

GC-MS Analysis, Anxiolytic and Anti-Epileptogenic Effects of *Combretum nigricans* Leaf Extract in Laboratory Animals

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Abstract Background: Disorders that interfere with the structural architecture and/or functional integrity of the central nervous system are generally described as central nervous system (CNS) disorders. Among the different types of CNS disorders are anxiety and seizure. Though a number of drug classes are currently available for the management of anxiety and epilepsy, different adverse effects and tolerance characterize the use of most of these agents especially after prolonged usage. This thus necessitates research aimed at developing newer agents with fewer adverse effects. **Aim/objective:** This study aims to evaluate the anxiolytic and anti-epileptogenic activities of *C. nigricans* leaf extract using acknowledged mice models. **Materials and method:** In the study, identification of bioactive constituents present in CNEE was carried-out using GC-MS analysis. Anxiolytic effect was evaluated using open field test (OFT), elevated plus maze (EPM) and light/dark box (LDB) models. Anti-epileptogenic effect was assessed using pentylenetetrazole (PTZ) induced seizure, while effect on motor co-ordination was assessed using a rotarod apparatus.

All pharmacological assessment was carried-out in mice with graded CNEE doses of 200 to 800 mg/kg. **Result:** Chromatographic analysis of CNEE using GC-MS shows the presence of 34 constituents including phytol. In the three models used to evaluate the anxiolytic effect, CNEE had a significant ($P < 0.001$) effect at 200 mg/kg. The extract increased time spent in light compartment in LDB model, increased time spent in open arm in EPM model and also increased the number of center square transverse in the OFT model. In PTZ-induced seizure, it delayed onset of seizure and also increased survival time. The extract did not however affect motor coordination in rotarod test. **Conclusion:** The study outcome revealed that *C. nigricans* leaf ethanolic extract has the potential for eliciting potent anxiolytic and anti-epileptogenic effects as shown in the animal models used for the study.

Keywords *Combretum nigricans*, GC-MS Characterization, Neurobehavioural Tests, Anxiety, Epilepsy, Motor Coordination

1. Introduction

Disorders that interfere with the structural architecture and/or functional integrity of the central nervous system are generally described as central nervous system (CNS) disorders [1]. A report by the World Health Organization (WHO) stated that over 1 billion people globally are suffering from one or multiple forms of CNS disorder hence has become a global health challenge [2]. Among the different types of CNS disorders are anxiety and seizure. Anxiety is a psychological phenomenon that results in harmful emotional experience. It is a common neurological disorder and it is estimated that about 322 million people globally are affected by anxiety [3]. It is an integral component of normal human response to potential danger or threat and is characterized by elevated arousal, expectancy as well as neuroendocrine and autonomic activation accompanied by specific behavioral pattern [4]. Though its actual etiology is unclear, several reports have implicated psychoactive agents, genetics and environmental factors as causes of anxiety [5]. Epilepsy is a neurological condition known to be characterized by excessive cerebral neurons firing hence resulting in series of repeated seizures and usually accompanied by partial or total loss of consciousness. Its etiology could be hereditary, or caused by head injury, neoplasm or could even be idiopathic [6]. Globally, about 61 out of every 100,000 people are suffering from epilepsy however, it is said to be more prevalent in low-income countries [7]. Some studies have shown that the pediatric and geriatric populations are at higher risk, while it has also been documented to be more prevalent in males than females [8-10]. Though a number of drug classes are currently available for the management of anxiety as well as epilepsy, different adverse effects and tolerance characterize the use of most of these agents especially after prolonged usage. This thus necessitates research aimed at developing newer agents with fewer adverse effects. Medicinal plants are known to have therapeutic effect against different ailments including neurological disorders hence, have become a major source for developing newer agents [11]. Among the medicinal plants used in Nigerian traditional medicine is *Combretum nigricans*. The plant is a small tree usually found in northern Nigeria, propagated by seeds and thrives in almost all types of soil. It is a versatile medicinal plant that is used to treat various ailments. Scientific chronicles show that in ethnomedicine leaves and stem bark are used to treat diarrhea, cough, jaundice, rheumatism, prurigo, malaria and diseases of the central nervous system [12, 13]. Previous scientific studies on the plant reveal that it possesses potent hypotensive and antimalarial activities in animals. Its cytotoxic, antileishmaniasis and antifungi activities in vitro have also been documented [14, 15]. However, there is a gap in knowledge with regards to the scientific basis for the ethnomedicinal application in management of CNS disorders such as anxiety and seizures. Therefore, this study aims to evaluate the anxiolytic and anti-epileptogenic activities of *C. nigricans* leaf extract

using acknowledged mice models.

