Cognitive enhancing properties of aqueous leaf extract of *Vigna unguiculata* in ketamine-induced memory damage in mice

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ABSTRACT

Conventional remedies for managing Alzheimer's disease (AD) and related cognitive deficits are not curative but relieve the symptoms and cause adverse side effects. Alternatively, use of herbal therapies to manage cognitive illnesses has increased substantially. *Vigna unguiculata* is commonly utilized for nutritional benefits and management of neurological disorders in herbal medicine. The present study evaluated cognitive enhancing potential of *V. unguiculata* leaf aqueous extract in mice with ketamine-induced AD-like cognitive deficits. Cognitive performance indicated by step-through latency was assessed using passive avoidance test. Anti-acetylcholinesterase (anti-AChE) and antioxidant potential of the extract were determined using brains of the test animals. Further, phytochemical constituents of the extracts were determined using LC-MS. Aqueous extract of *V. unguiculata* leaf demonstrated significant prowess in combating cognitive deficits in the test animals. This was evidenced by significantly higher (p<0.001) step-through latencies in extract-treated mice than the untreated cognitively damaged mice. Moreover, cognitively impaired mice given the studied extract exhibited significantly less (p<0.001) malondialdehyde levels and AChE activity than the negative control mice. This result confirmed antioxidant and anti-AChE properties of *V. unguiculata*, indicating its potential to attenuate oxidative stress in the brain and augment cholinergic transmission. Notably, some conventional therapies for cognitive disorders, especially AD, are AChE inhibitors. The studied extract contained phytocompounds such as flavonoids and phenolics with confirmed antioxidant and anti-AChE activities, thus, its cognitive enhancing efficacy could be attributed to these phytoconstituents. Collectively, this study upholds *V. ungiculata* usefulness in management of cognitive illnesses.

INTRODUCTION

Cognitive damage presents a major risk for development of neurological disorders such as Alzheimer's disease (AD), the most common type of dementia [1]. Alzheimer's disease is a multifactorial condition characterized by gradual memory decline, compromised cognitive functioning and personality changes resulting from neuronal damage in the frontal cortex and hippocampus [2]. The major neurochemical aberrations observed in AD condition include deterioration of cholinergic function in the central nervous system and imbalanced redox homeostasis [2]. Also, AD's pathogenesis is characterized by deposits of amyloid-β plaques and neurofibrillary tangles in the brain [3]. Some lines of research hypothesize that amyloid deposition in the brain is the fundamental etiology of AD [1] whereas others propose that tau hyperphosphorylation (pTau) is the principal pathogenic mechanism that initiates AD [4]. What is universally accepted is that all these factors are collectively involved in neuropathogenesis observed in AD [3].

Many approaches have been pursued to develop agents for managing and treating AD [5]. To some degree, these attempts have successfully produced several agents with efficacy in alleviating AD symptoms [5]. Some of the commonly used agents for

management of AD include memantine (N-Methyl-D-aspartate antagonist) [6], donepezil, galantamine, rivastigmine, and tacrine (acetylcholinesterase inhibitors) [7]. Unfortunately, clinical use of these drugs is associated with numerous side effects such as cardiovascular complications, muscle cramps, and urinary incontinence [7], among others. Further, the current pharmacotherapy for AD targets alleviating symptoms but cannot eradicate the disease and the cure for AD remains elusive [5]. In this regard, search for novel curative but safe anti-Alzheimer's agents remain an attractive area of vigorous research.

Several potential remedies are being investigated for alternative sources of AD therapy [5]. Markedly, there is considerable interest in using nutritionally bioactive foods as an imperial approach to prevent and manage cognitive disorders [8]. Application of nutritional agents to address neurodegenerative diseases has been unequivocally practiced [8,9]. Indeed, Hippocrates' philosophy notes that medical interventions which include patient's nutritional needs in treatment may boost recovery [8].

