**Combretum aculeatum** Vent (Combretaceae) hydroethanolic root bark extract attenuates D-galactose-induced cognitive impairment, oxidative, and hippocampi dysfunction in mice


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**Implication for health policy/practice/research/medical education**: The hydroethanolic extract of *Combretum aculeatum* reversed cognitive impairment by protecting the central nervous system against free radical damage. Therefore, *C. aculeatum* might be considered a potential therapeutic agent for neuropathologic diseases.


**Introduction**
Amnesia refers to the loss of memories, either partially or completely. It takes place when the complex neurobiological pathways associated with learning and information retention are damaged (1). Certain lesions in the brain usually cause neurological amnesia (2). These lesions may induce an inflammatory response and release free radicals that can contribute to cognitive disorders similar to Alzheimer’s disease (AD) (3). In excess, D-galactose induces the overproduction of free radicals, which can impair the antioxidant system and lead to amnesia due to inflammation of the brain (4). There is an imbalance between oxidizing species and antioxidants that causes stress, which damages lipids, proteins, and DNA, resulting in neuronal death (5). There are also numerous side effects and adverse events associated with...
drugs used to treat amnesia. Unfortunately, drugs provide only symptomatic relief to patients (6). This has prompted people, particularly in developing countries, to turn to medicinal plants as an alternative source. Amnesia can be treated with a variety of plants depending on where you live. *Daniellia oliveri,* for example, is used to treat diazepam-induced amnesia (7), and *Dichrocephala integrifolia* in the treatment of scopolamine-induced Alzheimer’s disease (8,9). *Combretum aculeatum* (Combretaceae) is abundant in the northern region of Cameroon. Its seeds and roots are used in the traditional treatment of schizophrenia. Previous studies on *Combretum aculeatum* leaf extracts have demonstrated their anti-inflammatory properties (10). This research aimed to determine the influence of treatment with a hydroethanolic mixture of *C. aculeatum* root on D-galactose-induced amnesia in mice.

### Materials and Methods

**Chemicals**
D-galactose, vitamin C, thiobarbituric acid, trichloroacetic acid, and epinephrine were ordered from Sigma-Aldrich (USA). All medications and mixtures were freshly formulated just before experimentation.

### Plant material

The root bark of *C. aculeatum* used for this study was harvested in May 2020 at Gassa. The plant was recognized and confirmed at the National Herbarium, where the reference sample was stored under code number 14900/H.N.C.

**Extraction**

*Combretum Aculeatum* root bark was shade-dried over ten days and ground to a powder. From the powder obtained, 1000 g was soaked in an ethanol/aqueous mixture (80:20) for 3 days. The extract obtained was passed through a filter paper. The filtrate collected was condensed in a rotary evaporator. The dust collected was scaled.

**Evaluation of bioactive content**

**Evaluation of total polyphenolic contents (TPC)**

All phenols in the hydroethanolic mixture of *C. aculeatum* were determined with the Folin-Ciocalteu reagent. In brief, 0.5 mL of plant mixture (10 g/L) was transferred to 1 mL of Folin-Ciocalteu. Five minutes later, 1 mL Na₂CO₃ (7.5%) was included in the mixture and hatched at 37 °C. At 750 nm, the absorbance was recorded (11).

**Estimation of total flavonoid contents (TFC)**

Flavonoids were predicted using the process of Mimica-Duckic et al (12). To summarize, 1 mL *C. aculeatum* was diluted with 1 mL of 10% ALCl₃, and 2 drops of 13M potassium acetate. The assemblage was stored at chamber ambient conditions. The absorptivity of the reactant composition was observed at 430 nm. Flavonoids were reported in units of mg quercetin equivalent (QE)/g dry extract.

### Di-phenyl-1-picryl-hydroxyl (DPPH) radical scavenging potential assessment

Spectrophotometric and DPPH tests were used to determine the trapping activity of DPPH (13). 0.2 mL of plant extract (10 g/L) was introduced into a 2 mL methanol solution of DPPH (1 mM). After 5 minutes mixing, the absorptivity of the final solution was noted at 517 nm. The proportion of radical scavenging potential was obtained as follows:

\[
\text{Inhibition} \% = \left( \frac{(A \text{ blank} – A \text{ sample})}{A \text{ blank}} \right) \times 100
\]

Where “A blank” is the absorbance of the control reaction and “A sample” is the absorbance of the test samples. Trolox equivalent antioxidant capacity (TEAC) and butylhydroxytoluene were used as standards.

