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Research Article

Neuroprotective Effect of a Nutraceutical Formulated with *Pouteria campechiana*'s and *Lannea microcarpa*'s Fruits on Alzheimer's Disease

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Abstract

Oxidative stress is a famous factor that may trigger Alzheimer's disease through the disruption of the redox balance to result to free radicals species, harmful for the brain. Fruits harbor antioxidant compounds that may preserve the brain against Alzheimer's disease. Some include phenolic compounds, unsaturated fatty acids and triterpenoids. This work was aimed at evaluating the neuroprotective activity of a nutraceutical made with *Lannea microcarpa*'s and *Pouteria campechiana*'s fruits on a model of oxidative stress-induced Alzheimer's disease rats. *Lannea microcarpa*'s ethyl acetate and *Pouteria campechiana*'s hexanic fractions were made by partitioning the hydroethanolic extract (20:80) of *Lannea microcarpa*'s fruit pulp and the ethanolic extract of *Pouteria campechiana*'s pulp fruit in ethyl acetate and n-hexane respectively. Antioxidant activities of both fractions were determined by DPPH and FRAP assays. A simplex lattice mixture design was done and the selected mixture was used as nutraceutical. Gas chromatography-mass spectrometry was performed to determine the phytochemical composition of fractions. $AlCl_3$ at 15 mg/kg bw was administered to 3 months rats; and the different treatments were administered every day by oral route. Behavioral assessment was carried out by Morris water maze and Open field tests. Malondialdehyde, nitric oxide, catalase, reduced glutathione, γ-aminobutyric acid, and acetylcholinesterase were determined in the brain. Red congo was used for brain analysis. Data were analyzed with SPSS 22.0 using one-way ANOVA and MINITAB 20.0. The mixture *L. microcarpa*'s fraction/*P. campechiana*'s fraction (62.5: 37.5) was selected. Major compounds detected in *Lannea microcarpa*'s and *Pouteria campechiana*'s fractions were phenolic compounds, triterpenoids and fatty acids. Treatment of animals with the nutraceutical increased the spatial and learning memory in the Morris water and open field mazes. Levels of malondialhedyde (1.969 ± 0.09) × 10^{-6} µM), nitric oxide (9.551 ± 0.296^{a} µM), acetylcholinesterase ((4.515 ± 0.268) × 10^{-6} µM/mg protein) reduced, and γ-aminobyturic acid (36.238 ± 1.833 µg/ml) increased. The non-treated group showed amyloid plaques and neurodegenerative features like cell pyknosis, cell vacuolation and neuron loss. Bioactive compounds contained in the nutraceutical could be targeted as acetylcholinesterase inhibitors and anti-amyloidogenic therapeutics.

Keywords

Lannea microcarpa, *Pouteria campechiana*, Antioxidant, Neuroprotective, Alzheimer's Disease

