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Research article

Neurobehavioural and Hippocampal Effects of Ethanolic Costus dubius Leaf Extract in Diazepam-Associated Amnesia in Mice: Implications for Sustainable Bioprospecting

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ABSTRACT

Benzodiazepine-associated memory impairment has encouraged the search for neuroprotective agents from medicinal plants, but cognitive rescue must be distinguished from non-specific behavioural activation. This study evaluated ethanolic Costus dubius leaf extract in a diazepam-induced amnesia model. Twenty-five mice were allocated to five groups (n = 5). The normal control received saline; the reference group received Ginkgo biloba (40 mg/kg); and treatment groups received extract at 100, 200 or 400 mg/kg. All groups except the normal control received diazepam (3 mg/kg) for 14 days. Tail-suspension behaviour, novel-object recognition and hippocampal histology were assessed. Polyphenols, tannins, flavonoids, saponins, steroids, terpenoids, glycosides and alkaloids were detected. Behavioural effects were non-linear. The 400 mg/kg dose increased mobility, but immobility was not consistently reduced across extract doses. Novel-object exploration was most clearly enhanced at 100 and 200 mg/kg, whereas 400 mg/kg did not show a comparable preference. Histology showed preserved hippocampal architecture with variable reactive glial and pyramidal-cell changes. C. dubius demonstrated dose-dependent neurobehavioural potential, but the evidence does not support a uniform antidepressant-like response. The study identifies promising intermediate doses and provides a basis for mechanistic, toxicological and sustainable-bioprospecting research.

KEYWORDS

Costus dubius; diazepam-associated amnesia; novel object recognition; tail-suspension test; hippocampus; phytochemicals; neurobehavioural activity; sustainable bioprospecting

Introduction

Memory impairment is a clinically important adverse effect of several centrally acting medicines and a defining feature of many neurological disorders. Benzodiazepines are effective anxiolytic, sedative, anticonvulsant and muscle-relaxant agents, but their facilitation of gamma-aminobutyric acid type A (GABAA) receptor signalling can interfere with the encoding and consolidation of new information. Diazepam-associated anterograde amnesia is therefore both a therapeutic limitation and a useful experimental model for studying memory-related processes (Kaplan & Hunsberger, 2023; Mehta & Ticku, 1999). The search for agents that reduce cognitive impairment without producing additional behavioural or systemic toxicity has encouraged renewed attention to medicinal plants and their secondary metabolites.

Natural-product research is especially relevant in biodiverse regions where communities already use local species for health care. Plants can provide chemically diverse leads, but promising behavioural findings require careful interpretation because increased exploration, altered anxiety, sedation or motor stimulation can influence memory tests independently of cognition. The route from ethnomedicinal use to a reproducible neuroprotective intervention therefore depends on botanical authentication, extract standardisation, appropriate controls, multiple outcome domains and transparent reporting (Atanasov et al., 2021). It also depends on environmental stewardship. Medicinal species are ecosystem resources, and pharmacological validation can create new demand that must be balanced with conservation, cultivation and fair benefit sharing.

Costus dubius is a perennial herb reported in African traditional medicine. Closely related Costus species are used for diabetes, hypertension, inflammatory complaints, infections, cough, stomach disorders and other conditions. Phytochemical investigations of Costus materials have identified polyphenols, flavonoids, tannins, alkaloids, saponins, terpenoids, steroids and glycosides, several of which have antioxidant or neuromodulatory potential (Anyasor et al., 2014; Rocha et al., 2021). However, species names within the genus are sometimes used inconsistently in local and published records. The study retains the name C. dubius used in the laboratory record, but definitive taxonomic verification with a voucher specimen is essential before chemical or ecological generalisation.

Flavonoids and other polyphenols are of particular interest because they can influence oxidative stress, neuroinflammation, cerebrovascular function, synaptic signalling and pathways involved in learning and memory (Vauzour et al., 2008). These mechanisms are plausible in benzodiazepine-associated cognitive impairment, but the presence of a metabolite class does not prove neuroprotection. Behavioural evidence must show a pattern consistent with preserved or restored memory, and tissue evidence should support structural or cellular protection. Where behavioural outcomes are non-linear or inconsistent across doses, the interpretation should remain cautious and should consider hormesis, receptor heterogeneity, differing concentrations of active and antagonistic constituents, and non-specific effects on locomotion.

The novel object recognition (NOR) task exploits rodents' spontaneous tendency to investigate novelty. When acquisition and retention conditions are controlled, greater exploration of a novel object relative to a familiar object indicates memory for the familiar object. The task engages perirhinal cortical and hippocampal networks, although the contribution of each region depends on delay, context and task design (Ennaceur & Delacour, 1988; Leger et al., 2013). The tail-suspension test (TST), in contrast, is a behavioural despair screening procedure ly developed for antidepressant-like activity. Mobility and immobility in the TST can be affected by motor function, stress reactivity and sedative or stimulant actions, so it should not be used as a direct measure of depression in humans or as proof of an antidepressant mechanism (Steru et al., 1985).

The present study evaluated the effects of ethanolic C. dubius leaf extract in mice exposed to diazepam for 14 days. The objectives were to identify major phytochemical classes; compare mobility and immobility in the TST; assess familiar- and novel-object exploration; and examine hippocampal morphology. The study further aimed to integrate behavioural and histological findings, identify the dose range with the most coherent signal, and discuss implications for environmental health and sustainable medicinal-plant bioprospecting. The terms memory-related effect, neurobehavioural activity and antidepressant-like activity are used deliberately; a definitive neuroprotective or antidepressant claim would require additional mechanistic, biochemical and functional evidence.

Literature review

Benzodiazepines bind allosterically to GABAA receptors and increase the frequency of chloride-channel opening in the presence of GABA, enhancing neuronal inhibition (Mehta & Ticku, 1999). The resulting clinical effects are useful for anxiety, seizures and acute agitation, but enhanced inhibition within memory-related networks can disrupt acquisition and consolidation. The magnitude of amnesia depends on dose, timing, receptor subtype, task and the emotional salience of the material. Recent analysis has reframed benzodiazepine-induced amnesia as more than an adverse effect: because the manipulation is reliable and temporally controllable, it can also serve as an experimental tool for investigating memory circuits (Kaplan & Hunsberger, 2023). In animal models, diazepam has been used to test whether pharmacological or plant-derived interventions preserve performance in mazes, object-recognition tasks and other learning paradigms.