**Ferric-reducing and antioxidant capacity assessment**

Anti-oxidation capacity of *C. aculeatum* was established using the ferric reducing antioxidant power (FRAP) method (14). The reaction was newly reformulated and combined at conditions 10:1:1 (v:v:v) for solutions A:B:C, where A = 300 mmol/L sodium acetate trihydrate in glacial acetic acid buffer (pH: 3.6); B= 2,4,6-Tri (2-pyridyl)-s-triazine (10 mM in 400 mM HCl), and C= ferric chloride (20 mM). Ascorbic acid was employed to generate a standard curve. Each extract (10 g/L) was relocated to a cuvette containing 2 mL FRAP solution, and after shaking, the optical density was taken at 593 nm. The FRAP of the extract was determined by the linear interpretability of a standard TEAC curve.

**Experimental animals**

Thirty juvenile albino mice from 8 to 12 weeks of age were used for this experiment. All mice were disposed of with free access to water. Animal treatment and care have been implemented in compliance with the criteria of the Bio-Ethics Commission (Reg N° FWA-IRB00001954) and line with the NIH-Care and a handbook for the use of lab animals (8th edition).

**Animals’ treatments**

Mice were divided randomly into six fractions of 5 animals each and submitted to the subsequent treatment scheme:

- **Group I** received vehicle and distilled water (SS 0.9% + DW 10 mL/kg);
- **Group II** took D-galactose (300 mg/kg) intraperitoneally.
- **Group III** received vitamin C (100 mg/kg) by gavage and served as the positive control
- **Fractions IV, V, and VI** received corresponding *C. aculeatum* mixture at the doses of 100, 200, and 400 mg/kg, by feeding for 21 days.
Behavioral studies

Y maze assessment
The Y tangle assessment estimates spatial working remembrance through instinctive behavioral changes in rodents (15). The maze consisted of an identical three-arm design (33 cm × 11 cm × 12 cm each), symmetrically separated at 120°. Each phase was punctuated by the passage of a mouse through the Y-maze for 8 minutes (16). The rate of spontaneous alternation was approximated by the mathematics relation below:

\[
\% \text{ Alternation} = \frac{\text{Number of alternations}}{\text{Total arm entries}} \times 100
\]

Radial arms maze (RAM) test
The RAM tangle is designed as a lab test for rodent reminiscence. The equipment consists of 8 arms, marked 1 to 8 (48 × 12 cm), starting from a central cylindrical platform (32.5 cm in diameter). The legs (1, 3, 5, and 7) were trapped and the instrument positioned 50 cm suspended higher up. The assessment was divided into two sections: a habituation phase (5 to 7 days) and a test phase (7 days). Each animal had to frequent the baited arms and not come back to them within the same trial (working reminiscence). They also had to develop the habit of avoiding the unbaited arms (reference remembrance). Mice had to use the skills developed during training to ingest the food hidden at the end of the trapped arms. A working remembrance defect was detected when the animal re-accessed a baited arm it had previously approached, while a reference memorial problem was registered with the condition that mice returned to the nonbaited branches (17). The session was closed either when all arms had been frequented, or when five minutes had been spent. Following each visit, the implement was disinfected to extract the remaining odor abandoned by the previous mouse.

Novel object recognized task (NORT)
The procedure was organized in three moments: habituation, inducement or training, and test step. To reduce worry during tests, mice were positioned on the tool with no objects, and permitted to investigate for 10 minutes. The following day, mice were housed in the same domain, but with two similar articles A1 and A2 in their company, which they had to explore for 5 minutes. Precisely one day after the previous phase, the mice were reintroduced to the same environment, but one of the targets has been changed to a new one (test phase). Exploration was only performed when the mice smelled or touched the items with their noses (18). The time spent in close contact with the “novel” gadget and the time spent in contact with the frequent gadgets (TF) was reported. After each run, the stage was rinsed with 70% ethanol to remove any residue. Recognition memory was measured by determining the discrimination index (DI) as indicated below:

\[
DI = \frac{TN - TF}{TN + TF}
\]

which DI = Discrimination index, TN = Time spent exploring the novel object, and TF = Time exhausted inspecting the intimate article.

