukeys's multiple comparison test).

Table 3: Effect of hydroethanol leaf extract of *C. afer* in the elevated plus maze test in mice

Treatment	Dose (mg/kg)	Time spent in open arms (sec.)	No. of entry into open arms	No. of head dips in open arms	No of entry into closed arms	Time spent in closed arms (sec.)
Distilled water	10 (mL/kg)	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.20	6.40 ± 1.20	4.86 ± 0.02
Diazepam	1	0.91 ± 0.16	1.20 ± 0.37	11.40 ± 1.56[a]	4.60 ± 0.87	3.61 ± 0.30[b]
Diazepam	3	0.30 ± 0.30	0.20 ± 0.20	2.20 ± 0.96	2.40 ± 0.40	4.33 ± 0.40
C. afer	25	0.03 ± 0.01[β]	1.20 ± 0.20	4.80 ± 0.86	5.20 ± 0.58	4.66 ± 0.05[α]
C. afer	50	0.18 ± 0.05[α]	2.00 ± 0.70	7.00 ± 0.44	5.00 ± 0.70	4.45 ± 0.12
C. afer	100	0.35 ± 0.09	3.40 ± 1.03[b]	9.60 ± 2.24	5.20 ± 1.56	4.47 ± 0.12
C. afer	200	0.34 ± 0.11	2.40 ± 0.40[a]	16.60 ± 5.04[c]	5.60 ± 1.03	4.39 ± 0.10

Values are Mean ± S.E.M. (n = 5). [a]p < 0.05, [b]p < 0.01, [c]p < 0.001 vs. distilled water; [α]p < 0.05, [β]p < 0.01 vs. diazepam 1 mg/kg (one-way ANOVA followed by Tukey's multiple comparison test).

Table 4: Effect of hydroethanol leaf extract of *C. afer* in the light/dark exploration test in mice

Treatment	Dose (mg/kg)	Latency of entry into dark box (sec)	Time spent in light box (sec)	Time spent in dark box (sec)	No. of rearings	No. of assisted rearings	No. of transitions
Distilled water	10 (mL/kg)	0.47 ± 0.04	0.73 ± 0.08	3.44 ± 0.17	1.40 ± 0.60	4.60 ± 0.50	4.60 ± 0.74
Diazepam	1	1.96 ± 0.78	3.72 ± 0.45[d]	0.45 ± 0.24[d]	1.20 ± 0.48	4.00 ± 0.70	6.00 ± 0.70
Diazepam	3	0.31 ± 0.13	0.24 ± 0.06	2.78 ± 0.44	0.20 ± 0.20	5.80 ± 2.08	2.00 ± 0.54
C. afer	25	0.72 ± 0.07	1.13 ± 0.12[γ]	2.88 ± 0.10[δ]	1.80 ± 0.58	18.60 ± 2.31[d,δ]	8.60 ± 0.50[b]
C. afer	50	0.38 ± 0.04	2.08 ± 0.49[α]	2.44 ± 0.47[γ]	1.40 ± 0.24	19.60 ± 1.20[d,δ]	10.40 ± 0.50[d,β]
C. afer	100	0.90 ± 0.13	2.15 ± 0.21[α]	2.47 ± 0.15[a,γ]	2.20 ± 0.20	14.80 ± 1.15[c,β]	10.80 ± 0.86[d,γ]
C. afer	200	1.44 ± 0.48	2.28 ± 0.25[α]	1.96 ± 0.21[α]	1.00 ± 0.31	16.20 ± 1.49[c,δ]	10.00 ± 0.70[d,β]

Values are Mean ± S.E.M. (n = 5). [a]p < 0.05, [b]p < 0.01, [c]p < 0.001, [d]p < 0.0001 vs. distilled water; [α]p < 0.05, [β]p < 0.01, [γ]p < 0.001, [δ]p < 0.0001 vs. diazepam 1 mg/kg (one-way ANOVA followed by Tukey's multiple comparison test).

In the forced swim test, *C. afer* (25-200 mg/kg) produced significant increases (p < 0.05, 0.01) in the latency of immobility with peak effect at 200 mg/kg (p < 0.01) relative to control. Imipramine also significantly (p < 0.0001) increased the latency of immobility relative to control. The extract significantly (p < 0.01, 0.001) reduced the cumulative duration of immobility with peak effect at 200 mg/kg (p < 0.001) compared to control. The effect of imipramine in reducing the cumulative duration of immobility was also significant (p < 0.05) relative to control (Table 5). Concerning the tail suspension test, the extract at 200 mg/kg elicited significant increase (p < 0.01) in the latency of immobility compared to control. The increase in the latency of immobility invoked by imipramine was non-significant (p > 0.05) relative to the control. *C. afer* elicited significant reduction (p < 0.05, 0.001) in total immobility time at 50 and 100 mg/kg compared to the control. Imipramine also caused significant reduction in total immobility time (p < 0.05) relative to the control (Table 6). Porsolt et al.[33] and Steru et al.[34] reported that both FST and TST are widely used behavioural despair procedures to evaluate novel antidepressant drugs. The immobility showed by animals is an adoption of passive response/defeat to stressful conditions.[35] In the FST, the extract at 25-200 mg/kg significantly increased the latency of immobility and decreased the total immobility time with peak effect at 200 mg/kg. In respect of TST, the extract at 200 mg/kg significantly increased the latency of immobility and reduced the total immobility time at 50 and 100 mg/kg. The peak effect of the extract on total immobility time was observed at 100 mg/kg. These effects suggest antidepressant-like activity by the extract. It is important to state that the extract did not elicit significant increase in locomotion and stimulant activity in the open field test except at the dose of 50 mg/kg.