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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease that affects the brain cortex and lead to memory loss. It is currently the most common dementia that affects people in the world, with a worldly prevalence of 50 million [1]. Predicting factors that may lead to the onset of AD are toughly understood. Nevertheless, oxidative stress (OS) through several pathways is reported as an enhancer of the disease. The mechanism underlying OS is the generation of free radicals harmful for the brain, ending up with AD's hallmarkers formation [2]. Commonly treatments used in AD are acetylcholinesterase inhibitors (AChEIs) such as donepezil, galantamine, and rivastigmine; and N-methyl-D-aspartate receptor antagonist (memantine). Due to the huge economic burden related to their use, and their inability to bring about a complete healing, other ways to overcome AD are highly recommended [1, 3]. In this line, food remains a good alternative. Besides the nutritive requirements necessary for body growth it provides, it also harbors health enhancers that are beneficial for the brain as they can prevent oxidative damage; and by so doing, enhance neuroprotection. Resveratrol is a polyphenol highly contained in fruits and nuts that has an activity against β-amyloid plaques formation [4]. Stigmasterol is a phytosterol capable of counteracting neuron damage and enhancing neurogenesis in the brain [5]. Sitosterol is a phytosterol that has been reported to improve cognitive decline in AD [6]. As shown above, these compounds act by different mechanisms in neuroprotection, so make a product that would contain varied types of these compounds might increase the product's efficacy on AD. *Lannea mcrocarpa*'s and *Pouteria campechiana*'s fruits are two interesting candidates. The first harbors phenolic compounds with antioxidant and anti-cholinesterasic properties [7]; while the second revealed the presence of carotenoids, triterpenoids and polyunsaturated fatty acids (PUFAs) with antioxidant and anti-amyloidogenic properties [7, 8]. These compounds may not be regularly available for human welfare since fruits are seasonal; hence they can be used to manufacture nutraceuticals. These refer to compounds contained in food that enhance health by preventing or curing diseases, beyond the food nutritive value [9]. In our previous studies, we found out that *Lannea microcarpa*'s flesh hydroethanolic extract had the highest antioxidant activity [10], and *Pouteria campechiana*'s flesh ethanolic extract had the best antioxidant activity [11] compared to the other extracts analyzed. This was related to bioactive phenolic compounds occurring in the extracts and conferring them a neuroprotective activity. Nevertheless, we lack knowledge on the specific compounds responsible for this activity in the extracts. Besides, we would like to find out whether the association of compounds from the two different fruits' extracts could increase the neuroprotective activity on AD's related parameters. This study was therefore designed to evaluate the neuroprotective activity of a nutraceutical made with *Lannea microcarpa*'s and *Pouteria campechiana*'s fleshes on AD's physiopathology.

2. Material and Methods

2.1. The Plant Material

2.1.1. Extraction in Solvents

Lannea microcarpa's hydroethanolic extract was obtained by macerating the fruit flesh powder in a mixture ethanol-water (80:20) (1w: 10v) for 48 hours; and *Pouteria campechiana*'s ethanolic extract by macerating the fruit flesh powder in ethanol (1w: l0v) for 48 hours. After the mixture was filtered with the *Whatman* paper No 4, the residue was once again macerated in the same solvent (1w: 6v) for 24 hours to increase the yield of extraction [12]. The filtrate obtained was air-dried at 45 ℃ for 48 hours an oven. The crude extracts obtained were each dissolved in ethanol, and then partitioned in a ternary solvent system composed of n-hexane, ethyl acetate and n-butanol. The n-hexanic filtrate was evaporated at 70 ℃, the ethyl acetate filtrate at 78 ℃, and the n-butanolic filtrate at 120 ℃ after the partition. The remaining residues obtained after the evaporation was air-dried in the oven at 45 ℃ to obtain the crude fractions.

2.1.2. Antioxidant Activity of the Fruits' Fractions

(i). DPPH Scavenging Test

This test is based on the transfer of hydrogen atom from an antioxidant compound to DPPH radical. The antioxidant power of a solution is observed when the blue-coloured solution turns to pale yellow [13]. The samples were prepared at different concentrations (2000 µg/ml, 1000 µg/ml, 500 µg/ml, 250 µg/ml, and 125 µg/ml). The percentage of inhibition of fruits' fractions was calculated using the following formula:

$$Inhibition\% = \frac{A_{DPPH} - A_{sample}}{A_{DPPH}} \times 100$$

The antioxidant capacity of fruits' fractions was expressed by the concentration of the fruit's fraction necessary to scavenge DPPH radical by 50%. This was determined from the equation y = ax + b obtained from the standard curve.

(ii). Ferric Reducing Antioxidant Power (Frap) Assay

This test is based on the transfer of electrons from the sample to Fe^{3+}-TPTZ (2,4,6-tripyridyls-triazine) complex. The latter generates a blue-coloured compound which maximally absorbs at 593nm. The fruit sample's capacity to reduce ferric ion to ferrous ion was expressed as Fe^{2+}

equivalent which are µM or FRAP value [14]. This was determined from the equation y = ax + b obtained from the standard curve.

2.1.3. Analysis of Compounds by Chromatography Coupled to Mass Spectrometry

Volatile compounds are separated based on their affinity with either phase. The more volatile compounds highly interact with the gas mobile phase and move faster through the column; whereas the less volatile compounds interact more with the silicone grease stationary phase and move slowly through the column. The detector generates different peaks of the compounds present in the sample based on their retention time (RT). Compounds that move rapidly in the column appear fast on the chromatogram and compounds that move slowly take more time to appear. The compound's name is given based on already available data of compounds' RT.

