Neuroprotective potential of the hydroethanolic extract of *Lannea microcarpa*'s fruit on oxidative stress induced Alzheimer's disease.

ABSTRACT

Background: Alzheimer's disease (AD) is the commonest neurodegenerative disease affecting about 33 million of people worldwide, with no efficient cure so far. Many studies have reported oxidative stress (OS) as an important factor involved in AD physiopathology. The imbalance between the production of prooxidants and antioxidant defense mechanisms, favours an overproduction of reactive oxygen species (ROS) which bring about brain cell impairment and nerve impulse transmission disruption, leading to memory loss. This work was aimed at evaluating the neuroprotective activity of *Lannea microcarpa*'s fruit on oxidative stress induced Alzheimer's disease rat model.

Method: Total phenolic, flavonoid and tannins contents, and antioxidant activity using DPPH scavenging and FRAP tests; were determined in the fruit extract. 28 female rats of 6 months were used. $AlCl_3$ at 10 mg/kg was administered four times a week by intraperitoneal route during 7 weeks. Morris water maze and eight arms radial maze were performed to assess behaviour. Total proteins, catalase activity, malondialdehyde, nitrite, reduced glutathione, and acetylcholinesterase activity were assessed. Brain histopathological examination was also performed.

Results: From previous studies, the fruit extract obtained was the hydroethanolic extract. The quantitative analysis of secondary metabolites showed TPC (40.961 ± 0.003 mg GAE/g extract), TFC (22.381 ± 0.003 mg CE/g extract), and TTC (10.64 ± 0.16 mg TAE/g extract). Antioxidant activity was determined by the capacity to scavenge DPPH radical (94.739 ± 0.207 %), and to reduce ferric ion (0.894 ± 0.036 mmol Fe^{2+}/ g extract). The administration of *Lannea microcarpa*'s fruit extract to rats decreased the escape latency in the Morris water maze, and the time to visit the maze's arms in the eight arms radial maze (EARM). It also increased catalase activity; but lowered malondialdehyde, nitrite and acetylcholinesterase activity, and prevented lesions in the brain.

Conclusion: The fruit's extract showed a significant effect on oxidative stress biomarkers and acetylcholinesterase actitivity. AD hallmarkers namely β-amyloid and Tau proteins were not evaluated because of lack of adequate equipments to sample the cerebrospinal fluid. Further analysis on the extract to determine the bioactive compounds could be of great interest.

Key words: *Lannea microcarpa*, oxidative stress, Alzheimer's disease, antioxidant activity, neuroprotective activity, aluminium.

1. Introduction

Alzheimer's disease (AD) is the commonest neurodegenerative disorder in aged people (65 years onwards). About 33 million of aged people are affected by AD in the world; and this number continuously grows **[1]**. Many studies have shown a close association between oxidative stress (OS) and AD. OS being a state of permanent imbalance between the production of prooxidants and the antioxidant defense mechanisms, with the balance in favour of prooxidants' production. This consequently triggers the generation of free radicals; which damage biomolecules such as nucleic acids, proteins, fats, and hinder some metabolic pathways **[2].** The brain particularly, is an organ that contains a high amount of fats especially polyunsaturated fatty acids that are susceptible to oxidation by free radicals **[3].** Moreover, other macromolecules especially DNA and proteins can be damaged or altered in the brain, leading consequently to brain impairment, disruption in nerve impulses' transmission as well as in metabolic pathways **[4]**. Treatments used in AD are mainly based on acetylcholinesterase inhibitors and N-methyl D-aspartate receptors antagonists. However, these treatments are high-priced and generate significant side effects. One way to overcome this disease is to prevent oxidative stress by providing the body sufficient antioxidants through food intake. Fruits are an important source of antioxidant compounds **[5, 6, 7]**. In this line, *Lannea microcarpa* is a plant that belongs to the family *Anacardiacea*, and produces fruits called african grapes which are used as food, medicines, fibre, dyestuff and fuel **[8]**. Extracts of *Lannea microcarpa*'s fruit have been demonstrated to have anti-inflammatory, antioxidant, antitumoral and anticancerinogen properties **[9, 10, 7]**. The protective potential of *Lannea microcarpa's* fruit (LMF) was evaluated on Parkinson's disease and it was found that LMF repaired locomotor deficits on paraquat-induced oxidative stress in *Drosophila melanogaster* by restoring antioxidant enzymes **[7]**. All these properties were related to the presence of secondary metabolites especially phenolic compounds in the fruit **[11]**. This work was therefore designed to evaluate the neuroprotective effect of *Lannea microcarpa*'s fruit extract on Alzheimer's disease through a model of aluminium-chloride induced oxidative stress rats. The fruit's extract is expected to restore antioxidant enzymes, and repair memory as well as brain lesions.

2. Material and methods

2.1.Plant material

2.1.1. Plant collection and secondary metabolites extraction

L. microcarpa's fruits were collected in Dschang in the West region of Cameroon and transported to the laboratory. Ripe fruits collected were thoroughly washed under running tap water and then peeled. The mesocarp was cut into small pieces and dried up for 72 hours at 45°C in a ventilated oven. Then it was coarsely powdered by using a blender. The powder was then macerated with a mixture ethanol-water (80:20) (hydroethanol) (50g in 500 ml) for 48 hours. After filtration, the residue was macerated in 300 ml for a few

hours to increase the yield of extraction **[12]**. The filtrate obtained was evaporated at 45°C for 48 hours in a ventilated oven. The crude extract obtained was kept at -4°C in the freezer for further analysis.

2.1.2. Secondary metabolites' determination in the fruit extract

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method **[13]**. Total flavonoid content (TFC) was determined using aluminum chloride method **[14]**. Total tannin content (TTC) was determined by Folin-Ciocalteu method **[15]**.

2.1.3. Evaluation of *in vitro* antioxidant activity

The antioxidant activity of *Lannea microcarpa*'s fruit extract (LMFE) was evaluated by DPPH radical scavenging test **[16]** and by ferric reducing antioxidant power (FRAP) assay to test the capacity of the extract to reduce ferric ion into ferrous ion **[12].**

2.2.Animal material

Female albino *Wistar* rats of 6 months weighing between 200 and 220 g were used. They were randomly allotted in 6 groups of 4 rats, and acclimatized to the standard laboratory conditions *ad libitum*.