Epidemiological studies have indicated that regular consumption of legumes is likely to reduce incidences of dementia [10]. More so, numerous studies have demonstrated invaluable significance of legume-based diets for management of cognitive disorders [9,11]. Among nutritionally appreciated leguminous foods, *V. unguiculata* is probably the most widely consumed [9]. Its leaf and seed are commonly used as food in the tropics (Africa) [12]. The plant is endowed with numerous proteins, minerals, vitamins and dietary fiber, enhancing its nutritive and health value [11,12]. Further, it contains various classes of secondary metabolites including flavonoids, unsaturated fatty acids, steroids, saponins, terpenoids, phenolics and alkaloids [9,12]. The pharmacological relevance of these compounds in neurological ailments is widely documented [11]. Some of the classes such as flavonoids have been reported to inhibit oxidative stress and reverse cholinergic decline, all of which are major drug targets for AD therapy [11].

Consequently, its medicinal utility in traditional therapy is exemplary. It is used to manage memory impairment, stomatitis, corneal ulcers and coeliac disease [12], among others. It's also used to relieve headaches, fever, antidote for snake bites and urinary schistosomiasis [13]. Besides, it is utilized to prevent menstrual pains, intestinal cramps, leucorrhea, diabetes and cardiac illness [14]. Locally, *V. unguiculata* is claimed to possess memory enhancing potency and is consumed traditionally for this purpose besides nutritional value [15]. Cowpea is given to school going children to improve their cognitive functions such as memory, attention and alertness [16]. Also, it is consumed for management of epilepsy, a recognized neurological disorder [17]. Further, *V. unguiculata* has been shown to express antioxidant, neuroprotective and cardioprotective properties [9,18], among others

Ethnopharmacological information [12], epidemiological evidence [10] and phytochemistry constituents [9,12] on *V. unguiculata* offer appealing background for its likely cognitive enhancing potential. More so, neuroprotective efficacy of *V. unguiculata* seed extracts has been demonstrated in Parkinson's disease [9]. Nevertheless, no evidence has been established on the efficacy of *V. unguiculata* against other cognitive disorders such as AD. Furthermore, previous studies on health benefits of *V. unguiculata* have majorly focused on seed preparations, yet the leaf is equally consumed as food and remedy for memory deficiencies in ethnomedicine [12]. Additionally, the promising cognitive improving effects of *V. unguiculate* leaf, noted in folkloric medicine, have not been analytically confirmed. As well, its probable cognitive enhancing mechanisms are yet to be established.

Given the elucidated gaps, this study aimed to evaluate cognitive enhancing properties of aqueous leaf extract of *V. unguiculata*. Further, in the view that oxidative dyshomeostasis and aberrant acetylcholine metabolism are major hallmarks of cognitive decline in AD [3], the study evaluated the potential of the studied extract to act as an antioxidant and anti-cholinesterase agent, preserving memory integrity in AD conditions.

MATERIALS AND METHODS

Sample collection and preparation of aqueous extract

Fresh leaves were sampled from *V. unguiculata* plants naturally growing in Namakuyu area, Muranga County, Kenya. A taxonomist authenticated the plant at Kenyatta University Herbarium, where a voucher specimen was labeled DJK (004). The collected samples were spread out under shade for drying at 25 ± 2^0C. Once completely dry, they were pulverized to a coarse powder and stored in dark, clean, clearly labeled dry plastic containers.

During extraction, 50 grams of the powdered sample was mixed with 500ml of distilled water heated to 100^0C in a water bath for 90 minutes. The treatment was conducted to mimic the folkloric preparation of *V. ungiuculata* leaf. The mixture was intermittently shaken to allow homogeneous dispersion. After that, it was cooled to 25^0C, decanted, and filtered. The filtrate was freeze-dried, and the obtained extract was preserved at 4^0C for further study.