For chromatography, 1g of the fruit fraction was mixed with 5 ml of methanol. Thereafter, the mixture was vigourously homogenized. The resulting solution was then used for chromatography. GC-MS apparatus was an Agilent 8890N GC coupled to a single Agilent 5977N quadrupole mass spectrometer. The GC was fitted with an Rtx-5MS capillary column (30 m x 0.25 mm ID x 0.25 µm df) composed of 5% diphenyl / 95% dimethyl poly siloxane stationary phase. The carrier gas used was Helium at a column flow rate of 1 mL/min. The inlet line temperature was 290 ℃ and the pressure 22.33 psi. An Agilent 7683 autosampler was used to inject 2 µL of the sample in the splitting mode 10:1. Oven temperature was initially set at 110 ℃, increased 10 ℃/min-no hold to 200 ℃, then increased at a rate of 5 ℃/min-12 min hold to a final temperature of 280 ℃. The total GC running time was 40.5 min. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV ionization energy. The MS inlet line temperature was set at 290 ℃, and the MS source temperature was at 250 ℃. The total MS running time was 40.5 min.

2.1.4. Mixture Design

(i). Type of the Mixture Design

The simplex lattice design was used. The sum of the proportions of both mixed components used was 100%. The components of the mixture were chosen based on compounds present in the fractions known to have antioxidant properties. The selected factors were *Lannea microcarpa*'s ethyl acetate fraction (LMEA) and *Pouteria campechiana*'s hexanic fraction (PCH). LMEA was targeted for fruit's availability and phenolic compounds' content. Likewise, PCH was selected based on its fruit's availability and phytosterols' content.

(ii). Choice of the Domain of Factors

The choice of the domain of variation of each factor was based on previous works performed as well as preliminary tests done in the laboratory (Table 1).

Table 1. *Definition of the experimental design.*

Factors	Range (%)	Lowest level (%)	Highest level (%)
LMEA	60 - 70	60	70
PCH	30 - 40	30	40

LMEA: *Lannea microcarpa*'s ethyl acetate fraction, PCH: *Pouteria campechiana*'s hexanic fraction.

(iii). Generation of the Matrix of Real and Coded Values

After the determination of the domain of variation of both factors, Minitab 20.0 was used to generate the number of assays according to the simplex lattice design based on two factors (Table 2).

Table 2. *Matrix of real and coded values obtained from a simplex lattice design mixture at two factors.*

Order Std	Order Assay	Type Pt	Blocks	LMEA	PCH
5	1	-1	1	47.5	52.5
3	2	0	1	55	45
4	3	-1	1	62.5	37.5
2	4	1	1	40	60
1	5	1	1	70	30

LMEA: *Lannea microcarpa*'s ethyl acetate fraction, PCH: *Pouteria campechiana*'s hexanic fraction.

(vi). Evaluation of Responses

Both fractions used in this study harbor bioactive compounds such as polyphenols, flavonoids, phytosterols, PUFAs and terpenoids; which may be dotted with significant antioxidant activity. The responses of this mixture were therefore generated by evaluating the antioxidant activity of the different assays by DPPH scavenging and FRAP assays.

(v). Formulation of the Nutraceutical

The mixture that gave the best response was used to formulate the nutraceutical. The paraffin oil was used to dissolve and stabilize the mixture of fractions at the concentration of 10 mg of mixture / ml of oil (10w: 1v).

2.2. The Animal Material

2.2.1. Allotment of Animals and Different Treatments Administered

Female *wistar* rats of 3 months weighing 180 to 200 g, were randomly allotted into 07 groups of 5 rats to start the experiment. In this model, the experiment lasted 15 weeks, and aluminum chloride ($AlCl_3$) 15 mg/kg b.w prepared in a saline solution 0.9% was administered five times per week by intraperitoneal injection to induce oxidative stress in rats except the normal control. The induction was done by administering $AlCl_3$ to rats over 8 weeks. One week was used to carry out behavioural assessment with the animals before the treatment starts. The fruits' fractions (50 mg/kg bw), the nutraceutical (50 mg/kg bw) and the excipient (5ml/kg bw) were then used to treat animals. Donepezil (0.35 mg/kg bw) was used as standard drug. They were treated by oral route every day for 6 weeks.