Biochemical studies

Brain sample preparation
The overall cerebellum was evacuated for biochemical and histological studies. The hippocampus was carefully excised and samples were merged with 10% (w/v) ice-cold 0.1M phosphate buffer (pH 7.4). The mixture was decanted by high-speed centrifugation, and the malondialdehyde (MDA) intensity and superoxide dismutase (SOD) activity were estimated.

Determination of MDA.
The thiobarbituric acid reaction method estimated the level of MDA lipids (19). 250 µL of the supernatant was added to 125 µL of 20% trichloroacetic acid in 250 µL of 0.67% thiobarbituric acid. The samples were kept at 95 °C for 15 minutes. After centrifugation, the reading was taken at 532 nm.

Determination of superoxide dismutase (SOD).
SOD activity was noted according to the process narrated by Misra and Fridovich (20). Briefly, 1660 µL of tampon carbonate (pH=10.2) was added to 140 µL of homogenate and 20 µL of epinephrine (0.3mM). The result of each sample was then noted at 480 nm at 30 and 90 seconds. SOD activity was then reported in units/mg of an organ.

Histological studies
After desiccating in ethanol and xylol, brain portions were embedded in paraffin. A series of gradually decreasing concentrations of alcohol was used to rehydrate deparaffinized sections. On the hippocampi, hematoxylin and eosin were applied. A digital camera and an optical microscope were used to photograph portions (Scientific, Haryana, India).

Statistical analysis
Results were presented in mean ± SEM form. All results were diagnosed by one-way ANOVA (Y-maze) and two-way ANOVA (RAM and NORT) tests, supplanted by Dunnett and Bonferroni post hoc assessment, individually. Results were expressive at P < 0.05.

Results
Calibration of TPC and TFC of the hydroethanolic extract of C. aculeatum
Qualitative analyses of the C. aculeatum hydroethanolic extract reported the prevalence of phenolic compounds, flavonoids, alkaloids, tannins, and steroids. Quantitative analyses revealed the strongest content of phenols

\[
\text{Determination of MDA.}
\]

\[
\text{Determination of superoxide dismutase (SOD).}
\]

\[
\text{Histological studies}
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\text{Statistical analysis}
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\text{Results}
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(724.15±7.01 mg GAE/g dry extract) and flavonoids (251.88±6.02 mg QE/g dry extract).

**In vitro antioxidant potential**
FRAP was more powerful than DPPH. High values of FRAP were an indication of the anti-oxidizing ability of *C. aculeatum* root bark extract. In support of this, there was a high proportion of suppression of FRAP (50.34±0.809). Meanwhile, the radical-scavenging activity of *C. aculeatum* (30.57±0.6) was not better than ascorbic acid and butylhydroxytoluene (75.45±0.7).

**In vivo activities**

**Effect of the hydroethanolic extracts of *C. aculeatum* on spatial working reminiscence in the Y tangle test**
Chronic treatment with D-galactose-induced subsequent low (*P<0.001*) spatial working memory in the negative control category as contrasted to the normal control fraction (79.4±2.67%). All performances were re-established in an effective manner by *C. aculeatum* extract at all examined doses, as well as the vitamin C group (Figure 1a). The experimental fractions exhibited a colossally expanded (*P<0.001*) spontaneous alternation at all doses when compared to the D-galactose non-treated fraction (Figure 1b).

**Effect of the hydroalcoholic extracts of *C. aculeatum* in the radial arms tangle task**
The effects of *C. aculeatum* on working remembrance mistakes and the reference remembrance failures are exposed in Figures 2a and 2b, respectively. Two-way ANOVA revealed a significant decrease (*P<0.001*) in working remembrance mistakes in the *C. aculeatum*-treated group contrasted to the D-galactose group on the 7th day of treatment (Figure 2a). Furthermore, throughout the test days, the reference memory mistakes were sequentially (*P<0.001*) lower than reference memory failures declined in the *C. aculeatum* (200 and 400 mg/kg) treated faction when matched with the amnesic but non-treated group (Figure 2b). Moreover, Figure 2c exposed that the time needed to ingest all the five baits was absolutely (*P<0.05*) diminished with the extract (200 and 400 mg/kg) compared with the amnesic class (Figure 2c).