Experimental animals

Male Swiss Albino mice (20 ± 2g) aged between 4-5 weeks were used in this study. They were obtained from Kenyatta University animal breeding facility and moved to the experimental room, where they were provided with standard rodent pellets and water *ad libitum*. The animals were housed at room temperature and natural night/day cycle. They were allowed to accustom this environment for a week prior to commencing the bioassays. All the animals were handled in accordance to the American Psychological Association's ethical guidelines on experimenting on laboratory animals [19]. Prior to conducting this study, ethical approval was granted by National Commission for Science, Technology, and Innovation *(NACOSTI)* (NACOSTI/P/22/17588*).

Randomly selected mice were assigned to 6 groups (n=5). Treatments were administered as follows (Table 1). Prior to treatment, the animals were starved overnight, after which extract dosages were orally administered to the respective experimental groups (D, E, F) and the reference drug (Donepezil) was given to the positive control mice as outlined above. Forty-five min later, 0.2ml of ketamine (15mg/kg bw) was intraperitoneally administered to all the groups except the normal control to initiate cognitive damage. The concentrations of the studied extract were selected through a pilot test and ketamine dosage was based on previous literature [20]. Saline was used to dissolve all treatments. The treatments were given daily for 3 days.

Table 1. Treatment protocol.

Group	Treatment
Group A (Normal control)	Normal saline
Group B (Negative control)	Ketamine (15mg/kg bw)
Group C (Positive control)	Donepezil (1mg kg^{-1} bw^{-1}) + Ketamine (15mg kg^{-1} bw^{-1})
Group D (Experimental group)	Plant extract (200mg kg^{-1} bw^{-1}) + Ketamine (15mg kg^{-1} bw^{-1})
Group E (Experimental group)	Plant extract (300mg kg^{-1} bw^{-1}) + Ketamine (15mg kg^{-1} bw^{-1})
Group F (Experimental group)	Plant extract (400mg kg^{-1} bw^{-1}) + Ketamine (15mg kg^{-1} bw^{-1})

Passive avoidance test

Passive avoidance test (PAT) was carried out for 6 consecutive days. The test used passive avoidance box with dimensions of 25cm x 20cm x 20cm [21]. The box comprised a brightly lit and dark compartment separated by a carton wall. The wall was fitted with a sliding door interconnecting the two chambers. An electric circuit (0.5 mA) was fixed on the surface of the dark chamber of the box.

The test was segmented into three phases: habituation, training and testing. During habituation, mice were introduced to the lit chamber of the passive avoidance box (PAB) and explored it for 20 seconds. Thereafter, the sliding door was opened, and the animals were allowed to roam in the lit and dark compartments for 300 seconds. The electric circuit was off during habituation.

After habituation, the animals were conditioned for memory development through a training session. Mice were individually positioned in the lit chamber of PAB which they explored for twenty seconds. Then, the sliding door was raised, and the mice were allowed to walk around the PAB for 300 seconds. Once the animal completely entered the dark partition with both hind legs, an electroshock of 0.5mA was administered for 10 seconds. The animals were restricted in the dark chamber for thirty seconds so that to associate the section with irritating stimuli.

Following training, memory retention testing was carried out for three consecutive days. Each mouse was administered with the respective treatment and transferred to the lit chamber of PAB for 20 seconds, after which the sliding door was opened. The animal was observed for 5 min while in PAB. The time taken (step-through latency) by the animal to enter the dark chamber with all the legs was recorded.

Between each mouse habituation, testing and training session, the box was carefully wiped with 70% ethanol to neutralize any lingering olfactory stimuli [22]. The experiments were conducted at 10.00 am throughout the study period (i.e., 24 h apart).

Ex vivo **biochemical assays**

On the 6th day of PAB test, the animals were euthanized, and their brains recovered. To prepare a homogenate, each mouse brain was mixed with 0.6ml sodium phosphate solution (pH 7.4, 0.1M) and centrifuged (12000 rpm) for 15 min at 4°C. The resultant homogenates were used to estimate levels of malondialdehyde (MDA) as described [23] and acetylcholinesterase (AChE) activity following the protocol outlined previously [24].