2. Materials and Method

2.1. Drugs and Chemicals

The following drugs and chemicals were used: pentylenetetrazole (SIGMA chemical company, United States); diazepam (Swiss parenterals Ltd, India). All drugs used were of the highest caliber, and daily fresh solutions were made from them before usage.

2.2. Plant Materials

The leaves of *Combretum nigricans* were collected by Mr. Mallam Muaza, Taxonomist, Herbarium unit, National Institute for Pharmaceutical Research and Development, Abuja, from its natural habitat in Abuja, Nigeria: The plant material was identified and authenticated with voucher number FHJ223 deposited at the Federal College of Forestry herbarium, Jos.

2.3. Extraction

The harvested leaves were rinsed in running water to remove dirt and then air-dried at normal room temperature for two weeks. The leaves after drying were pulverized using a mechanical grinder, then were macerated in ethanol (70%) at standard temperature for 72 h with routine shaking. The aftermath solution was filtered through a mesh sieve, cotton plugged funnel and filter paper (Whatmann No. 1). The resultant solution was concentrated at 40 °C.

2.4. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The samples were analyzed by GCMS-QP2010 SE (Shimadzu Japan) comprising Shimadzu QP-2010 GC with QP-2010 Mass Selective Detector (MSD), operated in the EI mode, electron energy of 70 eV, scan range of 45-700 amu, and Shimadzu GCMS solution data system. Column used was Agilent HP-5 MS fused silica capillary with 5% phenyl-methylpolysiloxane stationary phase, length was 30 m, internal diameter 0.25 mm, while thickness of film was 0.25 µm. Helium 99.999% was the carrier gas, while flow rate was 1.61 mL/min. The program employed for the oven temperature was 60-160 °C at 15 °C/minute and held at 160 °C for 1 minute, preceded by 160-280 °C at 20 °C/minute, and held at 280 °C for 2 minutes. Temperature of injection port was 250 °C, temperature of interface was 250 °C while ion source temperature was 200 °C. Sample that was dissolved in methanol was filtered through 0.45 µm and 1.0 µL was injected into the GC using autosampler and the split mode at a ratio of 25:1. Identification of individual constituents was by comparison of their mass spectra against known

compounds and NIST Mass Spectral Library. Percent composition of each chemical constituent is reported based on peak area.

2.5. Light Dark Box Test

This test was carried-out using a light dark box apparatus in accordance with the method described by Chu *et al.* [16] Thirty laboratory animals (swiss albino mice) of either sex were used during the study. The laboratory animals were randomized into five groups comprising of six animals each. The animals used for the study were treated with vehicle (distilled water), extract or standard drug. Group 1 received distilled water 10 ml/kg body weight p.o, groups 2 to 4 were administered CNEE 200, 400 and 800 mg/kg body weight p.o respectively, while group 5 was administered diazepam 0.5 mg/kg body weight i.p.

After 1 hour of oral administration or 30 minutes of i.p administration, each animal was placed on the apparatus for 5 minutes and their behavioural activity was observed and recorded.

2.6. Elevated Plus-Maze Test

This test was carried-out using an elevated plus-maze apparatus according to the method described by Komada *et al.* [17] Thirty laboratory animals (swiss albino mice) of either sex were used during the study. The experimental animals were randomized into five groups comprising of six animals each. The animals used for the study were treated with vehicle (distilled water), ethanolic extract of *Combretum nigricans* (CNEE) or standard drug (diazepam). Group 1 animals received distilled water 10 ml/kg body weight p.o, animals in groups 2 to 4 received CNEE 200, 400 and 800 mg/kg body weight p.o respectively, while group 5 animals were administered diazepam 0.5 mg/kg body weight i.p.