2.2.2. Behavioural Assessment

(i). Morris Water Maze

Rodents are warm-blooded animals that naturally dislike cold. So this test is based on the tendency of rodents to remember and deposit upon a visible platform put in a waterpool containing cold water. The Morris water maze (MWM) apparatus was set up as described by [15]. The waterpool was an apparatus of 60 cm in length with a diameter of 180 cm, divided into four quadrants with one containing a platform at its center, found 1 cm below the water level. Four different cues were found in the different quadrants, just above the water level to allow animals to easily find themselves during each passage. Each rat was given 60 s in the pool until it has found the platform. Before the test, all animals underwent two trials along which they were trained to develop spatial memory to easily recognize the position of the platform. During the training, each rat was given 90s to deposit on the platform, and 10 s once on the platform. Rats that did not succeed the task after 90 s, were delicately guided by the experimenter to the platform and were allowed 10 s upon it. A camera was used to record videos during the experiment and the data collected were the escape latency and the percent time spent in the quadrant containing the platform.

(ii). Open Field Test

The Open field test (OFT) was first described by [16] to evaluate anxiety in rodents. This test is based on the locomotion of animals in an open field where they cannot escape. The rate of animal's mobility in the field helps to evaluate anxiety and brain dysfunction as well. The open field apparatus was square-shaped measuring 70 cm in length with a height of 30 cm. At the floor base, the surface of the tool was marked by a central part (central square) and peripheral parts, and was covered with a transparent glass. During the test, each animal was placed at the central square of the tool, and was allowed 5 min to explore the apparatus. The passage of the animal was recorded by a camera. Data recorded were the number of defecations and the tendency to escape the field.

2.2.3. Sacrifice of Animals and Sample Collection

On the 106^{th} day, animals were sacrificed by ketamine/diazepam injection. The brain was collected by cervical dislocation. The brain after being removed from the animal was immerged and cleaned in physiological water (NaCl 0.9%) before it was kept in the fridge at 0 ℃.

2.2.4. Sample Preparation for Biochemical Analysis

The brain homogenate was made by crushing 0.5 g of the brain tissue in 5 ml of phosphate buffer (0.1 M, 7.4) prepared in NaCl 0.9%; using a porcelain mortar and pestle. Thereafter, centrifugation at 3000 g for 15 min was carried out and the filtrate was carefully collected using a micropipette. Brain homogenate collected was put in Eppendorf tubes and kept in the freezer at -20 ℃.

2.2.5. Biochemical Parameters

(i). Determination of Catalase Activity

Catalase activity was determined as described by [17]. The method is based on the reduction of dichromate prepared in acetic acid to chromic acetate when heated in the presence of hydrogen peroxide (H_2O_2) with the formation of perchloric acid as an unstable intermediate. The chromic acetate thus produced is measured colorimetrically at 570 nm.

(ii). Determination of Reduced Glutathione

Reduced glutathione (GSH) content was determined by the method described by [18]. GSH reacts with thiol reagent DTNB (5,5-dithiobis (2-nitrobenzoic acid)) to form GSSG and TNB yellow color chromophore (5-thionitrobenzoic acid), which has a maximal absorbance at 412 nm.

(iii). Determination of Malondialdehyde

Malondialdehyde (MDA) was determined according to the method described by [19]. Carbonylated compounds like MDA react with thiobarbuturic acid (TBA) to form pink chromophore compounds that have a maximum absorption at 532 nm. MDA is one of many end-products of lipid peroxidation. The test is based on the reaction of MDA with two molecules of TBA at high temperature (80-100 ℃) and low PH (pH 2).

(iv). Determination of Nitric Oxide

Nitric oxide was determined according to the method of [20]. Nitrite is reduced to nitrogen oxide using Griess Reagent I (sulphanilamide). Then, Nitrogen Oxide reacts with Griess

Reagent II (N-(1 Naphthyl) ethylene diamine dihydrochloride), forming a stable product that can be detected by its absorbance at 546 nm.