Characterization of secondary metabolites

Phytochemistry diversity of aqueous extract of *V. unguiculata* was explored using liquid chromatography coupled with tandem mass spectrometer (UPLC-MS/MS, Palo Alto, CA) following methods as described [25-26].

Statistical analysis

All the results were expressed as Mean ± SEM and presented in tables as well as graphs. Inferential analysis was done for passive avoidance tasks and ex vivo biochemical data. Normality was confirmed by Kolmogorov-Smirnov test, after which parametric one-way ANOVA was done for extract treated groups followed by fisher's post hoc test for multiple pairwise comparisons. Un-paired t test was done for comparison between two treatment groups. Means with p<0.001 (at 99% confidence interval) were statistically significant. All data were analyzed in Minitab v19.

RESULTS

Effects of aqueous leaf extract of *V. unguiculata* on passive avoidance

Effects of three doses (200, 300 and 400mg kg^{-1} bw^{-1}) of aqueous leaf extract of *V. unguiculata* were investigated in memory impaired mice. Testing was carried out for three consecutive days after habituation and training. Throughout the test period, animals in the negative control group revealed significantly reduced step-through latency ($P < 0.001$; Table 2) compared to all other treatment groups. On the other hand, the extract demonstrated cognitive remedial effects as it increased step-through latency in cognitively damaged mice. Universally, mice treated with 200 mg/kilogram bodyweight had the optimal cognitive improvement among the extract-treated animals as they exhibited the highest step-through latency across the test duration. On the 1st and 2nd days, 200mg/kg bw of the extract restored cognition to normalcy as animals treated with this dosage and normal control mice exhibited comparable ($p > 0.001$; Table 2) step-through latency. Furthermore, cognitive enhancing effects of the extract doses of 200 and 400mg/kg bw were similar on the 1st day of the test as revealed by comparable step-through latency ($p > 0.001$; Table 2). However, on the 2nd and 3rd day, the doses of the studied extract induced different cognitive enhancing effects as evidenced by statistically varying step-through latencies ($p < 0.001$; Table 2). On both days, 200 mg/kg bw, significantly induced the highest step-through latency while 300 mg/kg bw exerted the lowest step-through latency.

Effects of aqueous leaf extract of *V. unguiculata* on *ex vivo* acetylcholinesterase activity

The present study investigated effects of aqueous leaf extract of *V. unguiculata* on AChE activity in brain homogenates of ketamine-treated mice. All the extract concentrations and donepezil demonstrated prominent inhibition of AChE activity ($p< 0.001$; Figure 1). Extract dose of 400mg/kg bw of *V. unguiculata* demonstrated the highest anti-AChE efficacy though not significantly different from 300mg kg^{-1} bw^{-1} ($p > 0.001$; Figure 1). Further, 300mg kg^{-1} bw^{-1} of the extract and donepezil (reference drug) exerted statistically similar anti-AChE activity ($p > 0.001$; Figure 1). Extract dose of 200mg/kg bw exhibited the lowest anti-AChE activity (Figure 1).

Table 2. Effects of *Vigna unguiculata* aqueous leaf extract on step-through latency in mice after ketamine-induced cognitive impairment.

Groups	Treatment	Time (seconds)		
		Day 1	Day 2	Day 3
Group A (Normal control)	Normal saline	236.67±6.91[a]	272.37±5.81[a]	276.05±5.06[a]
Group B (Negative control mice)	Ketamine (15mg/kg bw) + Normal saline	130.16±6.17[d]	130.16±5.59[c]	67.22±5.22[e]
Group C (Positive control mice)	Ketamine (15mg/kg bw) + Donepezil (1mg/kg bw)	154.45±4.96[c]	132.9±4.64[c]	154.4±7.13[d]
Group D (Experimental group)	Ketamine (15mg/kg bw) + 200 mg/kg bw	206.69±6.81[ab]	257.98±5.97[a]	243.04±4.72[b]
Group E (Experimental group)	Ketamine (15mg/kg bw) + 300 mg/kg bw	160.19±5.66[c]	136.64±6.22[c]	160.19±7.84[d]
Group F (Experimental group)	Ketamine (15mg/kg bw) + 400 mg/kg bw	200.89±7.9[b]	196.69±4.63[b]	192.74±6.04[c]