An hour after oral administration or 30 minutes after i.p administration, each animal was placed in the apparatus for 5 minutes and their behavioural activity was observed and recorded: time spent in open compartment, time spent in closed compartment, time spent at the center and number of entries into the respective compartments.

2.7. Open Field Test

This test was carried-out using an open field apparatus in accordance with the method previously described by Tatem *et al.* [18] Thirty laboratory animals (swiss albino mice) of either sex were used during the study. The experimental animals were randomized into five groups comprising of six animals each. The animals used for the study were treated with vehicle (distilled water), ethanolic extract of *Combretum nigricans* (CNEE) or standard drug (diazepam). Group 1 animals received distilled water 10 ml/kg body weight p.o, animals in groups 2 to 4 received CNEE 200, 400 and 800 mg/kg body weight p.o

respectively, while group 5 animals were administered diazepam 0.5 mg/kg body weight i.p.

One hour after oral administration or 30 minutes after intraperitoneal administration, each animal was placed animal on the open field and observed for 5 minutes. Number of squares transversed number of rearing, and number of center square transversed was observed and documented.

2.8. Pentylentetrazole Induced Seizure Test

This study was carried-out following procedures previously described by Yuskaitis *et al.* [19] Thirty laboratory animals (swiss albino mice) of either sex were used during the study. The experimental animals were randomized into five groups comprising of six animals each. The animals used for the study were treated with vehicle (distilled water), ethanolic extract of *Combretum nigricans* (CNEE) or standard drug (diazepam). Group 1 animals received distilled water 10 ml/kg body weight p.o, animals in groups 2 to 4 received CNEE 200, 400 and 800 mg/kg body weight p.o respectively, while group 5 animals were administered diazepam 0.5 mg/kg body weight i.p.

One hour after oral administration or 30 minutes after intraperitoneal administration, PTZ 80 mg/kg body weight was administered to each mouse intraperitoneally. Onset of seizure, duration of seizure/survival time and mortality were observed and recorded.

2.9. Rotarod Test

The effect of the extract on motor co-ordination was evaluated using a rotarod apparatus rotating at a speed of 25 rev/min. Twenty-four laboratory animals (swiss albino mice) of either sex were used during the study. Animals for the study were selected based on their ability to remain on the rotarod for at least two consecutive 300s trials before the test day. The experimental animals were randomized into four groups comprising of six animals each. On test day, animals used for the study were treated with vehicle (distilled water) or ethanolic extract of *Combretum nigricans* (CNEE). Group 1 animals received distilled water 10 ml/kg body weight p.o, animals in groups 2 to 4 received CNEE 200, 400 and 800 mg/kg body weight p.o respectively.

After 1 hour of oral administration, each animal was placed on the rotarod for 300 s, and the latency to fall from the rotarod was measured.

2.10. Statistical Analysis

Data obtained from the study were presented as mean \pm standard error of mean (SEM). One way analysis of variance (ANOVA) and Dunnet's post hoc test were utilized to test for significance, $P < 0.05$ was deemed significant. The analysis was conducted using GraphPad Prism for windows (version 8.0).

3. Result

3.1. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The chromatographic analysis of CNEE using GC-MS

shows the presence of 34 constituents which are present in different quantities in the extract (Figure 1, Table 1). This includes 1-(+)-Ascorbic acid 2,6-dihexadecanoate; Octadec-9-enoic acid and phytol are the most abundant compounds present.