2.2.1. Allotment of animals

Group I served as normal control (CG) and was not treated. Group II served as negative control and was administered only a solution of $AlCl_3$ (NC). Group III received vitamin C at 200 mg/kg bw (Vc). Group IV received the standard drug donepezil at 0.30 mg/kg bw (DPZ). Group V was administered LMFE at 200 mg/kg bw (LM 200). Group VI was administered LMFE at 400 mg/kg bw (LM 400). All the groups were administered $AlCl_3$ 10 mg/kg bw prepared in a saline solution (0.9%) four times a week, except the normal control group that was instead administered the saline solution. The test lasted for 7 weeks. The two first weeks were used to administer only $AlCl_3$ to the groups except the normal control. Treatment with vitamin C, donepezil, and extract started on the third week.

2.2.2. Behavioural assessment

2.2.2.1.Morris water maze (MWM) test

MWM was used to assess memory in rats. The test was based on the capacity of rodents to remember and deposit upon a visible platform put in a waterpool containing cold water (22°C) after several trainings. Rodents are warm-blooded animals whose cold environment is an aversive condition. The waterpool was an apparatus of 60 cm in height with a diameter of 180 cm, divided into four quadrants with one containing a platform at its center, 1 cm below the water level. Four different cues were found in the different quadrants, just above the water level to allow the animal to easily find itself during each passage. Each rat was given 60 s in the pool until it finds the platform. The test was carried out three times during the experiment; and each

time, two trials were done before the test. The time taken to deposit on the platform (escape latency) and the time spent in the quadrant containing the platform were recorded by the aid of a camera as described by Morris and Pritchett **[17, 18]**.

2.2.2.2.Eight arms radial maze (EARM) test

This test was based on locomotion and remembrance capacity of rodents to visit targeted arms of the maze. The apparatus was a maze made up of eight arms (length: 60 cm; height: 50 cm). Each rat was given 5 min to explore the maze. The working memory (WM) was evaluated by counting the number of arms visited during the time allocated. The reference memory (RM), was evaluated by counting the number of arms containing food visited without repetitions. To assess the RM, rats were deprived from food for 12 hours, and a pinch of food was deposited in four arms of the maze. Two trials were performed before the test and the maze was cleaned with alcohol after each animal's passage. A camera was used to record a video during the test **[19]**.

2.2.3. Sacrifice of animals and determination of biochemical parameters

On the 50^{th} day, animals were sacrificed by ketamin injection. The brain was collected and each was divided into two. One was crushed in phosphate buffer (0.1 M; pH 7.4) 10% (w/v) to make homogenates and the other was used for histological analysis. Total proteins was determined by Biuret method **[20]**, reduced glutathione (GSH) by using Ellman reagent **[21]**, Acetylcholinesterase (AChE) activity by Ellman method **[22]**, Malondialdehyde (MDA) by thiobarbituric acid method **[23]**, catalase activity by using hydrogen peroxide **[24]**, and Nitrite content by using Griess reagent **[25]**. All reagents used were from analytical grade and supplied by Sigma Aldrich.

2.3.Brain histological analysis

The other part of brain collected for histopathological analysis, was stained with hematoxylin and eosin dyes as described by **[26].**

2.4.Statistical analysis

The data were expressed as mean ± standard deviation, and were analyzed by One-way Analysis of variance (ANOVA) using Duncan's test. The value of $p<0.05$ was considered statistically significant. Data were analyzed using Statistical Package for the Social Sciences (SPSS) software 22.0.

3. Results

3.1.Determination of secondary metabolites and *In vitro* antioxidant activity of the fruit extract

Quantitative analysis of some secondary metabolites contained in the extract has been done **(table 1).**

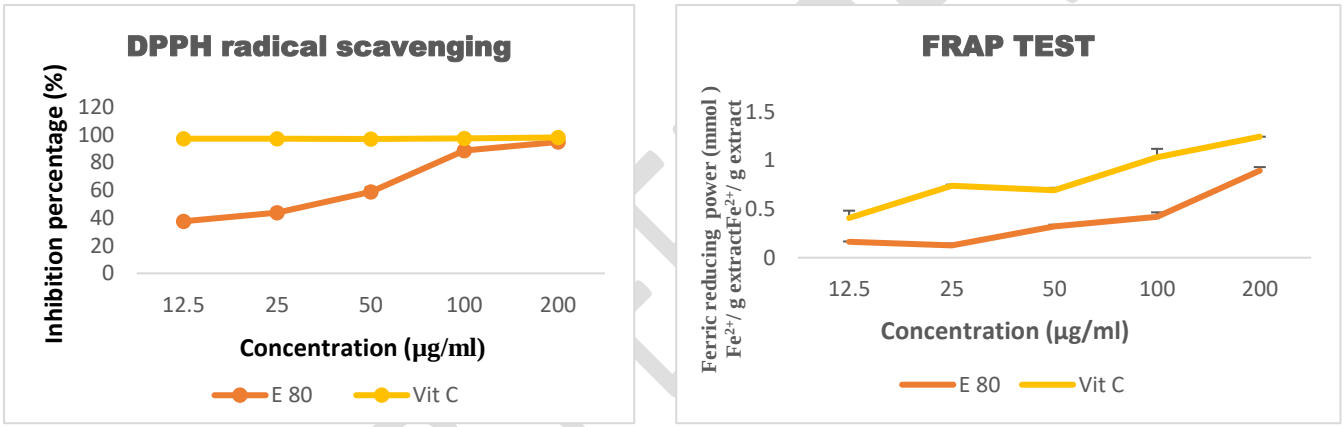
Table 1: secondary metabolites content of the fruit extract

Secondary metabolites	TPC (mg GAE/g extract)	TFC (mg CE/g extract)	TTC (mg TAE/g extract)
Content	40.961 ± 0.003	22.381 ± 0.003	10.64 ± 0.160

TPC: total phenolic content, **TFC**: total flavonoid content, TTC: total tannin content, **GAE**: gallic acid equivalent; **CE**: catechin equivalent; **TAE**: tannic acid equivalent.

3.2.Determination of *In vitro* antioxidant activity of the fruit extract

The general trend has shown that the antioxidant activity of the fruit extract increased with the extract concentration. Ascorbic acid was used as a standard molecule to better appreciate the extract antioxidant activity. From our findings, the percentage of inhibition of DPPH radical by the fruit extract was highest at the concentration of 200 µg/ml; and was 94.739 ± 0.207 %, a value close to that of the standard molecule (97.963 ± 0.437 %) **(fig. 1A)**. The standard and the fruit extract presented a similar trend when assessing antioxidant capacity to reduce ferric ion to ferrous ion. However, the activity of the standard was remarkably higher than that of the fruit extract at all concentrations. The highest activity of the fruit extract was noted at 200 µg/ml and was 0.894 ± 0.036 mmol Fe^{2+}/ g extract obtained **(fig. 1B)**.