Results are expressed as Mean±SE of mean. Means with a different superscript letter column-wise are significantly different ($p < 0.001$) tested by one-way ANOVA followed by Fisher's LSD
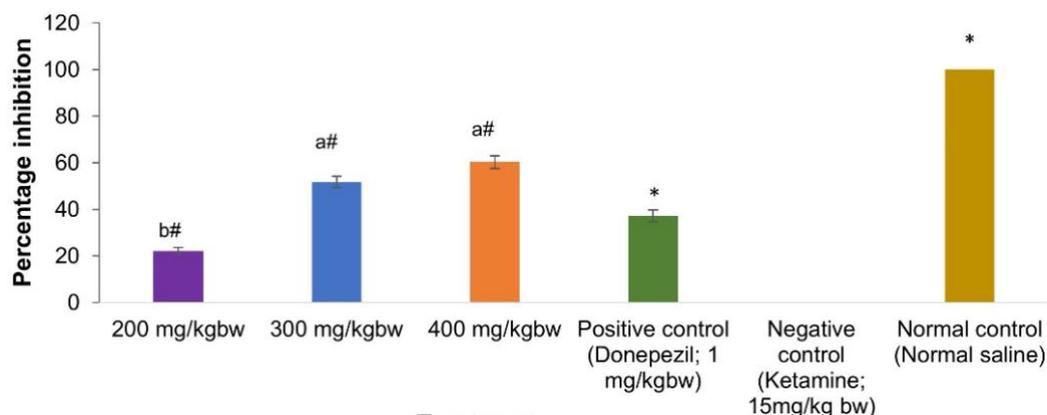

Figure 1. Percentage inhibition of acetylcholinesterase activity by *Vigna unguiculata* aqueous leaf extract in the brain of mice following ketamine-induced cognitive impairment. Bars represent means±standard error of mean. Alphabets denotes significant difference between concentrations of the extract; Bars with a different superscript alphabet are significantly distinct (one-factor ANOVA followed by Fisher's LSD; $p < 0.001$). Asterisk (*) denotes significant difference between the negative control (ketamine treated group) and the control groups (normal and positive control); Bar with asterisks shows significant difference between the negative control (ketamine treated group) and the control groups (normal and positive control) (Un-paired t test; $p < 0.001$). Hash (#) denotes significant difference between the negative control (ketamine treated group) and extract treated; Bar with hash shows significant different between the negative control (ketamine treated group) and extract treated (Un-paired t test; $p < 0.001$).

Effects of aqueous leaf extract of *V. unguiculata* on *ex vivo* MDA profile

It was observed that brains of negative control mice had the highest MDA level, which was significantly different ($p<0.001$; Figure 2) from all extract and donepezil-treated groups. Markedly, among all the tested doses of aqueous extracts of *V. unguiculata* leaf, brains of the mice given 300 and 400mg/kg bw had the lowest and comparable ($p>0.001$; Figure 2) MDA levels. Notably, the same doses restored MDA level to normal state as it was statistically similar to the levels observed in the normal control animals ($p>0.001$; Figure 2). Further, levels of MDA in the brains of mice given 200mg kg^{-1} bw^{-1} of the extract were comparable to the concentration of MDA in the brains of animals treated with the reference drug ($p>0.001$; Figure 2).