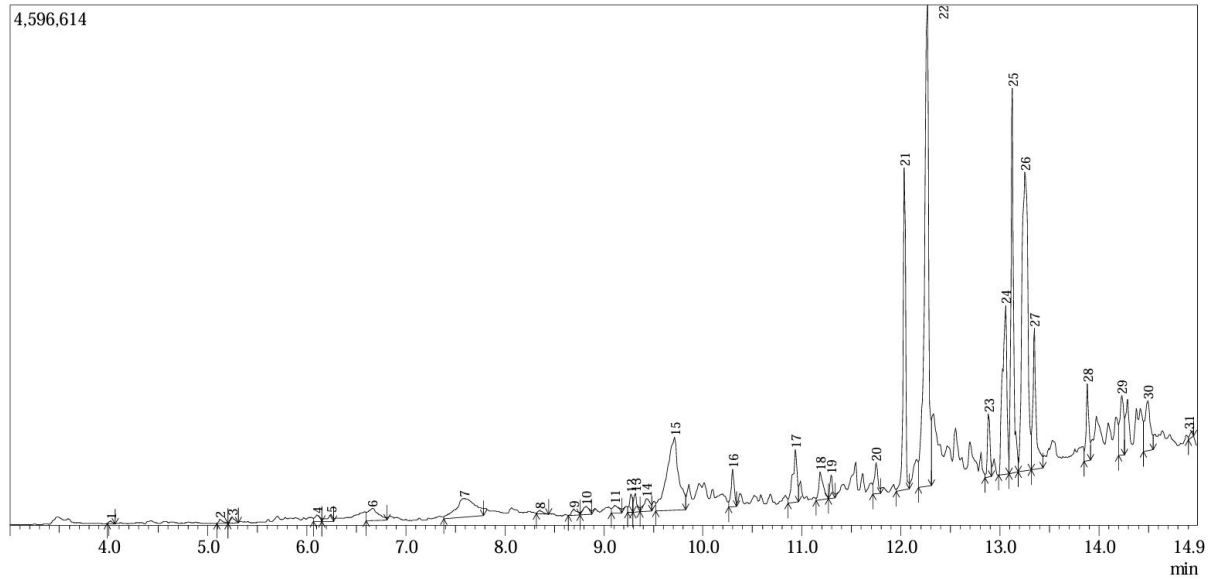


Figure 1. *Combretum nigricans* leaf ethanolic extract GC-MS chromatogram

Table 1. Chemical composition of *Combretum nigricans* ethanolic leaf extract

| Peak No. | Compound Name | Retention Time (min) | % Composition |
|----------|--|----------------------|---------------|
| 1 | Pantolactone | 4.013 | 0.10 |
| 2 | 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one | 5.125 | 0.14 |
| 3 | Benzoic acid | 5.244 | 0.34 |
| 4 | 3,3-Dimethylthietane | 5.701 | 0.26 |
| 5 | 3-Methoxy-2-methyl-4-pyridinol | 5.968 | 0.08 |
| 6 | Benzeneacetic acid | 6.104 | 0.20 |
| 7 | Nonanoic acid | 6.244 | 0.15 |
| 8 | 2(R),3(S)-1,2,3,4-Butanetetrol | 6.658 | 0.71 |
| 9 | 1,2,3-Benzenetriol | 7.603 | 3.15 |
| 10 | p-Thyrosol | 8.069 | 0.29 |
| 11 | Benzeneacetic acid, 4-hydroxy-, methyl ester | 8.694 | 0.35 |
| 12 | Bi-1,4-cyclohexadien-1-yl]-3,3',6,6'-tetrone, 4,4'-dihydroxy-2,2',5,5'-tetramethyl | 8.914 | 0.20 |
| 13 | Bicyclo[4.3.0]nonane, 2,2,6,7-tetramethyl-7-hydroxy- | 9.111 | 0.51 |
| 14 | 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- | 9.275 | 0.59 |
| 15 | Dodecanoic acid | 9.317 | 0.48 |
| 16 | beta.-D-Glucopyranoside, methyl | 9.715 | 7.18 |
| 17 | 8-Hydroxylinalool | 10.017 | 1.31 |
| 18 | 2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl | 10.300 | 1.08 |
| 19 | Tetradecanoic acid | 10.938 | 2.17 |