(v). Evaluation of Acetylcholinesterase Activity

Acetylcholinesterase (AChE) activity was determined according to the method of [21]. The substrate acetylthiocholine (ACh) is added to the sample containing the enzyme AChE. The reaction between ACh and AChE releases thiocholine (Ch) and acetic acid. The released thiocholine reacts with 5,5' dithiobisnitrobenzoate (DTNB) (Ellman reagent) to form a yellow chromatophore 5- thio 2- nitrobenzoic acid (TNB) compound that is highly absorbed at 412 nm.

(vi). Evaluation of GABA

The determination of gamma-aminobutyric acid (GABA) concentration in the biological samples was done calorimetrically according to the method of [22]. In basic conditions, the reaction between ninhydrin and gamma aminobutyric acid (GABA) gives rise to a purple colour, whose intensity is proportional to GABA concentration in the sample.

2.2.6. Histopathological Analysis

The brain was collected from each group of rats. It was then immerged in formaldehyde 10% (v/v) prepared in a saline solution 0.9%, and sent to the laboratory for analysis. Histopathological analysis of the brain was performed as described by [23]. The staining of brain tissue was done using a classic dye hematoxylin/eosin (HE) and then a specific dye red congo.

2.3. Statistical Analysis

All data were analyzed with SPSS 22.0. One-way analysis of variance (ANOVA) with Duncan test was used to compare the sample mean and to classify the treatments in case of significance ($P \leq 0.05$). A Post hoc analysis was also used to perform multiple comparisons between the means of two samples after One-ANOVA test was deemed significant. Minitab 20.0 was also used to establish the different mixtures of the mixture design.

2.4. Ethics Approval and Consent to Participate

All experiments carried out on animals in the course of this study were approved by the Animal house care of the University of Dschang (Cameroon); and were in accordance 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.

3. Results

3.1. Antioxidant Activity of the Fruits' Fractions

3.1.1. DPPH Scavenging Test

For the fruit *P. campechiana*, PCEA showed the lowest IC 50 (45.46 ±5.402 µg/ml), and for *L. microcarpa*, LMEA gave the lowest IC 50 (117.61 ±12.108 µg/ml) (Figure 1).