Diversity of phytochemicals in *V. unguiculata* aqueous leaf extract

The secondary metabolites identified in the studied extract are shown in Table 3. The compounds identified in the extract included oleic acid, octadecanoic acid, arachidic acid, catechin, pyrogallol, baicalein, beta-sitosterol, apigenin, luteolin, campesterol, stigmasterol, gallocatechin and quercetin. Mainly, the most concentrated compounds

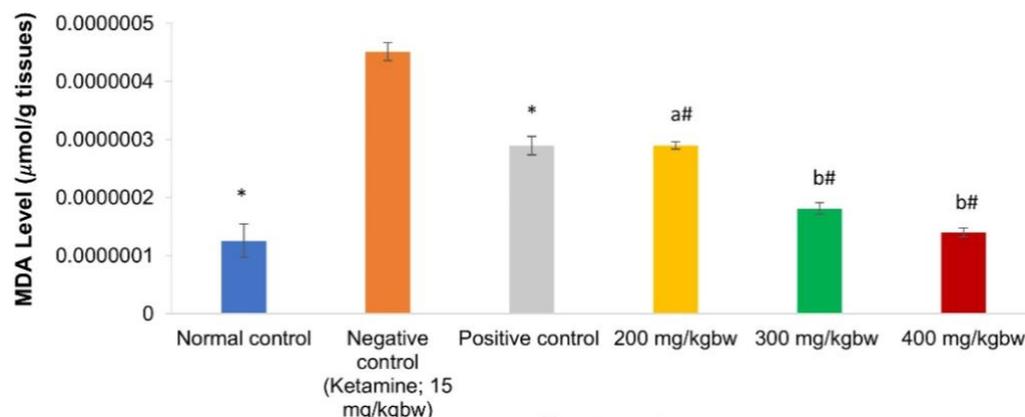were oleic acid and octadecanoic acid whereas the least concentrated were quercetin and arachidic acid.

Figure 2. Effects of *Vigna unguiculata* aqueous leaf extract on profile of malondialdehyde (MDA) in the brain of Mice following ketamine-mediated cognitive impairment. Bars represent means±standard error of mean. Alphabets denotes significant difference between concentrations of the extract; Bars with a different superscript alphabet are significantly distinct (one-factor ANOVA followed by Fisher's LSD; $p < 0.001$). Asterisk (*) denotes significant difference between the negative control (ketamine treated group) and the control groups (normal and positive control); Bar with asterisks shows significant difference between the negative control (ketamine treated group) and the control groups (normal and positive control) (Un-paired t test; $p < 0.001$). Hash (#) denotes significant difference between the negative control (ketamine treated group) and extract treated; Bar with hash shows significant different between the negative control (ketamine treated group) and extract treated (Un-paired t test; $p < 0.001$).

Table 3. Phytocompound diversity of aqueous leaf extract of *V. unguiculata.*

Compound name	Molecular formula	Chemical class	Concentration (ng/g)
Oleic acid	$C_{18}H_{34}O_2$	Fatty acid	138.5
Octadecanoic acid	$C_{18}H_{36}O_2$	Fatty acid	80.2
Catechin	$C_{15}H_{14}O_6$	Flavanol	77.5
Pyrogallol	$C_6H_6O_3$	Phenol	46.3
Baicalein	$C_{15}H_{10}O_5$	Flavone	41.9
Beta-Sitosterol	$C_{29}H_{50}O$	Phytosterols	20.3
Apigenin	$C_{15}H_{10}O_5$	Flavone	12.6
Luteolin	$C_{15}H_{10}O_6$	Flavonoid	10.7
Campesterol	$C_{28}H_{48}O$	Phytosterol	9.4
Stigmasterol	$C_{29}H_{48}O$	Phytosterols	7.7
Gallocatechin	$C_{15}H_{14}O_7$	Flavonol	5.3
Arachidic acid	$C_{20}H_{40}O_2$	Fatty acid	3.3
Quercetin	$C_{15}H_{10}O_7$	Flavonol	1.6

DISCUSSION

To recollect, this study evaluated cognitive improving effects of aqueous *V. unguiculata* in ketamine-treated mice. Ketamine induces memory dysfunction via mechanisms observed in Alzheimer's disease namely cholinergic dysfunction and oxidative dyshomeostasis [27]. It disrupts metabolism of major neurochemicals such as acetylcholine, leading to cholinergic decline [28]. Additionally, ketamine causes severe oxidative damage to the brain [29]. Through these events, ketamine is known to mediate hippocampal-associated memory deficits [28,29] typically observed in AD disease. Also, ketamine transiently increases tau hyperphosphorylation following clinical administration [30]. Thus, ketamine provided an adequate model for effectively initiating AD-like cognitive dysfunction as revealed by negative control animals which

showed decreased step-through latency, high MDA levels and acetylcholinesterase activity.