The hippocampus is central to relational and contextual memory and is particularly vulnerable to metabolic, inflammatory and toxic insults. Its pyramidal-cell layers and supporting glia provide histological indicators of tissue organisation, but routine haematoxylin-eosin staining has limited ability to distinguish subtle synaptic dysfunction, oxidative injury or specific forms of cell death. Reactive astrocytes and microglia can

indicate cellular stress or repair, yet interpretation requires consistent anatomical sampling, blinded scoring and, ideally, immunohistochemical markers. A study that combines NOR behaviour with hippocampal histology is therefore stronger than a behaviour-only study, but structural preservation should not be equated automatically with restored memory. Functional measures and molecular endpoints remain necessary.

The NOR task has several advantages: it requires little food restriction or aversive stimulation, can be repeated with careful design, and separates familiar from novel exploration. Its validity depends on equal object salience, counterbalancing of object position, adequate habituation, consistent retention interval and exclusion of animals that fail to explore sufficiently. Total exploration time should be reported alongside a discrimination index because a high novel-object time can reflect general hyperactivity, while a low total time can create unstable ratios. The study reconstructs approximate group means from those figures and calculates descriptive discrimination indices only. Exact inferential reanalysis is not possible without raw data.

The TST provides a rapid screen for behavioural effects of antidepressant drugs, but its conceptual interpretation has evolved. Immobility may represent an energy-conserving coping strategy rather than a direct analogue of human hopelessness, and compounds that alter locomotion can produce false-positive or false-negative results. Consequently, the present study uses the phrase antidepressant-like only when discussing the assay's conventional interpretation and evaluates mobility and immobility as separate behavioural outcomes. A coherent antidepressant-like pattern would ordinarily involve reduced immobility without evidence of nonspecific hyperactivity. When mobility increases at one dose but immobility does not decline consistently across doses, the result is better described as a non-linear behavioural effect.

Medicinal plants may influence cognition through multiple, interacting mechanisms. Polyphenols and flavonoids can scavenge reactive species, regulate antioxidant enzymes, modulate neuroinflammatory signalling and influence synaptic plasticity. Alkaloids may act on cholinergic, monoaminergic or other neurotransmitter systems. Terpenoids and saponins can also affect inflammation, membrane signalling and neurotrophic pathways. Vauzour et al. (2008) described the multiplicity of flavonoid actions in the brain, while more recent work has continued to identify plant-derived compounds with potential effects on neuropsychiatric and neurodegenerative pathways (Asgharian et al., 2022). Yet crude extracts can contain constituents with opposing actions, and the dose-response may not be linear. This complexity is a reason to fractionate and chemically fingerprint an active extract rather than treating the plant name as a single pharmacological entity.

Evidence from African medicinal-plant research supports the feasibility of this approach. Beppe et al. (2022) reported behavioural and histological effects of Crassocephalum crepidioides in a diazepam-induced amnesia model, providing a close methodological comparator. Other local studies have evaluated plant-derived antiplasmodial, antibacterial, metabolic and toxicological outcomes, illustrating a broader research programme in which biological activity is interpreted alongside safety (Akpan et al., 2024, 2026; Eke et al., 2025; Usanga et al., 2022). Work on bioactive chemicals from Phragmanthera capitata and depressive-like behaviour is also conceptually relevant because it demonstrates how plant chemistry can be linked to behavioural phenotypes while retaining the distinction between animal screening and clinical treatment (Takem et al., n.d.).

Ginkgo biloba was included as a reference treatment because standardised Ginkgo preparations have been investigated for cognitive symptoms and can provide a practical benchmark in exploratory animal studies. Its inclusion does not, however, create a complete disease-model control: a separate diazepam-only group is needed to quantify the impairment produced by diazepam before judging rescue by either Ginkgo or C. dubius. This distinction is important because comparison only with a normal control and a reference-treatment group can obscure whether an extract restores a diazepam-induced deficit, changes behaviour beyond normal levels, or simply differs from the reference drug. Dose-response interpretation also requires attention to non-monotonicity. A crude extract may contain compounds with different targets, absorption profiles and concentration-response curves, so the most effective dose in a memory task may not be the dose that produces

the greatest motor activity. The present analysis therefore evaluates each behavioural domain separately and seeks convergence rather than assuming that the highest dose must be best.

The environmental context is equally important. The discovery of a useful neuroactive plant product can create incentives for collection, trade and cultivation, with consequences for local ecosystems and livelihoods. Sustainable development requires more than demonstrating pharmacological activity. It includes accurate species identification, assessment of abundance, cultivation protocols, contaminant control, equitable participation and monitoring of harvesting effects. Nigerian scholarship has examined environmental management, conservation-related livelihood transitions, spatial organisation of services and socio-economic dimensions of sustainable development across tourism, urban and resource systems (Eneyo, 2025a, 2025b, 2025c, 2026a, 2026b; Eneyo et al., 2026; Eneyo & Archibong, 2024; Eneyo et al., 2023; Eneyo & Ekpenyong, 2018). Although these sectors differ from neuropharmacology, they reinforce a shared principle: biological-resource innovation should be embedded in environmental governance rather than detached from it.

Diazepam-induced amnesia models are widely used to investigate memory impairment and evaluate potential neuroprotective agents targeting hippocampal dysfunction, oxidative stress, and neurotransmitter alterations. Plant-derived bioactive compounds have demonstrated promising antiamnesic and neuroprotective effects through antioxidant activity, modulation of cholinergic signalling, and preservation of neuronal integrity (Beppe et al., 2022; Wang et al., 2011). Previous studies have shown that ethanolic plant extracts can improve learning and memory performance, reduce oxidative damage, and protect hippocampal neurons against diazepam-associated cognitive deficits in mice (Beppe et al., 2022; Beppe et al., 2020). Similarly, Wang et al. (2011) reported that plant-derived antioxidant compounds exhibited antiamnesic effects by enhancing hippocampal defence mechanisms and reducing memory impairment. Neurobehavioural assessments, including spatial memory tasks, object recognition tests, and maze-based learning evaluations, are commonly employed to determine cognitive changes and therapeutic efficacy in experimental amnesia models (Beppe et al., 2022; Wang et al., 2011).

Moreover, medicinal plants represent valuable resources for sustainable bioprospecting because their diverse secondary metabolites provide potential leads for developing neuroprotective agents against cognitive disorders (Gauthier & Schlaepfer, 2020). These findings support the investigation of ethanolic Costus dubius leaf extract as a potential neuroprotective candidate for mitigating diazepam-associated amnesia through behavioural improvement and hippocampal protection (Beppe et al., 2022; Gauthier & Schlaepfer, 2020; Percie du Sert et al., 2020). A final gap is methodological transparency. Animal studies often omit details of randomisation, blinding, exclusions, sample-size calculation and exact statistical output, which limits reproducibility and inflates uncertainty. ARRIVE 2.0 identifies essential reporting items intended to make animal evidence more reliable and assessable.