Table 1 continued

| | | | |
|----|---|--------|-------|
| 20 | Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-propenyl ester | 11.186 | 1.17 |
| 21 | 2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)- | 11.296 | 0.66 |
| 22 | 2-Pentadecanone, 6,10,14-trimethyl- | 11.541 | 1.97 |
| 23 | Pentadecanoic acid | 11.614 | 0.64 |
| 24 | Phthalic acid, isobutyl undecyl ester | 11.748 | 0.83 |
| 25 | Hexadecanoic acid, methyl ester | 12.035 | 8.52 |
| 26 | l-(+)-Ascorbic acid 2,6-dihexadecanoate | 12.271 | 22.74 |
| 27 | Phytol | 13.123 | 10.26 |
| 28 | Octadec-9-enoic acid | 13.256 | 17.69 |
| 29 | Octadecanoic acid | 13.349 | 5.15 |
| 30 | E-10,13,13-Trimethyl-11-tetradecen-1-ol acetate | 13.885 | 3.16 |
| 31 | 1-Heptatriacotanol | 14.232 | 2.17 |
| 32 | cis-9,10-Epoxyoctadecanoic acid | 14.291 | 2.46 |
| 33 | 1(2H)-Naphthalenone, octahydro-8a-methyl- | 14.496 | 3.19 |
| 34 | Carbamic acid, 2-(dimethylamino)ethyl ester | 14.945 | 0.13 |

3.2. Light Dark Box Test

Pre-treatment of the experimental animals with graded doses of CNEE as well as with the standard anxiolytic drug diazepam increased time spent in the light compartment compared to the control. The observed effect was not however dose-dependent with the extract as the lowest test dose (200 mg/kg) gave a better effect which was significant ($P < 0.001$). Time spent in dark compartment also decreased across the respective treated groups compared with the control (Figure 2).

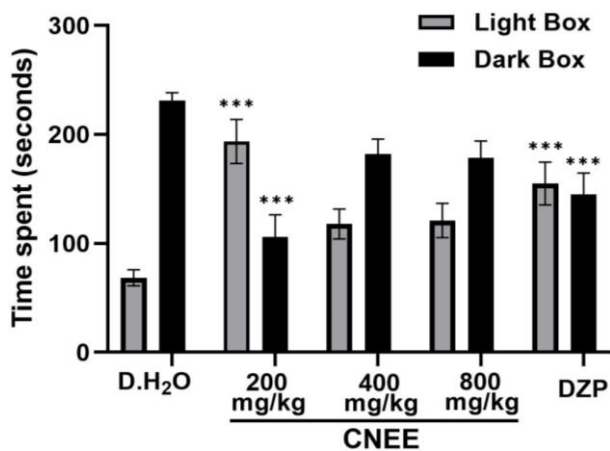


Figure 2. Anxiolytic effect of *Combretum nigricans* ethanolic extract in light/dark box test. Values presented as Mean \pm Standard Error Mean, where n=6, *significant at $P < 0.05$, **significant at $P < 0.01$, ***significant at $P < 0.001$, CNEE = *Combretum nigricans* leaf ethanolic extract, DZP = Diazepam, D.H₂O = Distilled water

3.3. Elevated Plus Maze Test

Pre-treatment of the experimental animals with 200 – 800 mg/kg doses of CNEE caused increased time duration spent in open arm with accompanying decrease in time duration spent in closed arm compared with control. However, CNEE 200 mg/kg elicited a better significant ($P < 0.001$) effect in the study (Figure 3a - b). This trend was similar in number of closed and open arms visits respectively.

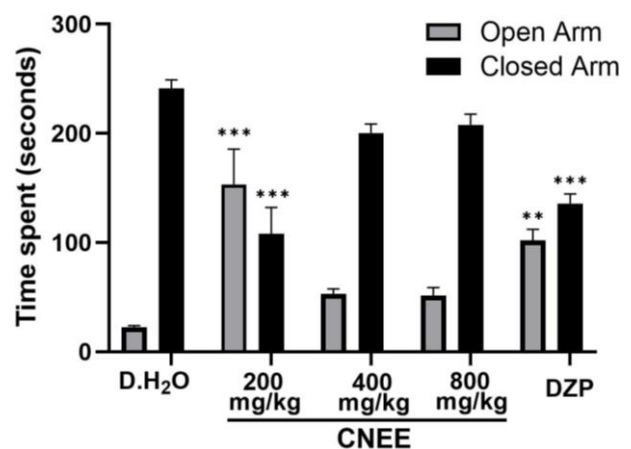


Figure 3a. Anxiolytic effect of *Combretum nigricans* ethanolic extract in elevated plus maze test (Time spent in each arm). Values presented as Mean \pm Standard Error Mean, where n=6, *significant at $P < 0.05$, **significant at $P < 0.01$, ***significant at $P < 0.001$, CNEE = *Combretum nigricans* leaf ethanolic extract, DZP = Diazepam, D.H₂O = Distilled water