A **B**

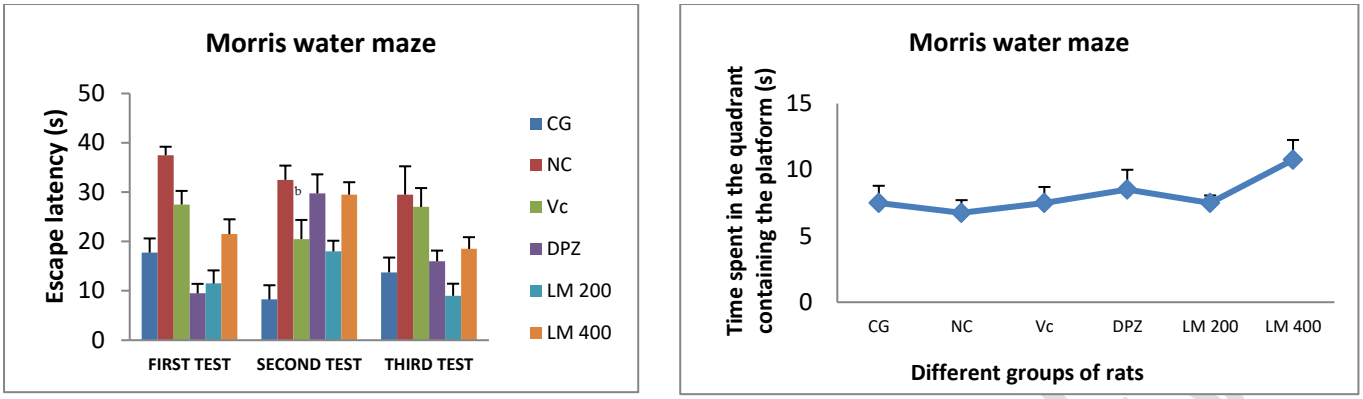
Fig. 1 Antioxidant activity of the fruit extract. **A**: Capacity of *Lannea microcarpa*'s fruit extract to inhibit 2,2-diphynyl-1-picrylhydrazyl (DPPH) radical. **B:** Ferric reducing antioxidant power (FRAP) of *L. microcarpa*'s fruit extract. **E 80**: hydroethanol extract (20:80).

3.3.Evaluation of the effect of the fruit's extract on memory

3.3.1. Effect of the fruit's extract on the escape latency and the time spent in the quadrant containing the platform during Morris water maze test

In the Morris water maze test, we used a water pool to evaluate the animal aptitude to remember and escape an aversive condition (cold water) by searching the platform where to deposit on in the water pool. We found out that for all the tests carried out, the non-treated group took the longest time to find the platform compared to the other groups (37.500 ± 2.886^{d} s). On the other hand, the group that was administered the extract at 200 mg/kg bw was fastest to find the platform, by having the lowest escape latency (EL) (9.000 ± 2.449^{a} s) **(fig. 2A)**. More to that, the animal tendency to remain in the quadrant containing the platform was also assessed, and the non-treated group spent the lowest time in the quadrant (6.750 ± 0.957 s), at the

difference of the group treated with the extract at 400 mg/kg bw that spent a longer time in the quadrant (10.750 ± 1.500 s) **(fig. 2B)**.



A **B**

Fig. 2 Effect of *L. microcarpa*'s fruit extract on learning ability. **A:** Effect of *L. microcarpa*'s fruit extract on escape latency during MWM test. **B:** Time spent in the quadrant with the platform by each group of rats during MWM test. **CG**: normal control that received only a saline water (0.9%) ; **NG**: negative group that was administered only $AlCl_3$; **Vc**: group that received vitamin C 200 mg/kg bw, **DPZ**: group that received the standard drug donepezil 0.35 mg/kg bw, **LM 200**: group that received the fruit extract at 200 mg/kg bw, **LM 400**: group that received the fruit extract at 400 mg/kg bw.

3.3.2. Effect of the fruit's extract on the reference and working memories during eight arms radial maze test

The fruit's extract at 400 mg/kg showed a better activity on the working memory (WM) (0.875 ± 0.275^{ab} arms visited/min) and the reference memory (RM) (0.325 ± 0.050^{ab} arms visited/min). These memories were higher than those of the non-treated group (WM: 0.750 ± 0.173^{a}, RM: 0.275 ± 0.095^{a} arms visited/min), but lower than those of the normal control (WM: 1.800 ± 0.282^{c} arms visited/min; RM: 0.650 ± 0.129^{c} arms visited/min) **(fig. 3)**.

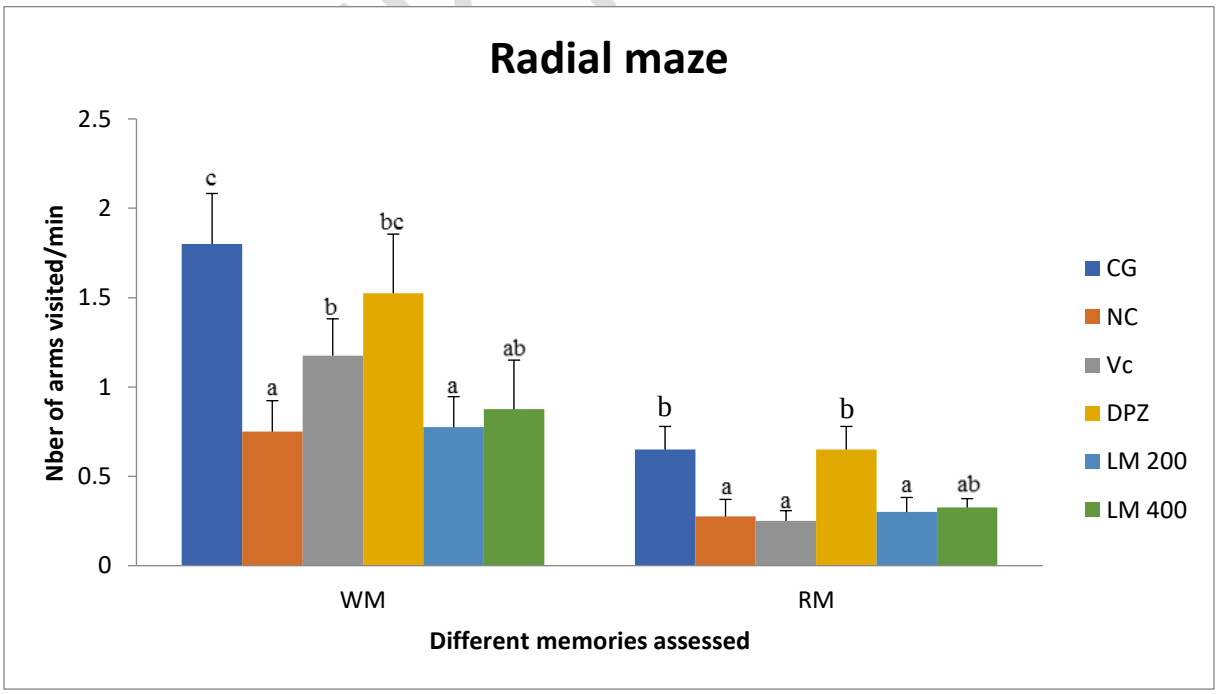
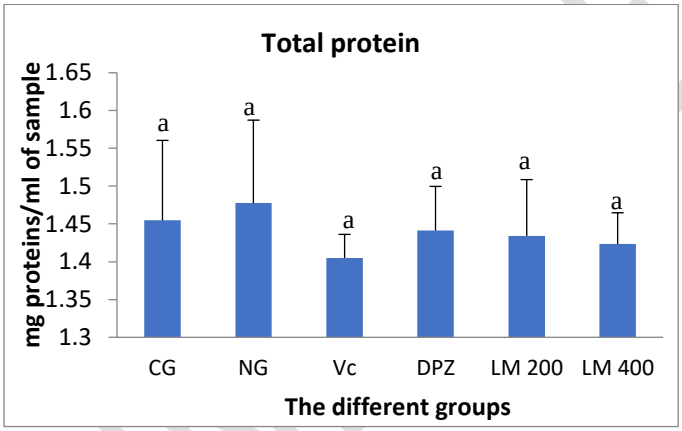