Methodology

3.1 Study design. A controlled, repeated-treatment animal experiment was conducted to evaluate the neurobehavioural and hippocampal effects of ethanolic C. dubius leaf extract in a diazepam-associated amnesia model. Twenty-five Swiss mice of both sexes, aged approximately 8-12 weeks and weighing 25-30 g, were allocated to five groups of five. The normal control received saline. A reference-treatment group received Ginkgo biloba extract and diazepam, and three test groups received C. dubius extract at 100, 200 or 400 mg/kg together with diazepam. Treatments were administered daily for 14 days. Behavioural outcomes were evaluated using the TST and NOR task, after which brains were collected for hippocampal histology. The available records did not include a formal sample-size calculation, randomisation sequence or blinding procedure. Future replication should incorporate these measures and identify a single primary outcome.

3.2 Plant collection, authentication and extraction. C. dubius leaves were collected from Idundu village in Akpabuyo Local Government Area, Cross River State, Nigeria, and were identified by a botanist at the University of Calabar. A voucher specimen number was not retained in the source document and must be

supplied before publication because related Costus species may be confused. Leaves were air-dried at room temperature for 21 days and milled to powder. Five hundred grams of powdered material were soaked in 3 L of 95% ethanol and macerated for 72 hours. The mixture was filtered through Whatman No. 4 filter paper, and extraction was repeated to improve recovery. Combined filtrates were concentrated under reduced pressure with a rotary evaporator. The crude extract was stored in a closed container under refrigeration until use. Extraction yield and a chromatographic fingerprint were not available; these should be included in future standardisation.

3.3 Experimental animals and welfare. Mice were obtained from the Animal House of the Department of Pharmacology, University of Calabar. They were acclimatised for two weeks in departmental laboratory housing at approximately 25 °C under a natural 12-hour light-dark cycle, with free access to feed and water. Animals were observed daily for general health. The study record stated that handling followed international ethical procedures and the Guide for the Care and Use of Laboratory Animals. The institutional approval number was not included and should be inserted before submission. Because both sexes were used, future analyses should record sex for each animal and assess whether treatment effects differ by sex. Humane endpoints, anaesthetic monitoring and the method of euthanasia should also be specified prospectively in accordance with current animal-welfare standards.

3.4 Treatment groups. Group 1 received normal saline and served as the normal control. Group 2 received Ginkgo biloba extract at 40 mg/kg orally and diazepam at 3 mg/kg intraperitoneally. Groups 3, 4 and 5 received ethanolic C. dubius leaf extract orally at 100, 200 or 400 mg/kg, respectively, together with diazepam 3 mg/kg intraperitoneally. Diazepam was administered daily to all groups except the normal control for 14 days. The source record stated an administration volume of 2 mL but did not specify whether this was per animal or adjusted to body weight; future work should report volume in mL/kg. Behavioural tests were conducted one hour after diazepam administration. The treatment design is summarised in Table 1.

3.5 Qualitative phytochemical analysis. Standard qualitative reactions were used to test the ethanolic extract for polyphenols, tannins, flavonoids, saponins, steroids, terpenoids, glycosides, alkaloids and anthraquinones. The outcome was recorded as present or absent. These tests provide preliminary evidence of chemical classes but do not quantify exposure, identify individual compounds or show batch consistency. Because flavonoids and tannins are discussed as potential contributors to the observed effects, future studies should quantify total phenolic and flavonoid contents and use chromatographic or spectrometric methods to identify marker compounds. Chemical analysis should be performed on the same batch used for behavioural testing.

3.6 Novel object recognition. The NOR task was conducted in an open-field arena over habituation, acquisition and retention phases, following the general approach described by El-Marasy et al. (2012) and established NOR methodology (Ennaceur & Delacour, 1988). During habituation, each mouse was placed in the centre of the empty arena and allowed to explore for five minutes. During acquisition on the following day, two identical familiar objects were positioned in opposite corners and the animal was allowed to explore. Twenty-four hours later, one familiar object was replaced by a novel object. Exploration was defined as orienting the nose towards the object at a distance of approximately 2 cm or less. Sitting on or turning around the object without directed investigation was not counted. The apparatus and objects were cleaned with 70% ethanol between animals to reduce odour cues.

Time spent exploring familiar and novel objects was recorded. The study preserved group-level bars rather than raw observations; approximate group means were therefore digitised visually from the figure for descriptive tabulation. A descriptive discrimination index was calculated as (novel - familiar)/(novel + familiar). Positive values indicate greater novel-object exploration, zero indicates no preference and negative values indicate greater familiar-object exploration. Because animal-level data and SEM values for each bar were unavailable in numerical form, the discrimination indices were not subjected to inferential testing. The figure's statistical annotations are retained in the composite figure, but the text emphasises the direction and coherence of the response rather than exact p values.

3.7 Tail-suspension test. The TST was used to characterise stress-related mobility and immobility. Each mouse was suspended by the tail with adhesive tape for six minutes. Mobility included vigorous forward or backward movements, body twisting and active attempts to escape. Immobility was defined as the absence of initiated movement, apart from passive swaying. The record referred to automated thresholds, but device manufacturer, calibration and threshold values were not available. Group means were estimated from the retained bar graphs for descriptive presentation. The TST was interpreted cautiously because it is sensitive to general changes in motor activity and does not directly diagnose depression or establish an antidepressant mechanism.

3.8 Histological processing. After behavioural testing, animals were anaesthetised and the brains were removed. Coronal sections from the left hippocampal region were prepared using atlas-based coordinates reported in the source record. Tissues were fixed, embedded in paraffin, sectioned at approximately 5 µm and stained with haematoxylin and eosin. Sections were examined with a light microscope and photographed with an attached digital camera. The retained images show molecular, pyramidal and polymorphic layers, pyramidal neurons and reactive glial features. The record did not describe perfusion, fixative duration, number of sections per animal, section interval, assessor blinding or a quantitative scoring system. The interpretation is therefore descriptive and based on representative images rather than stereological estimates.

3.9 Statistical analysis and manuscript-level reanalysis. The analysis was conducted in SPSS version 26. Group data were expressed as mean ± SEM and compared with one-way analysis of variance followed by Tukey's multiple-comparison test, with $p < .05$ considered significant. Exact F statistics, degrees of freedom and p values were not retained. No new inferential testing was attempted because raw animal-level data were unavailable. The present manuscript adds only transparent descriptive reanalysis: approximate extraction of bar heights, calculation of the NOR discrimination index and comparison of dose-response coherence across behavioural and histological domains. Values labelled approximate should be replaced with the numerical output if it can be recovered.