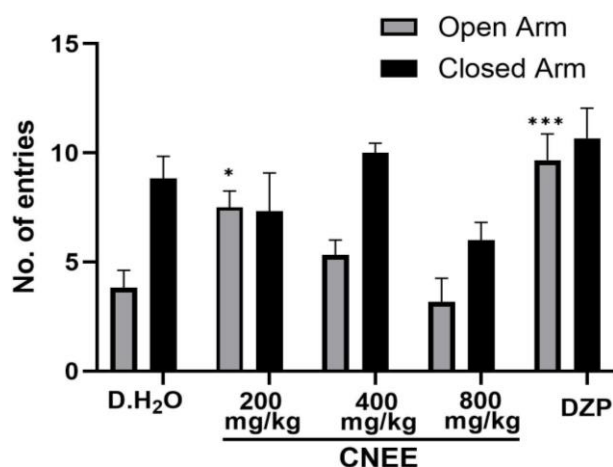


Figure 3b. Anxiolytic effect of *Combretum nigricans* ethanolic extract in elevated plus maze test (Number of entries into each arm). Values presented as Mean \pm Standard Error Mean, where n=6, *significant at $P<0.05$, **significant at $P<0.01$, ***significant at $P<0.001$, CNEE = *Combretum nigricans* leaf ethanolic extract, DZP = Diazepam, D.H₂O = Distilled water

3.4. Open Field Test

Administration of CNEE (200 – 800 mg/kg) to the experimental animals precipitated an increase in the center

squares transversed by the animals. This effect was not dose-dependent as CNEE 200 mg/kg gave a better effect which was significant ($P<0.001$) compared with the untreated group (control). This effect was slightly better than that elicited by the standard drug diazepam which also had a significant ($P<0.01$) effect compared with control (Table 2).

3.5. Pentylentetrazole Induced Seizure

Pre-treatment of the experimental mice with CNEE had a significant ($P<0.01$ – $P<0.001$) effect on both seizure onset and survival time. All test doses (200 – 800 mg/kg) of the extract used for the study delayed the time of seizure onset and increased the survival time when compared to control group. The highest dose used (800 mg/kg) had a better effect in the study. The standard drug diazepam also elicited a similar effect as the extract in the study (Table 3).

3.6. Rotarod Test

In the rotarod test, treatment of experimental mice with CNEE did not elicit any significant effect with regards to the time spent by the animals on the rotarod without falling when compared to the control (Table 4).

Table 2. Anxiolytic effect of *C. nigricans* ethanolic extract in open field test

| TREATMENT | DOSE (mg/kg Body Weight) | SQUARES TRANSVERSED | REARING | CENTER SQUARES TRANSVERSED |
|----------------------------|--------------------------|---------------------|------------------|----------------------------|
| Distilled H ₂ O | 10 ml/Kg | 46.60 \pm 7.33 | 14.40 \pm 2.16 | 0.40 \pm 0.24 |
| CNEE | 200 | 63.00 \pm 3.77 | 22.20 \pm 3.51 | 4.60 \pm 0.81*** |
| | 400 | 52.80 \pm 2.22 | 23.20 \pm 3.61 | 2.20 \pm 0.49 |
| | 800 | 42.60 \pm 8.16 | 15.60 \pm 2.77 | 2.40 \pm 0.68 |
| DZP | 0.5 | 70.00 \pm 13.51 | 20.60 \pm 4.34 | 3.60 \pm 0.81** |

Values presented as Mean \pm Standard Error Mean, where n=6, *significant at $P<0.05$, **significant at $P<0.01$, ***significant at $P<0.001$, CNEE = *Combretum nigricans* leaf ethanolic extract.