**Effect of the hydroalcoholic extract of *C. aculeatum* on spatial long-term reminiscence in the novel object recognition assessment**
Chronic treatment of D-galactose substantially (*P<0.001*) reduced the inspection time and discrimination index of the negative control group versus the normal control fraction. In normal mice, *C. aculeatum* extract and vitamin C significantly (*P<0.001*) enlarged the separation index, and the inspection time was noted in the novel item task (Figure 3). The investigation time of the novel gadget was seriously (*P<0.001*) expanded in mice treated with all doses of *C. aculeatum* compared to the D-galactose-treated class (Figure 3a). The extract of *C. aculeatum* significantly increased the discrimination index from -0.24±0.04 in the D-galactose-treated group to 0.60±0.2, 0.67±0.2 and 0.65±0.2 (*P<0.001*) in mice treated with *C. aculeatum* (Figure 3b).
Influence of the hydroalcoholic extract of *Combretum aculeatum* on lipid peroxidation (MDA) and SOD activity.

Estimation of MDA level and superoxide dismutase activity

Chronic treatment with D-galactose significantly raised the incidence of lipid peroxidation, as indicated by MDA values, in hippocampal homogenates versus normal controls (*P* < 0.001). *Combretum aculeatum* pre-treatment lowered lipid peroxidation in hippocampal homogenates in groups treated with all *Combretum aculeatum* extract doses, similarly as in the faction that took vitamin C (100 mg/kg), compared with the D-galactose treated fraction (*P* < 0.001) (Figure 4a). Concerning SOD activity, the D-galactose-induced amnesia class substantially (*P* < 0.001) reduced SOD activity in the negative control class when contrasted to the normal control. Pretreating with all doses of *Combretum aculeatum* seriously (*P* < 0.001) expanded SOD activity when compared with the D-galactose-generated amnesia faction. The vitamin C-treated faction also exhibited a substantial (*P* < 0.001) increase in SOD potential (Figure 4b).

Impacts of the hydroalcoholic extract of *Combretum aculeatum* on hippocampi histological sections

The analysis of hippocampal tissue slices showed that D-galactose administration decreased cell population densities in the hippocampal horn (CA3) and dentate gyrus hilum compared with the normal control. This consideration was inverted by pretreatment with *Combretum aculeatum* and vitamin C (Figure 5).

Discussion

D-galactose-induced nerve damage is a model used to study memory decline related to aging and oxidative stress (21). The emphasis of the present research was to determine the impact of the administration of *Combretum aculeatum* hydroethanolic extract root bark on D-galactose-induced amnesia in mice. Y tangle is motivated by animals’ instinct to explore ecosystems they are placed in for the first time. This test mainly assesses spatial memory for hippocampus-dependent tasks (21). We observed spontaneous alternating training during spatial memory tasks. Memory-impaired animals, on the other hand, will not be able to recall the arm they visited previously and will have a very low alternation rate (22). Pre-treatment with the extract resulted in a substantial (*P* < 0.001) expansion in this percentage contrasted to the D-galactose-treated category. Onaolapo et al (23) related that an elevation in spontaneous instability correlated with an improvement in short-term reminiscence, while a decline reflected a reduction in working reminiscence. This result suggests that the hydroethanolic extract of *Combretum aculeatum* root bark could improve spatial working memory in mice. The radial tangle assessment is a well-appreciated model for getting measures of working and reference remembrance in rodents (24). Treating animals with the extract dropped learning memory in a significant way (*P* < 0.001), by reversing the proportion of working and reference remembrance mistakes balanced with the class of untreated amnesic mice. Chinnala et al (25) showed evidence that reducing errors in working remembrance and reference perception was linked to an improvement in awareness in amnesic mice. The reduction in the proportion of mistakes in working remembrance in mice treated with the extract, at least partially, confirms the results obtained in the Y maze. These observations suggest that the extract stimulates both short-term memory and reference memory. Rodents have an innate instinct to investigate new objects, which makes object recognition useful in assessing long-term memories (26). D-galactose substantially (*P* < 0.001) reversed the time to explore the novel article and the discrimination index in the negative control faction contrasted with the normal control category.