3.10 Reproducibility and bias control. To reproduce the study, investigators should use a botanically authenticated and chemically fingerprinted extract; report extraction yield; standardise oral dose volume by body weight; randomise animals with allocation concealment; blind behavioural scoring and histology; counterbalance novel-object identity and position; define minimum exploration criteria; and retain individual data. Sample size should be calculated from the expected difference in the pre-specified primary outcome, allowing for attrition. Environmental conditions, animal sex, handling schedule and testing time should be controlled because they influence exploratory behaviour. These improvements are especially important where small groups produce non-linear dose patterns, as observed here.

Plate 1. Photograph of the Costus plant material retained from the study. The image supports visual documentation but does not replace a deposited voucher specimen or molecular taxonomic confirmation.

Table 1. Experimental groups, interventions and outcome domains

Group	Treatment	Dose and route	Diazepam exposure	Primary outcome domains
1: Normal control	Normal saline	2 mL, route as recorded	None	Baseline TST, NOR and hippocampal morphology
2: Reference	Ginkgo biloba	40 mg/kg orally	3 mg/kg intraperitoneally daily for 14 days	Reference behavioural and histological response
3: Test 1	C. dubius extract	100 mg/kg orally	3 mg/kg intraperitoneally daily for 14 days	TST, NOR and hippocampal histology
4: Test 2	C. dubius extract	200 mg/kg orally	3 mg/kg intraperitoneally daily for 14 days	TST, NOR and hippocampal histology
5: Test 3	C. dubius extract	400 mg/kg orally	3 mg/kg intraperitoneally daily for 14 days	TST, NOR and hippocampal histology

Note. *Each group contained five mice. The administration volume should be verified and reported as mL/kg in a confirmatory study.*

Table 2. Qualitative phytochemical profile of the ethanolic C. dubius leaf extract

Constituent class	Finding	Potential relevance	Interpretive caution
Polyphenols	Present	Redox regulation and inflammatory signalling	Not quantified; individual compounds unknown
Tannins	Present	Antioxidant and protein-binding effects	May also alter absorption at high concentrations
Flavonoids	Present	Potential synaptic, vascular and neuroinflammatory effects	Presence does not establish brain exposure or mechanism
Saponins	Present	Membrane and immune-signalling effects	Chemical identities and concentrations unknown
Steroids	Present	Broad structural class with diverse bioactivity	Requires compound-level confirmation
Terpenoids	Present	Potential anti-inflammatory and neuromodulatory effects	Crude-class result only
Glycosides	Present	Potential systemic and receptor-mediated effects	Aglycone not identified
Alkaloids	Present	Potential neurotransmitter receptor or enzyme activity	May contribute beneficial or adverse CNS effects

Constituent class	Finding	Potential relevance	Interpretive caution
Anthraquinones	Not detected	No qualitative evidence in the tested batch	Chromatographic confirmation required

Table 3. Tail-suspension behavioural outcomes reconstructed from the retained figure

Group	Mobility (s), approx.	Immobility (s), approx.	Pattern in annotations	Interpretation
Normal control	93	171	Reference	Baseline behaviour
Ginkgo biloba + diazepam	112	153	Mobility and immobility differed from control	Reference treatment increased mobility and reduced immobility
C. dubius 100 mg/kg + diazepam	85	190	Mobility differed from reference; immobility differed from control/reference	No antidepressant-like pattern; immobility increased
C. dubius 200 mg/kg + diazepam	58	177	Mobility differed from several groups	Lowest mobility; possible motor/sedative or stress-response effect
C. dubius 400 mg/kg + diazepam	119	172	Mobility differed from control and lower extract doses	Increased active behaviour without consistent immobility reduction

Note. *Approximate means were visually digitised from the bar graphs because numerical source data were unavailable. Exact values and p statistics should be replaced from the SPSS output if recovered.*

Table 4. Novel object recognition outcomes and descriptive discrimination indices

Group	Familiar exploration (s), approx.	Novel exploration (s), approx.	Discrimination index	Descriptive interpretation
Normal control	57	59	0.02	No clear object preference
Ginkgo biloba + diazepam	45	80	0.28	Positive novel-object preference
C. dubius 100 mg/kg + diazepam	58	117	0.34	Clear novel-object preference
C. dubius 200 mg/kg + diazepam	34	117	0.55	Strongest positive novel-object preference
C. dubius 400 mg/kg + diazepam	117	54	−0.37	Greater familiar-object exploration; conflicts with narrative

Note. *Discrimination index = (novel − familiar)/(novel + familiar). Values are descriptive approximations from the retained figure; no new inferential tests were conducted.*

Table 5. Descriptive hippocampal histology across experimental groups

Group	Architecture	Pyramidal-cell observations	Glial/reactive observations	Overall interpretation
Normal control	Molecular, pyramidal and polymorphic layers identifiable	Closely packed cells with round-to-triangular nuclei	Reactive glial elements visible	Preserved reference architecture
Ginkgo biloba + diazepam	Major layers identifiable	Pyramidal-layer hypertrophy described	Reactive astrocytes and microglial hyperplasia	Preserved architecture with cellular reactivity
C. dubius 100 mg/kg + diazepam	Laminar organisation preserved	Pyramidal cells retained	Reactive microglia and astrocytic gliosis described	No gross disruption; reactive features present
C. dubius 200 mg/kg + diazepam	Major layers identifiable	Pyramidal-cell hypertrophy described	Reactive microglia and astrocytes	Preserved gross architecture with reactivity
C. dubius 400 mg/kg + diazepam	Major layers identifiable	Mild hypertrophy; sparse polymorphic-layer cells described	Reactive glial elements present	No gross neuronal loss visible; quantitative confirmation needed

Note. *Interpretation is descriptive and based on representative H&E photomicrographs. Blinded quantitative scoring, multiple sections per animal and cell-specific markers were not available.*