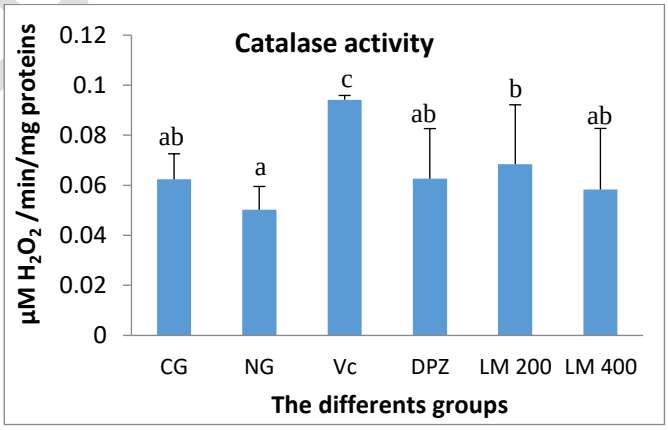
Fig. 3 Effect of *L. microcarpa*'s fruit extract on memory during EARM test**. CG**: normal control that received only a saline water (0.9%) ; **NG**: negative group that was administered only $AlCl_3$; **Vc**: group that received vitamin C 200 mg/kg bw, **DPZ**: group that received the standard drug donepezil 0.35 mg/kg bw, **LM 200**: group that received the fruit extract at 200 mg/kg bw, **LM 400**: group that received the fruit extract at 400 mg/kg bw.

3.4.Evaluation of the effect of the fruit's extract on some biochemical parameters

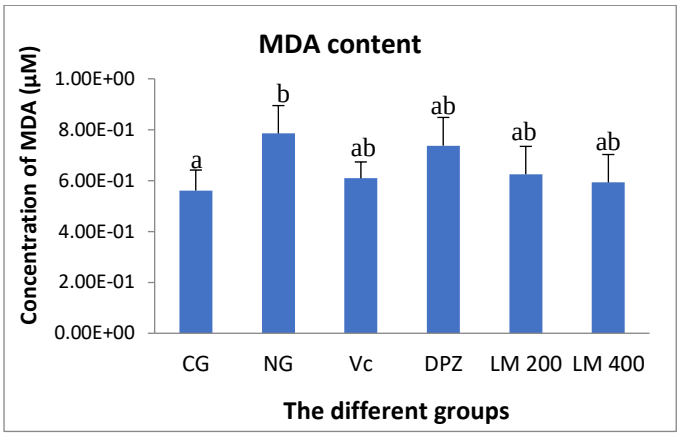
Total protein concentration was significantly the same in all the groups. Neither the administration of $AlCl_3$, nor the treatment of animals with the extract or standard drugs has changed protein concentration **(fig. 4A)**. The administration of the fruit extract at 200 mg/kg bw has significantly increased catalase activity (0.068 ± 0.023^{b} µM H_2O_2 /min/mg proteins) compared to the control group; while in the non-treated group that received only $AlCl_3$, catalase activity was significantly low **(fig. 4B)**. Reduction in MDA level was more significant in the group LM 400 (0.592 ± 0.109^{ab} µM) among the groups treated with the extract, whereas it was highest in the non-treated group (0.785 ± 0.109^{b} µM) **(fig. 4C)**. The evaluation of LMFE on nitrite content showed that the treatment with the extract has not significantly impacted nitrite concentration. However, the group treated with the standard drug DPZ showed a significant reduction in nitrite concentration **(fig. 4D)**. The group treated with LM 200 (1.838 ± 0.199^{b} mol GSH/mg proteins) showed significantly the same GSH concentration with the control group, whereas GSH concentration in the non-treated group was reduced (1.438 ± 0.199^{a} mol GSH/mg proteins) **(fig. 4E).** AChE activity was highest in the negative control group (4.277 ± 0.318^{c} µM/min/mg proteins), and lowest in the group treated with LM 200 (2.200 ± 0.202^{a} µM/min/mg proteins) **(fig. 4F)**.