Table 3. Anti-epileptogenic effect of *C. nigricans* ethanolic extract in pentylentetrazole induced seizure

| TREATMENT | DOSE (mg/kg Body Weight) | SEIZURE ONSET (Seconds) | SURVIVAL TIME (Seconds) |
|----------------------------|--------------------------|-------------------------|-------------------------|
| Distilled H ₂ O | 10 ml/Kg | 31.67 \pm 1.12 | 65.50 \pm 5.81 |
| CNEE | 200 | 72.00 \pm 6.43*** | 148.5 \pm 18.00** |
| | 400 | 64.50 \pm 5.32** | 218.80 \pm 17.71*** |
| | 800 | 86.17 \pm 6.68*** | 253.30 \pm 11.65*** |
| DZP | 0.5 | 84.50 \pm 6.76*** | 1800.00 \pm 0.00*** |

Values presented as Mean \pm Standard Error Mean, where n=6, *significant at $P<0.05$, **significant at $P<0.01$, ***significant at $P<0.001$, CNEE = *Combretum nigricans* leaf ethanolic extract, DZP = Diazepam.

Table 4. Effect of *C. nigricans* ethanolic extract on motor coordination in Rotarod test

| TREATMENT | DOSE (mg/kg Body Weight) | TIME SPENT WITHOUT FALLING (S) |
|----------------------------|--------------------------|--------------------------------|
| Distilled H ₂ O | 10 ml/Kg | 300.0 \pm 0.00 |
| CNEE | 200 | 300.0 \pm 0.00 |
| | 400 | 291.7 \pm 8.33 |
| | 800 | 286.7 \pm 13.33 |

Values presented as Mean \pm Standard Error Mean, where n=6, CNEE = *Combretum nigricans* leaf ethanolic extract

4. Discussion

This study evaluated the anxiolytic and anti-epileptogenic potentials of *C. nigricans* ethanolic leaf extract using acknowledged animal models. Findings from the study indicate that CNEE has anxiolytic activity, as shown by the behavior of experimental mice pre-treated with the extract in the light/dark box, elevated plus maze and open field tests, and also possesses an anti-epileptogenic effect, as revealed by the pentylenetetrazole induced seizure in mice. Light/dark box, elevated plus maze and open field test are three most utilized rodent models employed in screening potential anxiolytic agents [20].

Anxiety in the light/dark model is generated through a conflict between the tendency of exploring and innate aversion to areas that are brightly illuminated. This can be evaluated basically by the time spent in the light compartment [21]. Drug-induced increase in time spent in the light compartment by experimental animals may be considered as reflection of the anxiolytic activity of such drug. The study results revealed that the test doses of CNEE increased time spent in the light compartment compared with the control. This effect was not dose-dependent as the lowest test dose (200 mg/kg) gave a better significant ($P < 0.001$) effect. This thus suggests that the extract has a potent anxiolytic effect.

Anxiety indices in the elevated plus maze test include the time spent in the open arms and the number of entries as well [22]. Standard anxiolytic agents like benzodiazepines are known to elicit marked effect in this test model [23]. This was also observed in this study as diazepam significantly ($P < 0.01$, $P < 0.001$) increased time spent in open arm and number of entries as well when compared to control. CNEE 200 – 800 mg/kg increased time spent in open arm and decreased time spent in closed arm when compared to control. CNEE 200 mg/kg elicited a better effect in the study. This trend was similar in the number of visits to the open and closed arms respectively.

The open field test is a widely accepted behavioural model for unconditioned anxiety. Parameters such as rearing, number of squares traversed and number of visits to the center are used as indices for anxiety in this model [24]. Potent anxiolytic agents have been reported to increase visit to the center and as well increase rearing in this model when compared to control (untreated) [25]. In this study, administration of CNEE (200 – 800 mg/kg) to the experimental animals precipitated an increase in the center squares transverse and rearing by the animals when compared with control (water). CNEE 200 mg/kg had the highest effect in the study (significant at $P < 0.001$). This effect was slightly better than that elicited by the standard drug diazepam (significant at $P < 0.01$) when compared with control (water). Observations from the study indicate a clear anxiolytic effect of CNEE at a 200 mg/kg dose, revealing that the extract elicits marked anxiolytic-like behavioural activities in experimental mice on exposure to

light/dark box, elevated plus maze and open field tests.