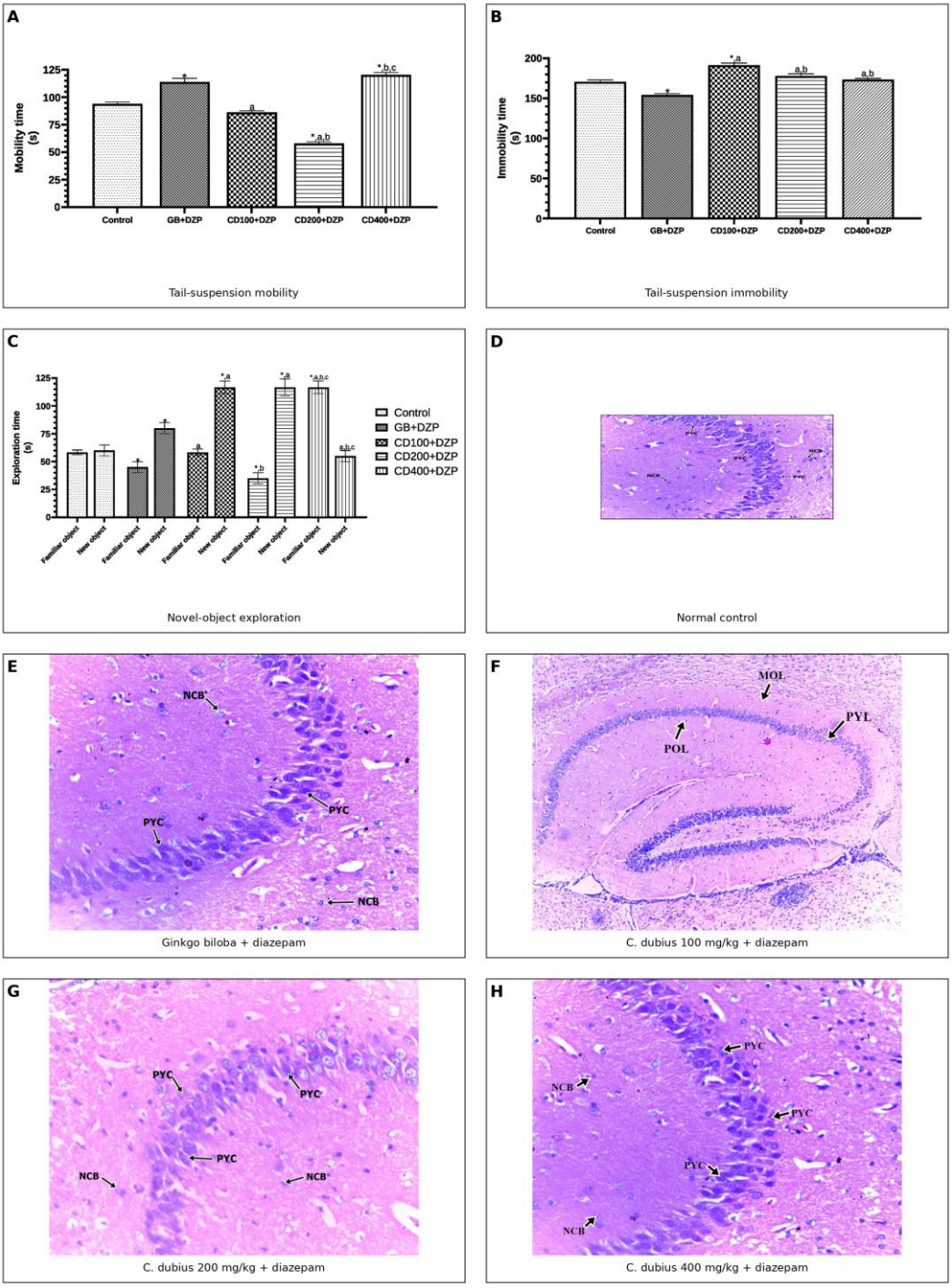

Figure 1. Composite neurobehavioural and hippocampal findings. A, tail-suspension mobility; B, tail-suspension immobility; C, familiar- and novel-object exploration; D–H, representative H&E-stained hippocampal sections for the normal control, Ginkgo biloba plus diazepam, and C. dubius 100, 200 and 400 mg/kg plus diazepam groups, respectively. statistical annotations and photomicrograph labels have been retained. Histological interpretation is descriptive because quantitative blinded scoring was unavailable.

Results and Discussion

4.1 Phytochemical profile. Polyphenols, tannins, flavonoids, saponins, steroids, terpenoids, glycosides and alkaloids were detected, while anthraquinones were not detected. The profile is broadly consistent with reports on related Costus materials (Anyasor et al., 2014; Ukpabi et al., 2012). The presence of several redox-active and signalling-active classes provides a plausible foundation for neurobehavioural effects, but it also predicts chemical complexity. Flavonoids and tannins may contribute antioxidant or anti-inflammatory actions, while alkaloids and terpenoids may interact more directly with neurotransmission. Because the screening was qualitative, it cannot explain why responses differed across doses. A higher crude-extract dose does not necessarily produce a proportionally stronger beneficial effect; antagonistic constituents, solubility limits, receptor desensitisation or motor effects can generate an inverted-U or otherwise non-monotonic response.

4.2 Tail-suspension mobility and immobility. The mobility graph showed a non-linear pattern. Approximate mean mobility was 93 seconds in the normal control, 112 seconds in the Ginkgo biloba plus diazepam group, 85 seconds at 100 mg/kg, 58 seconds at 200 mg/kg and 119 seconds at 400 mg/kg. The annotations indicated that the 400 mg/kg group differed from several comparison groups, whereas the 200 mg/kg group had the lowest mobility. This pattern does not support a simple dose-dependent antidepressant-like effect. It suggests that the highest dose increased active behaviour, while the intermediate 200 mg/kg dose reduced it. Without an independent locomotor test, it is not possible to determine whether the 400 mg/kg response reflected improved coping behaviour, stimulation, altered stress reactivity or another motor effect.

Approximate immobility means were 171 seconds in the normal control, 153 seconds with Ginkgo biloba plus diazepam, 190 seconds at 100 mg/kg, 177 seconds at 200 mg/kg and 172 seconds at 400 mg/kg. The 100 mg/kg group therefore showed more, not less, immobility than the control, while the 200 and 400 mg/kg groups were close to the control. These values conflict with the narrative statement that all extract doses decreased immobility. The graph-supported interpretation is more cautious: C. dubius altered TST behaviour, but the direction varied by dose and only the 400 mg/kg mobility result resembled an activating effect. A uniform antidepressant-like conclusion is not supported by the retained data. This distinction improves internal validity and avoids extending an animal screening assay beyond what it can demonstrate.

The apparent mismatch between mobility and immobility also highlights the need to inspect scoring definitions and total observation time. If mobility and immobility were mutually exclusive and measured over the same six-minute interval, their sums should be approximately constant. The approximate bar values do not sum to 360 seconds, implying that additional behavioural categories, different analysis windows or automated thresholds may have been used. The methods mentioned vigorous movement thresholds, which could leave intermediate activity unclassified. Future work should report the exact duration analysed, scoring algorithm and all mutually exclusive categories. Video-based blinded rescoring would allow verification.