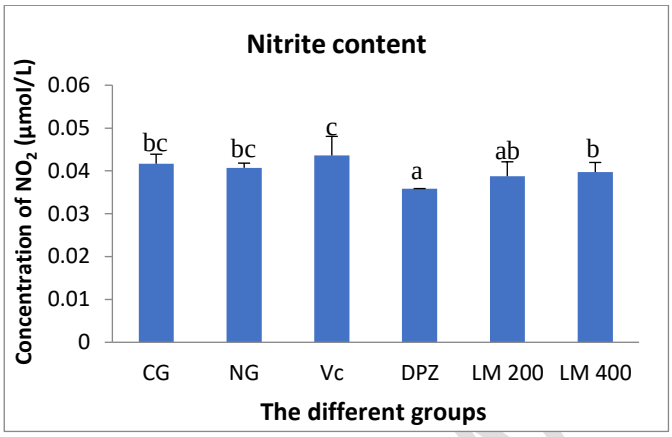


A

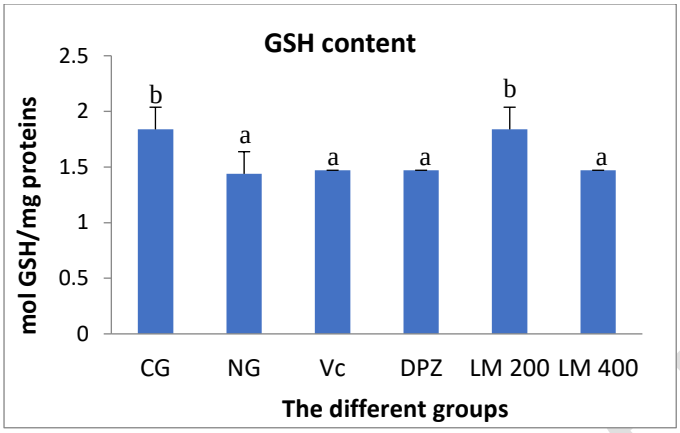


B

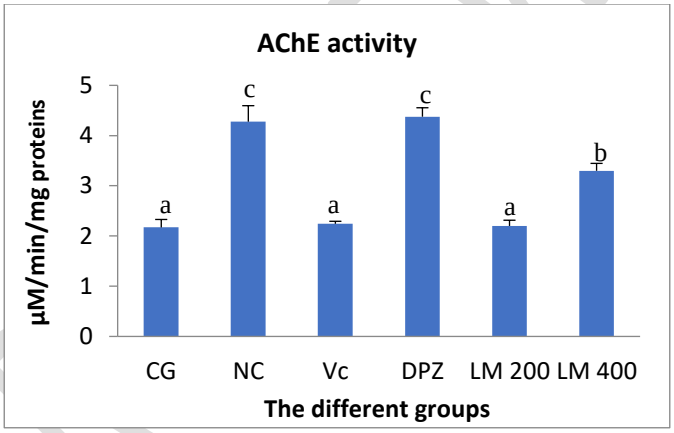




C



D



E **F**

Fig. 4 A: Effect of *L. microcarpa*'s fruit extract on total proteins content. **B**: Effect of *L. microcarpa*'s fruit extract on catalase activity. **C**: Effect of *L. microcarpa*'s fruit extract on MDA content. **D**: Effect of *L. microcarpa*'s fruit extract on nitrite content. **E**: Effect of *L. microcarpa*'s fruit extract on GSH content. **F**: Effect of *L. microcarpa*'s fruit extract on acetylcholinesterase activity. **CG**: normal control that received only a saline water (0.9%) ; **NG**: negative group that was administered only $AlCl_3$; **Vc**: group that received vitamin C 200 mg/kg bw, **DPZ**: group that received the standard drug donepezil 0.35 mg/kg bw, **LM 200**: group that received the fruit extract at 200 mg/kg bw, **LM 400**: group that received the fruit extract at 400 mg/kg bw.

3.5.Brain histopathological analysis

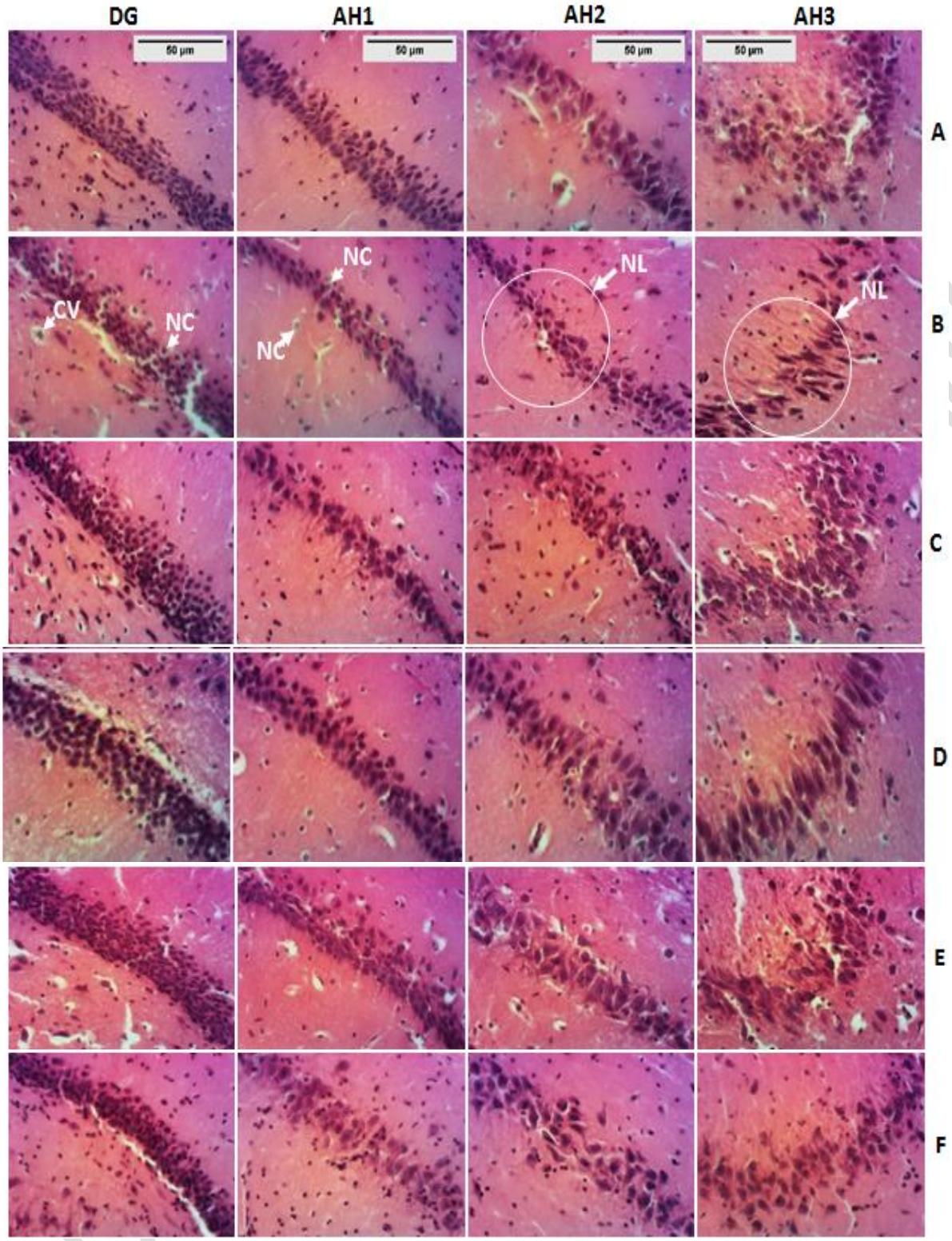


Fig. 5 Brain histopathological analysis. **A** = Control group **; B** = Negative control **; C** = Positive control vit C **; D** = Positive control donepezil **E and F :** Extracts 200 and 400 mg/kg bw **; DG** = Dentate Gyrus **; AH 1, 2, 3** = Ammon's horn 1, 2 et 3 **; CV** = Cell body vacuolation **; NC** = Neuron cytolysis **; NL** = Neuron loss.