Passive avoidance test, a fear-driven avoidance test [31], was used to evaluate memory recovery after extract intervention. Passive avoidance technique is extensively used to assess ability of animals to learn and recall aversive stimulus (electrical foot-shock) in the dark chamber of PAB. The test is conceptualized on the basis that rodents innately prefer dark to lit environment [31]. Therefore, they would naturally occupy the dark chamber of passive avoidance box. However, the dark chamber provided hostile stimuli through electroshock, hence cognitively functioning mice would recall aversive stimuli based on memory acquired during training and hesitate to re-enter the dark chamber. This technique has been widely used to assess cognitive functionality in rodents [8].

Treatment of cognitively impaired mice with aqueous extract of *V. unguiculata* profoundly reversed memory decline induced by ketamine as revealed by increased step-through latency. Memory formation involve processes such as acquisition, retention and retrieval of memory in hippocampus [32]. Increased step-thorough latency indicated that the extract-treated mice recalled to avoid aversive stimuli in the dark chamber where they experienced electric shocks [21,31]. Hence, cognitive improving effects of the extract reflect its efficacy to enable recall of the information developed during training.

Biochemical evaluation illuminated on the potential mechanisms through which *V. unguiculata* enhanced cognition. AChE is a recognized of biomarker for cognitive functionality [33,34]. The extract demonstrated anti-acetylcholinesterase efficacies in brains of cognitively damaged mice. Assay of acetylcholinestarase activity is widely utilized to screen for agents with cognitive enhancing potential. Elevated acetylcholinesterase activity is associated with cognitive decline [35–37]. AChE breaks down acetylcholine (ACh), a key neurotransmitter that acts on cholinergic receptors to mediate signal transmission in the synapse [38]. Reduced levels of ACh results in decline of cholinergic neurotransmission which produces memory deficits observed in AD patients [38]. Indeed, modulation of cholinergic neurotransmission has received considerable attention because of its direct correlation with AD [38]. Inhibition of AChE, prevents degradation of ACh resulting in rescue of cholinergic transmission ultimately correcting cognitive dysfunction [39]. Currently, novel AChE inhibitors are being sort for development of the most effective agents to relieve cognitive consequences observed in AD [39]. The outcome of the present study demonstrated that aqueous leaf extract of *V. unguiculata* could salvage cholinergic decline through inhibition of AChE activity. Thus, the extract is a promising candidate for management of neurological disorders such as AD.

In the present study, *V. ungiuculata* reversed ketamine mediated oxidative harm as revealed by low levels of malondialdehyde in brain homogenates. Malondialdehyde is a biomarker for oxidative imbalance in cellular systems [40]. Level of MDA in brain homogenates are assayed to estimates levels of oxidative stress in the brain. High MDA levels reveals elevated levels of oxidative stress in the brain [41–43]. Oxidative stress is a prominent mechanism that has been implicated in progression of neurological disorders including AD [44]. The brain is highly susceptible to oxidative assault by free radicals due to higher oxygen consumption, increased metabolism, rich content of oxidizable polyunsaturated fatty acids and relatively low antioxidant mechanism [8]. Oxidative stress damages brain cell molecular components (lipids, DNA, RNA), resulting in their apoptosis, eventually compromising learning and memory

development processes [8,44]. Thus, overexpression of oxidative radicals may result in abnormal neuronal signaling in the hippocampus [44].

Antioxidant substances have been shown to limit pathogenesis and progression of neurological disorders. Indeed, antioxidant intake has been linked with low incidence of dementia [45]. As such, antioxidant substances have been considered as a promising avenue for diminishing the rate of initiation and progression of cognitive disorders [45]. Thus, the current study confirmed the potential of *V. unguiculata* in antioxidant therapy for neurological disorders like AD.