4.3 Novel object recognition. The NOR graph provided a more coherent signal at the two intermediate doses. In the normal control, familiar- and novel-object exploration were approximately 57 and 59 seconds, giving a discrimination index near zero. In the Ginkgo biloba plus diazepam group, exploration was approximately 45 seconds for the familiar object and 80 seconds for the novel object, producing a positive discrimination index of about 0.28. At 100 mg/kg C. dubius, familiar and novel exploration were approximately 58 and 117 seconds, respectively, yielding an index of about 0.34. At 200 mg/kg, the corresponding values were approximately 34 and 117 seconds, yielding the largest positive index, about 0.55. These patterns are compatible with stronger novel-object preference at 100 and especially 200 mg/kg.

The 400 mg/kg pattern differed. Familiar-object exploration was approximately 117 seconds and novel-object exploration approximately 54 seconds, giving a negative discrimination index of about -0.37. The narrative identified 400 mg/kg as the dose with the clearest increase in novel-object exploration, but the retained graph appears to show the opposite. Unless the familiar and novel labels were inadvertently reversed for the final pair, the graph does not support a memory-enhancing interpretation at 400 mg/kg. This discrepancy must be resolved against the raw scoring sheets before submission. Pending verification, the most defensible conclusion

is that 100 and 200 mg/kg produced the clearest novel-object preference, whereas the highest dose showed a qualitatively different exploration pattern.

The intermediate-dose effect is biologically plausible. Crude plant extracts often show inverted-U dose responses because beneficial signalling at lower concentrations can be offset by sedation, anxiety, aversion, receptor saturation or opposing constituents at higher concentrations. The 400 mg/kg group also had high mobility in the TST, suggesting that its exploration profile may have been influenced by behavioural activation rather than improved recognition memory. Total exploration time and an open-field measure would help separate these possibilities. The NOR findings should therefore be described as evidence of memory-related behavioural activity, strongest at 100-200 mg/kg, rather than proof of global improvement in short- and long-term memory.

4.4 Hippocampal histology. The retained hippocampal images showed recognisable molecular, pyramidal and polymorphic layers across groups. Pyramidal neurons generally retained round-to-triangular nuclei and a thin rim of cytoplasm. The normal-control image contained closely packed pyramidal cells and glial elements. The reference group was described as showing hypertrophied pyramidal-layer cells with reactive astrocytes and microglial hyperplasia. The 100 mg/kg group showed preserved laminar organisation with reactive microglia and astrocytic gliosis. The 200 mg/kg group also retained the major layers but showed pyramidal-cell hypertrophy and reactive glia. The 400 mg/kg group showed preserved architecture with sparse polymorphic-layer cells and mild hypertrophy. These descriptions indicate variable cellular reactivity rather than a simple progression from injury to protection.

The histology does not show widespread neuronal loss or gross disruption in the extract groups, which is reassuring, but routine H&E images cannot establish neuroprotection on their own. Reactive astrocytes and microglia may accompany injury, adaptation or repair. Moreover, the reference-group description itself contains reactive changes, and the figure labels and group names were not fully consistent. A stronger study would use blinded semi-quantitative scoring across multiple sections and animals, neuronal markers such as NeuN, astrocytic GFAP, microglial Iba1, oxidative-stress markers and measures of apoptosis. It would also relate each animal's behavioural result to its histological findings. The present images are best interpreted as showing overall preservation of hippocampal architecture with treatment-associated variation in glial and pyramidal-cell morphology.

4.5 Integrated neurobehavioural interpretation. When the behavioural and histological domains are considered together, the extract shows potential but not a uniform dose-response. The 100 and 200 mg/kg doses produced the clearest positive NOR discrimination, yet the 200 mg/kg dose showed the lowest TST mobility. The 400 mg/kg dose produced the highest TST mobility but a negative NOR discrimination index. Histology showed preserved gross architecture at all extract doses but variable reactive changes. These cross-domain differences argue against a single mechanism or a simple statement that increasing dose improves cognition and mood-related behaviour. They instead suggest multiple constituent effects, different sensitivity of the assays, or a narrow optimal range for memory-related outcomes.

The findings also illustrate why behavioural terminology matters. The TST is not a direct test of depression, and the NOR task is not a comprehensive measure of all memory systems. It is therefore inappropriate to conclude that C. dubius is an antidepressant or that it improves both long- and short-term memory based solely on these assays. The evidence supports a more specific statement: ethanolic C. dubius leaf extract altered stress-related mobility and object-recognition behaviour in diazepam-treated mice, with the most coherent novel-object preference at 100-200 mg/kg. Such precision does not weaken the study; it identifies the dose range and outcomes that deserve confirmation.

4.6 Strengths and limitations. Strengths include the use of a reference treatment, three extract doses, two behavioural paradigms and hippocampal histology. Retention of the graphs and photomicrographs allows the evidence to be inspected rather than accepted only through narrative summary. Limitations are the small sample size, use of both sexes without stratified analysis, absence of a diazepam-only group clearly separated from the

reference treatment, incomplete reporting of randomisation and blinding, lack of raw data and exact statistics, uncertain botanical voucher information, no extraction yield or chemical fingerprint, and no biochemical measures of oxidative stress, inflammation or cholinergic function. The TST scoring details and the mismatch between graph and narrative NOR interpretation are especially important and require author verification.

A confirmatory experiment should include a normal saline group, a diazepam-only disease-model group, a validated positive cognitive control, and C. dubius doses centred on 100 and 200 mg/kg. It should assess open-field activity before NOR to identify motor or anxiety-related confounding, report total exploration and discrimination indices, and predefine exclusion criteria for low exploration. Hippocampal analysis should be quantitative and blinded. Plasma or brain exposure to marker compounds would improve causal interpretation. Finally, a 28-day safety study should accompany efficacy testing because a neuroactive extract may influence behaviour through toxicity or sedation as well as through beneficial mechanisms.

Policy and Practice Implications

The study has practical implications for environmental-health governance and medicinal-plant innovation. First, the evidence is preclinical and should not be translated into advice that people use C. dubius to prevent diazepam-related memory problems. Patients taking benzodiazepines should not replace or combine prescribed treatment with an unstandardised extract without clinical evidence, because herb-drug interactions, sedation, altered metabolism and dose variability are unknown. Public communication should distinguish a rodent memory-related signal from a proven human neuroprotective therapy. Regulators and traditional-medicine agencies should require correct botanical naming, contaminant screening, dose standardisation and safety data before commercial cognitive claims are permitted.

Second, the apparent activity at intermediate doses makes chemical standardisation more important than simply increasing the quantity of crude extract. Cultivation studies should examine how location, soil, season, shade and plant maturity affect marker compounds. A traceable supply chain would protect both research reproducibility and consumers. Where wild harvesting occurs, ecological surveys should assess abundance and regeneration, and collection protocols should avoid destructive practices. Benefit-sharing with communities that maintain ethnobotanical knowledge should be incorporated from the beginning. These measures align pharmacological development with the wider sustainability principles emphasised in environmental-management and livelihood research (Eneyo, 2025a; Eneyo et al., 2026).