4. Discussion

The determination of organic compounds in a biological sample depends upon numerous factors among which the nature of the solvent used. This refers to the ability of the solvent to form bonds with a compound

that is being extracted (solvent polarity) **[27]**. In this study, the hydroethanol extract (20:80) extracted significant quantities of phenolic compounds, flavonoid and tannin. This obviously showed that the mixture ethanol/water 80:20 is suited for secondary metabolites' extraction from *Lannea microcarpa*'s fruit. This result goes in line with that of **[28]** who showed that the hydroethanol (30:70) had the highest TPC and TFC during the determination of secondary metabolites from *Pouteria campechiana*'s fruit.

Antioxidant activity in plant extracts is usually attributed to phenolic compounds. They act by donating protons and electrons to reduce oxidant compounds into non-toxic compounds. The antioxidant activity shown by the fruit extract could be related to the amount of phenolic compounds occurring in. These compounds possess antioxidant properties which reduce the risk of oxidative damage mediated by free radicals **[29, 30, 27]**. In the similar way, the hydroethanolic extract of *Pouteria campechiana*'s fruit showed the highest antioxidant activity when assessing DPPH radical scavenging and ferric ion reducing power by using ethanol, mixture ethanol-water (70:30) and water as solvents **[28]**.

Aluminium (Al) is a heavy metal that possesses a neurotoxic effect on the brain when administered at a high dose. It brings about oxidative damage in the brain by binding to negatively charged phospholipids containing polyunsaturated fatty acids, rending them susceptible to oxidation with a great chance to generate reactive oxygen species (ROS). This disturbs the cell membrane permeability, and triggers the formation of AD hallmarkers namely neurofibrillary tangles (NFT) and β-amyloid plaques (βAP), which both hinder nerve impulse transmission, leading to cognitive impairment **[31, 32]**. This could explain the low remembrance aptitude noticed in the non-treated group when evaluating the memory in the water maze and the radial maze. They were slow to find the platform in the waterpool and to identify the arms containing food in the radial maze. Unlike the non-treated group, the groups administered with the extract showed a better performance during these tests. Bioactive compounds especially phenolic compounds present in *Lannea microcarpa*'s fruit extract (LMFE), may act as antioxidant, by limiting oxidative damage caused by Al and brain injury as well. This result is similar with that of **[6]**.

Al is a heavy metal that is involved in free radicals' production; which consequently promote biomolecules' damage **[33, 5, 34]**. That is why in this study, the effect of LMFE was assessed on proteins, antioxidant enzymes, MDA and nitrite which are OS biomarkers.

Malondialdehyde: the brain is a vital organ made up of polyunsaturated fatty acids. A significant increase of MDA in the non-treated group compared to the normal control group could be due to reaction of Al with fatty acids. Al may promote ROS production through catalytic reactions like Fenton reactions, where Al converts anion superoxide ($O^{2-\bullet}$) and hydrogen peroxide (H_2O_2) to hydroxyl radical (HO^-) species, destabilizing conjugated bonds; leading to lipid peroxidation **[35]** with release of final oxidative products such as carbonyls, peroxinitrites, and MDA within neurons **[36, 6].** However, treating animals with LMFE at a dose of 400 mg/kg has significantly lowered MDA production, showing that the extract may contain

bioactive compounds that reduce lipid peroxidation in living tissues. **[5]** reported a decrease in MDA when assessing *Anacardium Occidentale*'s fruit extract on AD. In the similar way, **[6]** also found a decrease in MDA when assessing neuroprotective effect of *Raphia hookeri*'s fruit on the brain.

Reduced glutathione (GSH): the conversion of H_2O_2 to water requires a peroxidase which works with glutathione. Reduced glutathione (GSH) is a tripeptide that serves as an antioxidant in living organisms. So concentration of GSH is an important marker of OS **[37].** In this study, the highest concentration of GSH shown by the group that received the extract at 200 mg/kg bw could be related to the fact that the extract harbored bioactive compounds like flavonoid that triggered GSH production as a response of Al administration . This therefore means that exposure of the negative control to Al without treatment increased the level of reactive species, which required more GSH to hinder their production **[38, 4]**.

Catalase activity: the significant increase in catalase activity seen in the groups treated with the standard drug and vitamin C showed that DPZ used in AD treatment has an action against OS. The treatment of animals with LMFE at 200 mg/kg has stimulated catalase production compared to the non-treated group. Catalase is an enzyme that highly occurs in peroxisomes where it detoxifies living tissues by converting H_2O_2 to H_2O and O_2; reason why it is considered as one of the powerful enzymes of the antioxidant system **[39]**. Giving that the extract has increased catalase activity, it is then thought to contain phenolic-like compounds known to be implicated in OS **[6].** These bioactive compounds are reported to act through several mechanisms including expression of genes responsible for the secretion of antioxidant enzymes like catalase, superoxide dismutase (SOD) and peroxidase **[40] ,** reduction of OS incidence by neutralizing free radicals through redox reactions **[41]**.

Nitrite: in this study, vitamin C triggered an increase of nitrite concentration, while LMFE triggered a decrease in nitrite in $AlCl_3$-OS induced rats. Vitamin C is a powerful antioxidant doted of a two-face mechanism. The most common is the elimination of excess reactive species by increasing the concentration of endogenous antioxidant enzymes such as catalase, peroxidase, and SOD **[42, 43]**. Another mechanism is the increase of reactive species to destroy harmful cells or molecules to clear the living tissue. In a neuropathology like AD, ascorbic acid may increase nitrite production to break down β-amyloid proteins, a hallmarker involved in AD pathogenesis **[44]**. Phenolic compounds in the extract could have limited nitrite production since nitrite is a nitrogen reactive species which is generated as consequence of oxidative processes in the living tissue.

Acetylcholinesterase activity: many researchers have reported a low concentration of acetylcholine (ASCh) in AD caused by an intensive activity of AChE which breaks down ASCh to acetic acid and choline before it performs its functions **[45]**. However, ASCh is an important neurotransmitter involved in memory process**.** That is why the effect LMFE on AChE activity was also assessed. The lower AChE activity caused by LMFE intake in the brain compared to the non-treated group that did not receive LMFE, showed that LMFE

at 200 mg/kg, remarkably reduced AChE activity to prolong nervous transmission mediated by ASCh. Moreover, LMFE contains phenolic compounds which are known to play a significant role in the memory process. In this study, LMFE is suggested to hamper free radicals' production in the brain, enhance ASCh production, and reduce AChE activity. A similar result was reported by some authors when assessing the neuroprotective effect of some fruits' extracts such as *Anacardium occidentale*'s fruit extract, *Raphia hookeri*'s fruit extract on AChE activity **[46, 47]**.