Cognitive remedying prowess of *V. unguiculata* may be ascribed to its phytochemistry constituents, as many findings have reported their therapeutic neuroprotective effectiveness. Anti-AChE and antioxidant potency of the majority of the compounds identified in the extract have been documented including pyrogallol [46], quercetin [47], catechins and [48], apigenin [49], campesterol, β-sitosterol, stigmasterol, octadecanoic acid [50], and oleic acid [51]. The antioxidant mechanisms have been shown to involve increased activity of endogenous antioxidant enzymes such as catalase, glutathione S-transferases, superoxide dismutase and glutathione peroxidase [52]. Also, phenolics and flavonoids could have inhibited oxidative stress via their hydroxyl groups, which can directly scavenge oxidative radicals [52].

Alongside the two neuroprotective mechanisms confirmed in this study, the compounds reported in the studied extract have been shown to improve cognition via additional mechanisms. For instance, flavonoids like apigenin and baicalein are known to increase levels of brain-derived neurotrophic factor, a neurotrophin that aids in neuronal proliferation and survival [53]. More so, apigenin is known to down-regulate production of βA precursor protein-cleaving enzymes, presenilin 1 and 2 proteins, which are genetic risk factor of AD disease [49]. Baicalein, luteolin, oleic acid and catechin has been revealed to inhibit Aβ-stimulated damage of neurons [48]. Additionally, these compounds as well as campesterol and phytosterols (sitosterol, campesterol and stigmasterol), have been demonstrated to inhibit neuroinflammation, a major mechanism that accelerates AD neurodegeneration [54]. Furthermore, oleic acid has been shown to promote neuronal clustering, production of the axonal growth-associated protein-43 and axonal growth, thus acting as a neurotropic factor [55]. Therefore, the cognitive improving properties of *V. ungiuculata* reflect the mechanism of action of its neuroprotection associated phytochemicals.

It is worth noting that cognitive ailments like AD are multifactorial disorders with mixed molecular etiologies [5]. As such, this complicates choice of the most effective targets to hit while developing therapies for neurological disorders [1]. New approaches seek to design therapeutic cocktails or single molecule directed to multiple drug targets in cognitive diseases [56]. Vigorous research is ongoing in search of multimodal agents for management and treatment of cognitive disorders [56]. In the present study, *V. unguiculata* demonstrated dual-modal efficacy; anti-AChE and antioxidant, through which it remedied cognitive dysfunction. Also, through review of previous studies, the extra contained secondary metabolites with diverse neuroprotective mechanisms. Taken together, it's conceivable that *V. unguiculata* could enhance cognition via multiple targets. This study provides evidence that supports the promising prospects of *V. unguiculata* for development of multi-functional therapy for cognitive ailments.

CONCLUSION

The aqueous extract of *V. unguiculata* reversed ketamine-induced cognitive impairment in mice. The mechanism of cognitive enhancement by the extract was partly attributed to activation of cholinergic system by inhibiting AChE activity. Also, the extract alleviated cognitive decline via antioxidant mechanisms reducing ketamine-initiated oxidative damage in the brain. The cognitive enhancing outcomes of the extract may have been exerted by the phytochemicals present in the extract. The phytochemicals potency to block oxidative stress and enhance cholinergic neurotransmission has been documented. Thus, the present study confirms cognitive enhancing properties of aqueous extract of *V. unguiculata* observed in traditional use of this plant. This study presents *V. unguiculata* as a promising agent for management of AD as well as other related cognitive disorders. The present proposes bioassay-guided studies on cognitive enhancing potential of phytochemical constituents of *V. unguiculata.* Also, further studies are required to confirm cognitive enhancing efficacy of *V. unguiculata* in other models.

AUTHOR CONTRIBUTIONS

DJK, AMI and MPN were involved in the conception, design and performed the experiments. They also contributed to writing of the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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