The PTZ-induced seizure is an acute seizure model and is generally accepted as an effective way for the initial evaluation of suspected antiepileptic agents [26]. PTZ elicits excitotoxicity in experimental mice with the hippocampus being among the most vulnerable region for lesions resulting from this effect. Parameters such as time of onset of clonic and tonic-clonic are the commonly used indices for the PTZ-induced seizure model. Agents that prevent or delay the onset of these forms of seizure in experimental animals are inferred to possess potent antiepileptogenic activity [27, 28]. Observations from the study revealed that CNEE had significantly ($P < 0.01$ – $P < 0.001$) delayed seizure onset and increased survival time of the experimental mice when compared to control group. CNEE 800 mg/kg had the highest effect in the study. The standard drug diazepam also delayed the onset of seizure and prevented mortality of the animals.

Rotarod tested a classical experimental model employed in evaluating pharmacologically active compounds for peripheral neuromuscular blockade and effect on motor coordination in experimental animals [29]. The time spent by the treated animals on the apparatus before fall is used to score the effect of the test agent. Longer latency time before fall is an indication of better coordination in the examined animals and lack of muscle relaxant effect as well [30]. In the rotarod test, treatment of experimental mice with CNEE did not elicit any significant effect with regards to the time spent by the animals on the rotarod without falling when compared to the control. Hence, it can be inferred that the extract does not have an effect on motor coordination. Preliminary qualitative study on the phytochemicals present in the extract that reported somewhere else revealed alkaloids, phenols, saponins, tannins and terpenoids are present in CNEE [31]. Previous reports have revealed that these classes of phytochemicals possess potential of eliciting different effects on the central nervous system [32, 33].

Previous reports have revealed that alkaloids and phenols elicit potent central nervous system activity which can be attributed to their high affinity for benzodiazepine-binding site present in Gamma-aminobutyric acid (GABA) receptors. Binding to and activation of this receptor site leads to hyperpolarization of neuronal membrane due to the influx of chloride ions [34]. The resultant hyperpolarization thus leads to therapeutic effects such as anxiolytic and anti-epileptogenic effects. It has also been reported that plant-derived tannins and phenolic compounds could elicit potent anxiolytic activity by GABA_A and 5-HT_{1A} receptors up-regulation, with an accompanying increase in serotonin, dopamine, norepinephrine, brain-derived neurotrophic factor (BDNF), while serum cortisol level is decreased [35]. In this study, GC-MS analysis to identify specific compounds present the extract revealed the presence of 34 constituents in different quantities in the extract including 1-(+)-Ascorbic acid 2,6-dihexadecanoate; Octadec-9-enoic

acid and phytol which are the most abundant constituents. A report by Costa *et al.* [36] revealed that phytol, an acyclic terpenoid, could elicit pharmacological effects on the central nervous system including anxiolytic and anti-epileptogenic activities. This however was opined not to be via the GABAergic pathway but may be by interaction with neurotransmitter systems like noradrenergic, serotonergic and glutamatergic systems. A study published elsewhere by the authors showed that the extract acute oral toxicity dose is greater than 2000 mg/kg in mice [31]. This suggests that the extract may possess a high therapeutic index and could be a candidate worthy of consideration for possible clinical application.

5. Conclusions

The study outcome revealed that *C. nigricans* ethanolic leaf extract has the potential for eliciting anxiolytic and anti-epileptogenic effects as shown in the animal models used for the study. These pharmacological effects observed may be attributed to the array of bioactive compounds present in the extract. It may thus be considered as a candidate for further research aimed at possible exploitation of these proven pharmacological benefits demonstrated.

6. Future Perspectives

The results of this study provide clues for possible future investigations. First, the isolation and further studies of the key bioactive compound responsible for the anxiolytic and anti-epileptogenic activities observed during the study will represent an advance toward its potential clinical use. This should include studies aimed at determining the mechanism by which it produces the physiological activities observed in the study. Additionally, in-depth studies to elucidate its toxicity profile will be important. Second, a synergistic trial with currently used agents such as benzodiazepines and barbiturates at reduced doses could be considered. If effective, this could help reduce the likelihood of serious side effects and dependence on these agents. Finally, data-oriented translational studies can also be recommended for possible clinical application in the future.

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