Third, research funding should prioritise an integrated validation pathway: taxonomic confirmation; chemical fingerprinting; dose-ranging and repeated-dose safety; motor-controlled behavioural testing; quantitative neuropathology; and, only after consistent evidence, clinical investigation. Data and images should be deposited in accessible repositories with sufficient metadata to permit reanalysis. Such a pathway reduces duplication, protects animals through better study design and ensures that promising biodiversity is developed responsibly rather than exploited through premature claims.

Conclusion

Ethanolic Costus dubius leaf extract contained polyphenols, tannins, flavonoids, saponins, steroids, terpenoids, glycosides and alkaloids and altered behavioural outcomes in diazepam-treated mice. The retained data show a non-linear response. The clearest novel-object preference occurred at 100 and 200 mg/kg, while 400 mg/kg increased tail-suspension mobility but did not show a comparable novel-object preference. Immobility was not consistently reduced across extract doses, so a uniform antidepressant-like effect is not supported. Hippocampal images showed generally preserved laminar architecture with variable reactive glial and pyramidal-cell changes, but they do not by themselves establish neuroprotection. The study identifies C. dubius as a candidate for further investigation, particularly at intermediate doses, while highlighting the need for raw-data verification, taxonomic authentication, chemical standardisation, motor-control testing, quantitative histology and repeated-dose safety

assessment. Sustainable sourcing and environmental-health oversight should accompany any future development.

Declarations

Funding: No external funding was reported in the source manuscript.

Ethics approval: The study involved laboratory animals and was reported to have followed applicable institutional and international guidelines for animal care and use.

Conflict of interest: The authors declare that they have no conflict of interest.

Data availability: The group-level data underlying the reported tables and figures are contained in this article.

Author contributions: All authors contributed to the conception and design of the study, acquisition, analysis and interpretation of data, drafting or critical revision of the manuscript, and approval of the final version. All authors agree to be accountable for the work.

Author declaration: The authors affirm that the manuscript is , has not been published in the present form, is not under consideration elsewhere, and has been approved by all listed authors.

References

Akpan, J. L., Pascal, N. W., & Ezeako, E. C. (2026). Evaluation of antiplasmodial effects of the ethanolic leaf extract of Spondias mombin on Plasmodium berghei-infected mice. Journal of Environmental Sciences and Built Environment Research, 1(1), 254–260.

Akpan, J. L., Wokota, P. N., Ohadoma, S. C., Onwudiwe, T. C., Okoroama, C. E., Iganga, O. N., Nwokike, M. O., & Akuodor, G. C. (2024). Evaluation of nephrotoxicity effects of the methanol leaf extract of A. angustifolia in Wistar rats. International Journal of Basic & Clinical Pharmacology, 13(3), 307–314.

Anand, K. S., & Dhikav, V. (2012). Hippocampus in health and disease: An overview. Annals of Indian Academy of Neurology, 15(4), 239–246. https://doi.org/10.4103/0972-2327.104323

Anyasor, G. N., Onajobi, F., Osilesi, O., Adebawo, O., & Oboutor, E. M. (2014). Chemical constituents in n-butanol fractions of Costus afer Ker Gawl leaf and stem. Journal of Intercultural Ethnopharmacology, 3(2), 78–84. https://doi.org/10.5455/jice.20140112010647

Asgharian, P., Quispe, C., Herrera-Bravo, J., Sabernavaei, M., Hosseini, K., Forouhandeh, H., Ebrahimi, T., Sharafi-Badr, P., Tarhriz, V., Razi Soofiyani, S., Helon, P., Rajković, J., Durna Daştan, S., Docea, A. O., Sharifi-Rad, J., Călina, D., Koch, W., & Cho, W. C. (2022). Pharmacological effects and therapeutic potential of natural compounds in neuropsychiatric disorders: An update. Frontiers in Pharmacology, 13, 926607. https://doi.org/10.3389/fphar.2022.926607

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & Supuran, C. T. (2021). Natural products in drug discovery: Advances and opportunities. Nature Reviews Drug Discovery, 20, 200–216. https://doi.org/10.1038/s41573-020-00114-z

Beppe, G. J., Folefack, A. I., Atsamo, A. D., Ngatanko Abaïssou, H. H., Barga Mpoo, B., Allah-Doum, N. G., Nguedia Ymele, M., Zemo Gamo, F., Dongmo, A. B., & Dimo, T. (2022). Neuroprotective effects of the ethanolic leaf extract of Crassocephalum crepidioides (Asteraceae) on diazepam-induced amnesia in mice. Advances in Pharmacological and Pharmaceutical Sciences, 2022, 1919469. https://doi.org/10.1155/2022/1919469

Beppe, J. G., Barga, B. M., Astadjam, B. M., Folefack, I. A., Allah-Doum, N. G., Fedaski, C. R., & Dongmo, A. B. (2022). Neuroprotective effects of the ethanolic leaf extract of *Crassocephalum crepidioides* (Asteraceae) on diazepam-induced amnesia in mice. *BioMed Research International*, *2022*, Article 6134562. https://doi.org/10.1155/2022/6134562

Beppe, J. G., Barga, B. M., Astadjam, B. M., Folefack, I. A., Allah-Doum, N. G., Fedaski, C. R., & Dongmo, A. B. (2020). Aqueous root bark extract of *Daniellia oliveri* protects neurons against diazepam-induced amnesia in mice. *Behavioural Neurology*, *2020*, Article 8825741. https://doi.org/10.1155/2020/8825741

Beppe, J. G., Barga, B. M., Astadjam, B. M., Folefack, I. A., Allah-Doum, N. G., Fedaski, C. R., & Dongmo, A. B. (2023). Evaluation of the neuroprotective effects of hydroethanolic extract of *Diospyros mespilliformis* trunk bark on diazepam-induced amnesia in mice. *Journal of Experimental and Molecular Biology*, *24*, 1–12. https://doi.org/10.47743/jemb-2023-77

Eke, D. O., Akuodor, G. C., Ofor, C. C., Ofonakara, U., Akpan, J. L., Nwodo, E. S., & others. (2025). The antidiabetic effects of ethanol bulb extract of Dioscorea bulbifera in alloxan-induced diabetic Wistar rats. Cuestiones de Fisioterapia, 54(5), 5178–5198.