Administration of sulpiride, metergoline and prazosin with *C. afer* 200 mg/kg produced significant increase (p < 0.05, 0.01) in the duration of immobility compared to extract (200 mg/kg) only treated group. Yohimbine did not significantly impact (p > 0.05) on the effect of the extract on duration of immobility (Figure 1). The hypothesized theory of depression predicts depletion in the levels of serotonin, noradrenaline and/or dopamine.[36]

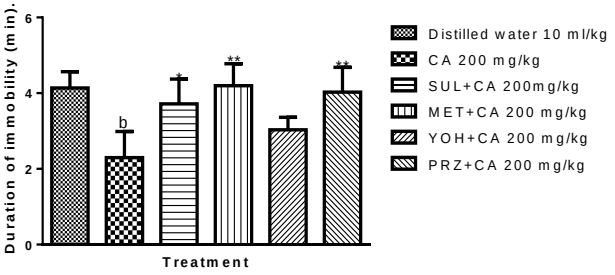

Figure 1: Elucidation of possible mechanism(s) of antidepressant-like effect of *C. afer* in the FST. Values are Mean ± S.E.M. (n=6). [b]p < 0.01 vs. distilled water; [*]p < 0.05, [**]p < 0.01 vs. *C. afer* 200 mg/kg (one-way ANOVA followed by Tukey's multiple comparison tests).

Table 5: Effect of hydroethanol leaf extract of *C. afer* in the forced swim test in mice

Treatment	Dose (mg/kg)	Latency of immobility (min.)	Total duration of immobility (min.)
Distilled water	10 (mL/kg)	0.64 ± 0.05	3.85 ± 0.46
Imipramine	20	2.06 ± 0.10[d]	2.31 ± 0.40[a]
C. afer	25	1.30 ± 0.18[a]	1.75 ± 0.25[c]

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C. afer	50	1.39 ± 0.19[b]	1.76 ± 0.27[c]
C. afer	100	1.30 ± 0.08[a]	1.82 ± 0.26[b]
C. afer	200	1.50 ± 0.17[b]	1.49 ± 0.29[c]

Values are Mean ± S.E.M. (n=5). [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$, [d]$p < 0.0001$ vs. distilled (one-way ANOVA followed by Dunnett's multiple comparison test).

In this study, the role of biogenic amines in the antidepressant-like activity of *C. afer* was investigated using the FST model. The contribution of the serotonergic system in the antidepressant-like activity of the extract was probed using metergoline (5-HT_2 receptor antagonist) and the drug was found to reverse the anti-immobility effects of the extract. Inhibition of 5-HT_2 receptor uptake plays a major role in the antidepressant-like effect of conventional antidepressants in FST.[37]

Massive dopamine neuron deficit and low dopamine and its metabolites levels have been found in depression.[38-40] In this study, it was observed that sulpiride (dopamine D_2 receptor antagonist) significantly reversed the anti-immobility activity of the extract. Depression has also been associated with the down-regulation of adrenergic neurons.[41] Prazosin (α_1-adrenoceptor antagonist) in this study blocked the anti-immobility activity of the extract, but yohimbine (α_2-adrenoceptor antagonist) did not. These findings suggest the involvement of α_1-adrenoceptor, dopamine D_2 and 5-HT_2 activities enhancement in the antidepressant-like activity of *C. afer*.

Murtala *et al.*[17] reported the presence of flavonoids, steroids, glycosides, phenols, alkaloids, terpenoids and tannins in the hydroethanol leaf extract of *C. afer* while reporting total antioxidant capacity, total phenols and total flavonoids values of 31.78±0.24 mg/100 g ascorbic acid equivalent, 17.60±0.97 mg/100 g gallic acid equivalent and 25.34±0.64 mg/100 g quercetin equivalent, respectively. Anyasor *et al.*[19] reported the presence of alkaloids, saponins, diterpenes, triterpenes, phytosterol, phlobatannins, phenols, flavonoids and tannins. Some of these compounds have been associated with the anxiolytic and antidepressant activities of medicinal plants.[42, 43]

GC-MS analysis revealed hexadecanoic acid ethyl ester (peak #18); (E)-9-octadecenoic acid ethyl ester (peak #16); 13-octadecenal (Z) (peak #25); octadecanoic acid, 2-hydroxy-1,3-propanediyl (peak #26); octadecanoic acid, 2-hydroxy-1,3-propanediyl (peak #19); and octadecanoic acid (peak #20) as the most abundant components of the plant extract (Figure 2).