The dentate gyrus (DG) and three regions of the Ammon's horn (AH) were stained. Cell body vacuolation, neuron cytolysis, and neuron loss were more significant in the non-treated group compared to other groups. This shows the neurotoxic effect of Al, which has severally been reported by researchers **[31, 32]**. However, the groups that were administered LMFE at 200 and 400 mg/kg showed less neurodegenerative features compared to the non-treated group; suggesting that LMFE contains bioactive compounds with anti-neurodegenerative properties.

5. Conclusion

Treatment of AD rats model with LMFE increased antioxidant enzymes' concentration on one hand, and reduced free radicals' formation as well as acetylcholinesterase activity on the other hand. LMFE also prevented the brain from damage. LMFE at a dose 200 mg/kg showed a more significant neuroprotective potential compared to the dose 400 mg/kg. However, further investigations on LMFE will allow us to have a clear understanding on mechanisms used by bioactive molecules with neuroprotective activity found in the extract.

Ethics approval and consent to participate

All experiments carried out on animals in the course of this study were approved by the Institutional Ethical Committee of the University of Dschang (Cameroon); and were in concordance with the internationally accepted standard ethical guidelines for laboratory animals use and care as stipulated in the guidelines of the European Union Institutional Ethics Committee on Animal Care (Council EEC 86/609/EEC of the 24th November 1986). All sections of this report are in line with ARRIVE Guidelines for reporting animal research **[48].**

Data availability

The supporting information file contains the data that was utilized to support this study's findings.

REFERENCES

1. Syed Fahad Javaid, Clarissa Giebel, Moien Khan AB, Muhammad Jawad Hashim. Epidemiology of Alzheimer's disease and other dementias: rising global burden and forecasted trends. *F1000 Research*. 2022; 10:425.
2. Shibata, N., Kobayashi M. The role for oxidative stress in neurodegenerative diseases. *Brain Nerve*. 2008; 60:157–70.
3. Niki, E. Lipid peroxidation: physiological levels and dual biological effects. *Free Radical Biology and Medicine*. 2009; 47:469-484.
4. Hatice, B.A.Ş. The effects of free radicals on aging process. *Current Trends Biomedical Engeering and Biosciences*. 2018; 13:76-78.
5. Bhagyasree P., Kalyani G. Neuroprotective effect of Anacardium occidentale (cashew apple fruit) against aluminum toxicity: an experimental study on cognitive dysfunction and biochemical alterations in rats. *Asian Journal of Pharmaceutical and clinical Research*. 2017; 10:164-169.
6. Doungue, H.T., Kengne, A.P.N., Womeni, H.M. Antioxidant and neuroprotective activities of the mesocarp of Raphia hookeri fruit against aluminum chloride-induced neurotoxicity in rats. *Food and Nutrition Sciences*. 2020; 11:396-415.
7. Oloyede, H.O.B., Adeleye, A.O., Ariyo, F.A., Salawu, M.O., Ganiyu, A.I., Yusuf, B.O. Protective potential of Lannea microcarpa fruit pulp extract on paraquat-induced oxidative stress and locomotor deficits in Drosophila melanogaster. *Biokemistri*. 2020; 32:2020.
8. Muhammad, S., Hassan, L.G., Umar, K.J., Sani, N.A. African grapes (Lannea microcarpa) fruits: the nutritional compositions. *International Journal of Basic, Applied and Innovative Research*. 2018; 7:121–128.
9. Picerno, P.T., Mencherini, R., Della-Loggia, Meloni, M., Sanogo, R. An extract of Lannea microcarpa: Composition, activity and evaluation of cutaneous irritation in cell cultures and reconstituted human epidermis. *J. Pharm. Pharmacol.* 2006; 58:981-988.
10. Bationo, J.H., Hilou, A., Savadogo, P.W., Nacou, O.G. Content of polyphenolics constituents and the antioxidant and antimicrobial activities of extracts from leaves and fruits of Lannea microcarpa Engl. & K. Kraus (Anacardiaceae). *Current Research Journal of Biological Sciences*. 2012; 4:290-296.
11. Eude Oré, Adédiran Goudégnon, Fifanou, Vodouhê G, Gérard Nounagnon Gouwakinnou, Valère Kolawolé Salako, Madjidou Oumorou. Ethnic and generational differences in traditional knowledge and cultural importance of *Lannea microcarpa* Engl. & K. Krause in Benin's Sudanian savannah. *Bois et forêts des tropiques*. 2017 ; 334:49.
12. Iqbal, K.A., Salim Lim, L.B.L. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of Goniothalamus velutinus (Airy Shaw) from Brunei Darussalam. *Journal of King Saud University-Science.* 2015.

13. Chaovanalikit, A., Wrolstad, R.E. Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. *Journal of food Sciences*. 2004; 69:67–72.
14. Dewanto Veronica, Xianzhong Wu, Adom Adom, K., Rui Hai Liu. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry*. 2002; 50:3010– 4.
15. Govindappa, M., Naga Sravya, S., Poojashri, M.N., Sadananda, T.S., Chandrappa, C.P. Antimicrobial, antioxidant and in vitro anti-inflammatory activity of ethanol extract and active phytochemical screening of Wedelia trilobata (L.) Hitchc. *Journal of Pharmacognosy and Phytochemistry*. 2011; 3:43-51.
16. Abdulwahab, N.Z., Shahar, S., Abdullah-Sani, H., Pihie, A.H., Ibrahim N. Antioxidant, antibacterial and antiviral properties of Goniothalamus umbrosus leaves methanolic extract. *African Journal of Microbiology and Research*. 2011; 5:3138-3143.
17. Morris, R. Developments of a water maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*. 1984; 11:47-60.
18. Pritchett David, Taylor Amy, M., Christopher Barkus, Sandra Engle, J., Nicholas Brandon, J., Trevor Sharp, Russell Foster, G., Paul Harrison, J., Stuart Peirson, N., David Bannerman, M. Searching for cognitive enhancement in the Morris water maze: better and worse performance in D-amino acid oxidase knockout ($Dao^{-/-}$) mice. *European Journal of Neuroscience*. 2016; 43:979–989.
19. Olton, D.S., Samuelson, R.J. Remembrance of places passed: spatial memory in rats. *Journal of experimental psychology*. 1976; 2:97-116.
20. Gornall, A.G., Bardawill, C.J., David, M.M. Determination of serum proteins by means of the Biuret reaction. *Journal of Biology and Chemistry*; 1949; 177:751-766.
21. Ellman, G.L. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophys*ics. 1959; 82:70-77.
22. Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology and Physiology*. 1961; 7:84-90.
23. Schmedes, A., Helmer, G. A new thiobarbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides seledctively as a measure of lipid peroxidation. *Journal of the American Oil Chemists' Society*. 1989; 66:6.
24. Sinha Asru, K. Colorimetric assay of catalase. *Analytical Biochemistry*. 1972; 47:389–394.
25. Szonntagh, E.L. Colorimetric azo dye methods for the atmospheric analysis of nitrogen dioxide; historical development, Honeywell Inc. Fort Washington, Pennsylvania, USA, Presented by Prof. Dr. F. Szabadvary. 1979; 207-215.