The hippocampus and cortex are closely engaged in the combination of preference for novel items and the increase in discrimination index (27). Pre-treatment of animals with all doses of *Combretum aculeatum* significantly
Antioxidant and cognitive effects of *C. aculeatum* 

(P<0.001) increased the novel gadget exploration time and discrimination index. The results suggested that *C. aculeatum* extract reduced the neurotoxicity of D-galactose and had a memory effect. The nervous system is subjected to oxidative stress because it is made up of predominantly fat and requires a lot of energy but above all because the metabolic rate is higher compared to the other cells (28). A repeated chronic administration of D-galactose in the negative control group significantly (P<0.001) increased the MDA levels compared with the D-gal but untreated class. Variously, mice treated with extract indicated substantial reversal (P<0.05) in MDA levels versus the negative control. Minimization in the amount of MDA in the animals' hippocampal homogenates suggests that the extract may defend the nervous system against lipid damage. In another direction, pre-treatment with all doses of the extract increased SOD and catalase (CAT) in mice given the extract contrasted with the negative control faction.

The elevation in SOD and CAT levels after all mice were treated with the extract and the diminish in MDA concentration certified the antioxidant value of the extract. Phenolics have redox properties that allow them to adsorb and neutralize free radicals, singlet, and triplet oxygen quenching, or peroxide decomposition (29). Free radicals produce oxidative stress in vivo that can lead to oxidative modifications of biological formations such as lipids, proteins, and DNA, and can result in degenerative diseases such as AD (30). Previous studies on the impact of antioxidants *in vitro* from *C. aculeatum* extract have demonstrated its increased capacity to reduce iron (FRAP). Studies have shown a direct relationship between antioxidant activities, the capacity to limit iron levels, and the total phenolic compounds of some plant extracts (31). Various species of the Combretaceae genus are well known for their antioxidant potential (32). This effect may explain the neuroprotective action of *C. aculeatum* obtained in this study, which would be due to certain phenolic compounds such as polyphenols and flavonoids in the extract that have been shown to possess strong antioxidant power (33). Nerve cells ensure the transmission of information through one of their properties, which is conductivity (34). More importantly, the histological study showed a diminish in the mass of

![Figure 5. Histopathological studies of hippocampi sections of (A) normal-control, (B) D-galactose treated, (C) D-galactose+ vit C treated, (D) D-galactose+ CA (100 mg/kg) treated, (E) D-galactose + CA (200 mg/kg) treated, and (F) D-galactose + CA (400 mg/kg) treated animals. The hippocampus lesions were assessed microscopically at 100X magnification. CA1- CA3: cornu ammonis area 1-3; DG: dentate gyrus. Note: The arrows indicate the areas of the cell that have been disorganized.](http://www.herbmedpharmacol.com)
CA3 neurons in the D-galactose-treated class expanded to the normal control faction. On the contrary, all doses of the extract resulted in the restructuring of all hippocampal formations. This property could be attributed to the flavonoids contained in this plant. Flavonoids can preserve, using their ability to alter intracellular signals that promote cell viability (35).

**Conclusion**

This study established that different doses of the extract of *C. aculeatum* effectively improved memory processes and restored the antioxidant status of the brain, which could be caused by an enlargement in the quantity of MDA and an acceleration in SOD activity and CAT. These results showed that the extract of *C. aculeatum* possesses amnesic effects, which may be partially due to its antioxidant potential mediated by its high total phenolic and total flavonoid compounds.

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**Authors’ contribution**

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**Conflict of interests**

The authors confirm that there is no border dispute associated with the divulgation of this paper.

**Ethical considerations**

The study was confirmed by the Ethics Committee of the Faculty of Sciences of the University of Maroua (Ref. N°14/0261/ Um/D/FS/VD-RC), according to the guidelines of Cameroonian bioethics committee (reg N°. FWA-IRB00001954).

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**References**


11. Hamad KM, Sabry MM, Elgayed SH, El Shabrawy AR, El-Fishawy AM, Abdel Jaleel GA. Anti-inflammatory and phytochemical evaluation of *Combretum aculeatum* Vent
Antioxidant and cognitive effects of *C. aculeatum*