Pandey *et al.*[44] reported oxidative stress and a low level of antioxidant enzymes in patients with depression. It has also been reported that some inflammatory mediators are higher in people with depression compared with normal subjects.[45] Some medicinal plants have been reported to exhibit antidepressant activity by reducing oxidative stress and inflammatory mediators.[45,46] Adeoye-Isijola *et al.*[47] reported the antioxidant activity of hexadecanoic and octadecanoic acids present in medicinal plants. Based on these findings, the antidepressant-like effect exerted by the extract may also be due to synergistic antioxidant activities of hexadecanoic acid and octadecanoic acid in *C. afer*.

No mortality was recorded at extract oral dose of 2 g/kg. The animals elicited some degree of inactivity, dumbness and reduced movement within 2 h post-treatment. No signs of delayed toxicity and mortality were observed 2 weeks post-treatment. Since no mortality was observed at the oral dose of 2 g/kg, the extract can be considered as safe.[48] The i.p. LD_{50} was estimated to be 298 mg/kg. At the higher doses, animals showed signs of writhing, increased respiration and paw licking.

Table 6: Effect of hydroethanol leaf extract of *C. afer* in tail suspension test in mice

Treatment	Dose (mg/kg)	Latency of immobility (min.)	Total duration of immobility (min.)
Distilled water	10 (mL/kg)	0.76 ± 0.19	3.01 ± 0.48
Imipramine	20	1.67 ± 0.54	1.72 ± 0.14[a]
C. afer	25	1.40 ± 0.13	2.12 ± 0.19
C. afer	50	1.98 ± 0.38	1.68 ± 0.42[a]
C. afer	100	1.78 ± 0.36	0.88 ± 0.06[c]
C. afer	200	2.26 ± 0.20[b]	1.99 ± 0.20

Values are Mean ± S.E.M. (n=5). [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ vs. distilled water (one-way ANOVA followed by Dunnett's multiple comparison test).

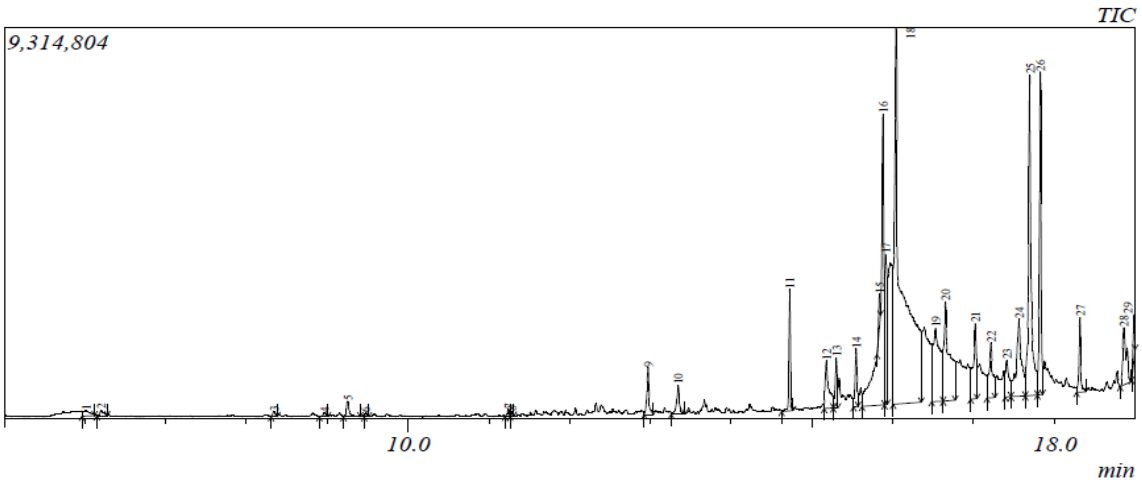

Figure 2: GC chromatogram of hydroethanol leaf extract of *C. afer* displaying 29 peaks.

Conclusion

The hydroethanol leaf extract of *Costus afer* possesses anxiolytic and antidepressant activities possibly by enhancing serotonergic, dopaminergic and adrenergic systems in the brain. This suggests potential usefulness of *C. afer* hydroethanol leaf extract as remedy for anxiety and depression conditions. Further work is on-going using a bioactivity guided fractionation approach to identify specific phytomolecules responsible for the reported anxiolytic and antidepressant activities observed in this study.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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