26. Suvarna Kim, Christopher Layton, John Bancroft, D. Bancroft's theory and practice of histological techniques, 9th edition. *Elsevier*. 2019; 557.
27. Nawaz Haq, Muhammad, Aslam Shad, Najiha Rehman, Hina Andaleeb and Najeeb Ullah. Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Brazilian Journal of Pharmaceutical Sciences*. 2020; 56:7129.
28. Nurul Hidayah, Sani Nurlaela Fitriansyah, Diah Lia Aulifa, Siska Dewi, Windania Barkah Sekolah Tinggi Farmasi. Determination of total phenolic, flavonoid content and antioxidant activity of Campolay (Pouteria campechiana (Kunth) Baehni) extract, 2nd Bakti Tunas Husada-Health Science International Conference (BTH-HSIC 2019). *Advances in Health Sciences Research*. 2019; 26.
29. Brewer, M.S. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Compr Rev Food Sci Food Saf*. 2011; 10:221-47.
30. Coles, L. Functional foods: the connection between nutrition, health, and food science. New Jersey: CRC Press. 2013.
31. Yoshida, H., Yoshimasu, F. Alzheimer's disease and trace elements, Nippon Rinsho. 1996; 54:111-116.
32. Prashar, Y., Gill, N., Perween, A. Protective effect of Lagenaria siceraria in reversing aluminium chloride induced learning and memory deficits in experimental animal model. *International Journal of Recent Advances in Pharmaceutical Research*. 2014; 4:87-104.
33. Martyn, C.N., Barker, D.J.P., Osmond, C., Harris, E.C., Edwardson, J.A., Lacey, R.F. Geographical relation between Alzheimer's disease and aluminium in drinking water. *The Lancet*. 1989; 33:59-62.
34. Kengne, A.P.N., Rodrigue Tidjong, Dieudonné Kuate, Doungue, H.T., Takam, P.N., Inelle Makamwé. Protective effect of ethanolic extract and raw juice of Solanum betaceum on aluminum induced oxidative stress and associated memory deficits in rats. *Investigational Medicinal Chemistry and Pharmacology*. 2019; 2:27.
35. Angelova, P.R., Esteras, N., Abramov, A.Y. Mitochondria and lipid peroxidation in the mechanism of neurodegeneration: finding ways for prevention. *Med. Res. Rev*. 2021; 41:770–784.
36. Singh Anju, Ritushree Kukreti, Luciano Saso, Shrikant Kukreti. Oxidative Stress: a key modulator in neurodegenerative diseases. *Molecules*. 2019; 24:1583.
37. Wu, G. Glutathione metabolism and its implications for health. *J Nutr*. 2013;134:489-92.
38. Deponte, M. Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. *Biochim. Biophys. Acta*. 2013; 1830:3217–3266.
39. Pongsavee Malinee. Effects of ERCC5 rs 751402 Polymorphism on oxidative stress and the impact of curcumin on catalase activity in breast carcinogenesis. *Asian Pacific Journal of Cancer Prevention*. 2022; 23: 2065-2069.

40. Mercader, A.G., Duchowicz, P.R., Fernández, F.M., Castro, E.A., Bennardi, D.O., Autino, J.C., Romanelli, G.P. QSAR prediction of inhibition of aldose reductase for flavonoids. *Bioorgan. Med. Chem*. 2008; 16:7470–7476.
41. Nijveldt, R.J., Van Nood, E., Van Hoorn, D.E.C., Boelens, P.G., Van Norren, K., Van Leeuwen, P.A.M. Flavonoids: a review of probable mechanisms of action and potential applications. *Am. J. Clin. Nutr*. 2001; 74:418-425.
42. Kook, S.Y., Lee, K.M., Kim, Y., Cha, M.Y., Kang, S., Baik, S.H., Lee, H., Park, R., Mook-Jung, I. High-dose of vitamin C supplementation reduces amyloid plaque burden and ameliorates pathological changes in the brain of 5XFAD mice. *Cell Death and Disease*. 2014; 5:1083.
43. Aditya Marianti, Fransisca Widhi Mahatmanti. Synergetic effect of chitosan and vitamin C on the oxidative enzyme status in rat exposed to lead acetate, Acta Scientiarum. *Biological Sciences*. 2018; 40:41869.
44. Sampaio Isabella, Felipe Domingues Quatroni, Paula Maria Pincela Lins, Alessandro Nascimento, S., Valtencir Zucolotto. Modulation of beta-amyloid aggregation using ascorbic acid. *Biochimie*. 2022; 200:36-43.
45. Goschorska Marta, Izabela Gutowska, Irena Baranowska-Bosiacka, Katarzyna Piotrowska, Emilia Metryka, Krzysztof Safranow, Dariusz ChlubekInfluence of acetylcholinesterase inhibitors used in Alzheimer's disease treatment on the activity of antioxidant enzymes and the concentration of glutathione in THP-1 macrophages under fluoride-induced oxidative stress. *Int. J. Environ. Res. Public Health*. 2019; 16:10.
46. Tonnies Eric, Trushina Eugenia. Oxidative Stress, synaptic dysfunction, and Alzheimer's disease. *Journal of Alzheimer's Disease*. 2017; 57:1105–1121.
47. Tagne Tueguem Géradin Joel, Anne Pascale Nouemsi Kengne, Hermine Tsafack Doungue, Michel Pegui Kemtsop, Julius Oben Enyong. Evaluation of the effect of Pouteria Campechiana's fruit powder and ethanolic extract on aluminum-chloride induced Alzheimer's disease. *Journal of Food Science and Nutrition Research*. 2020; 3:083-091.
48. Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M., Altman, D.G. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol. 2010; 8: e1000412.