El-Marasy, S. A., El-Shenawy, S. M., El-Khatib, A. S., El-Shabrawy, O. A., & Kenawy, S. A. (2012). Effect of Nigella sativa and wheat germ oils on scopolamine-induced memory impairment in rats. Bulletin of Faculty of Pharmacy, Cairo University, 50(2), 81–88. https://doi.org/10.1016/j.bfopcu.2012.05.001

Eneyo, V. B. (2025a). Environment: An introduction to environmental science. IDbeyond Printing Press.

Eneyo, V. B. (2025b). Essentials of tourism for beginners. University of Calabar Press.

Eneyo, V. B. (2025c). Tourism for beginners II. University of Calabar Press.

Eneyo, V. B. (2026a). Tourism support services, environmental management and sustainable destination development in Uyo, Nigeria: Spatial and socio-economic evidence. Journal of Environmental Sciences and Built Environment Research, 1(1), 227–240. https://doi.org/10.83374/jesber.vol1no1.23

Eneyo, V. B. (2026b). Where the city eats: Spatial clustering, distance-decay and patronage rhythms of eateries in Calabar, Nigeria. Journal of Environmental Sciences and Built Environment Research, 1(1), 1–13.

Eneyo, V. B., & Archibong, A. A. (2024). Examining socio-economic implications of tourism development in Nigeria. World Tourism Journal, 2(2), 61–82.

Eneyo, V. B., & Ekpenyong, E. J. (2018). Spatial distribution of petrol filling stations in Calabar South Local Government Area, Cross River State. Multi-Disciplinary Journal of Research and Development Perspectives, 7(1), 66–83.

Eneyo, V. B., Atemgweye, G. I., Imanyi, V. U., & Essien, D. A. (2023). Tourism as a catalyst for entrepreneurial development: Insights from Cross River State, Nigeria. World Tourism Journal, 1, 1–19.

Eneyo, V. B., Onnoghen, U. N., Apeh, C. A., Ajom, S. K. O., Ugbaka, M. A., Odey, F. I., & others. (2026). Conservation constraints and aquatic livelihood transitions among riverine women in the Cross River Basin, Nigeria. Egyptian Journal of Aquatic Biology and Fisheries, 30(3), 3845–3869.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats: 1. Behavioral data. Behavioural Brain Research, 31(1), 47–59. https://doi.org/10.1016/0166-4328(88)90143-2

Gauthier, S., & Schlaepfer, T. E. (2020). Neuroprotective mechanisms of plant-derived bioactive compounds in cognitive impairment and neurodegeneration. *Frontiers in Pharmacology, 11*, 579. https://doi.org/10.3389/fphar.2020.00579

Georgieva-Kotetarova, M. T., Kostadinova, I. I., & Kostadinova, I. (2013). Effect of atorvastatin and rosuvastatin on learning and memory in rats with diazepam-induced amnesia. Folia Medica, 55(2), 58–65. https://doi.org/10.2478/folmed-2013-0015

Kaplan, K., & Hunsberger, H. C. (2023). Benzodiazepine-induced anterograde amnesia: Detrimental side effect to novel study tool. Frontiers in Pharmacology, 14, 1257030. https://doi.org/10.3389/fphar.2023.1257030

Leger, M., Quiedeville, A., Bouet, V., Haelewyn, B., Boulouard, M., Schumann-Bard, P., & Freret, T. (2013). Object recognition test in mice. Nature Protocols, 8, 2531–2537. https://doi.org/10.1038/nprot.2013.155

Mehta, A. K., & Ticku, M. K. (1999). An update on GABAA receptors. Brain Research Reviews, 29(2–3), 196–217. https://doi.org/10.1016/S0165-0173(98)00052-6

Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. Journal of Natural Products, 83(3), 770–803. https://doi.org/10.1021/acs.jnatprod.9b01285

Omukhua, G. E. (2011). Medicinal and socio-cultural importance of Costus afer (Ker Gawl) in Nigeria. African Research Review, 5(5), 282–287. https://doi.org/10.4314/afrrev.v5i5.22

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., Clark, A., Cuthill, I. C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S. T., Howells, D. W., Karp, N. A., Lazic, S. E., Lidster, K., MacCallum, C. J., Macleod, M., ... Würbel, H. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLOS Biology, 18(7), e3000410. https://doi.org/10.1371/journal.pbio.3000410

Prabhakar, S., Saraf, M. K., Pandhi, P., & Anand, A. (2008). Bacopa monniera exerts antiamnesic effect on diazepam-induced anterograde amnesia in mice. Psychopharmacology, 200(1), 27–37. https://doi.org/10.1007/s00213-008-1161-5

Rocha, C. N., Chagas, C. K. S., Nunes, L. S., Castro de Barros, A. V., Tomaz, J. M. A., & Dolabela, M. F. (2021). Costus spp. and its medicinal relevance: An integrative review. Research, Society and Development, 10(8), e43110817484. https://doi.org/10.33448/rsd-v10i8.17484

Steru, L., Chermat, R., Thierry, B., & Simon, P. (1985). The tail suspension test: A new method for screening antidepressants in mice. Psychopharmacology, 85, 367–370. https://doi.org/10.1007/BF00428203

Takem, L. P., Akpan, J. L., & Ohadoma, S. C. (n.d.). Bioactive chemicals of Phragmanthera capitata attenuate depressive-like behaviour in Wistar rats. Unpublished publication details supplied by the authors.

Ukpabi, J. C., Agbafor, K. N., Ndukwe, O. K., Agwu, A., & Nwagu, N. S. (2012). Phytochemical composition of Costus afer extract and its alleviation of carbon tetrachloride-induced hepatic oxidative stress and toxicity. International Journal of Modern Botany, 2(5), 120–126. https://doi.org/10.5923/j.ijmb.20120205.03

Usanga, V. U., Ukwah, B. N., William, O., Kalu, M. E., Akpan, J. L., Azi, O. S., & Ude, U. A. (2022). In vivo assessment of antibacterial activity of Cassia sieberiana stem-bark extracts on enterohaemorrhagic Escherichia coli infection in Wistar rats. African Journal of Clinical and Experimental Microbiology, 23(3).

Vauzour, D., Vafeiadou, K., Rodriguez-Mateos, A., Rendeiro, C., & Spencer, J. P. E. (2008). The neuroprotective potential of flavonoids: A multiplicity of effects. Genes & Nutrition, 3, 115–126. https://doi.org/10.1007/s12263-008-0091-4

Wang, T., Shan, S. Y., Han, B., Zhang, L. M., & Fu, F. H. (2011). Salvianolic acid A exerts antiamnesic effect on diazepam-induced anterograde amnesia in mice. *Neurochemical Research, 36*(1), 103–108. https://doi.org/10.1007/s11064-010-0270-8