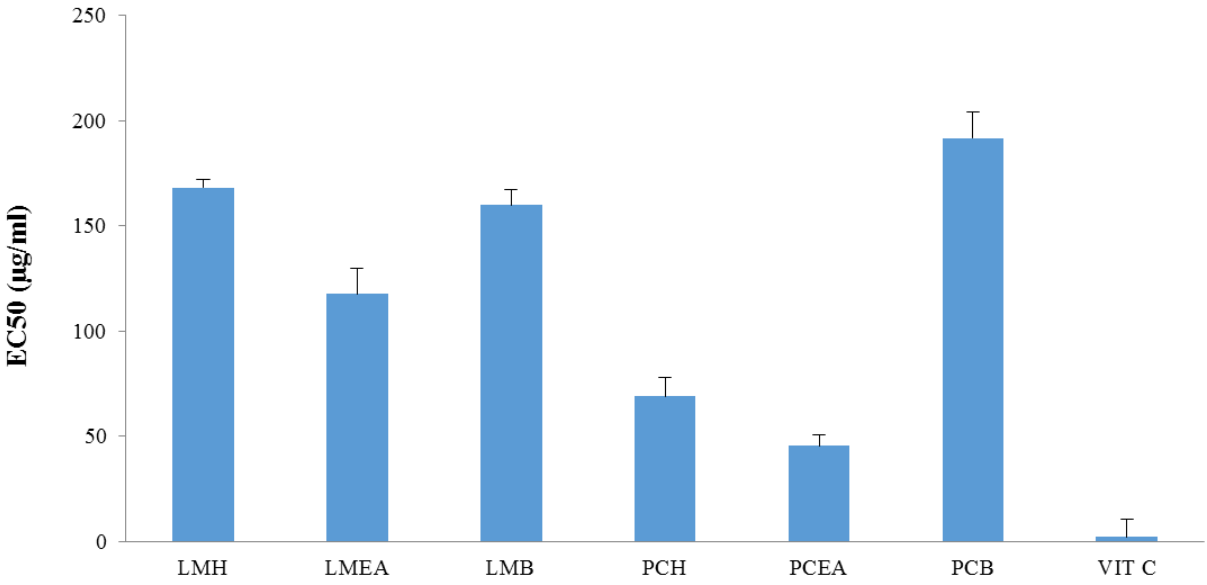

Figure 1. *Capacity of plants' fractions to scavenge DPPH radical.*

LMH: *Lannea microcarpa*'s hexanic fraction, LMEA: *Lannea microcarpa*'s ethyl acetate fraction, LMB: *Lannea microcarpa*'s butanolic fraction, PCH: *Pouteria campechiana*'s hexanic fraction, PCEA: *Pouteria campechiana*'s ethyl acetate fraction, PCB: *Pouteria campechiana*'s butanolic fraction, VIT C: Vitamin C.

3.1.2. Ferric Reducing Antioxidant Power (Frap) Assay

For the fruit *P. campechiana*, PCH showed the highest FRAP value (0.256 ± 0.057 µM Fe^{2+}), and for *L. microcarpa*, LMEA gave the highest IC 50 (0.164 ± 0.09 µM Fe^{2+}) (Figure 2).

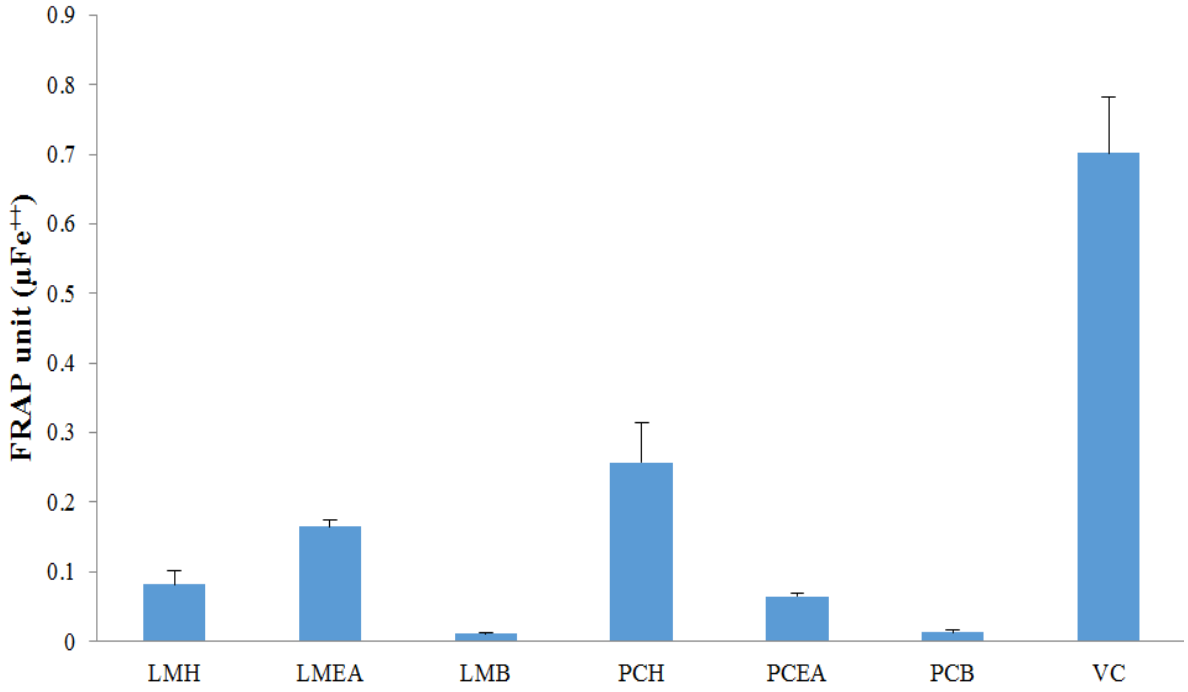

Figure 2. *Capacity of fruits' fractions to reduce ferric ion.*

LMH: *Lannea microcarpa*'s hexanic fraction, LMEA: *Lannea microcarpa*'s ethyl acetate fraction, LMB: *Lannea microcarpa*'s butanolic fraction, PCH: *Pouteria campechiana*'s hexanic fraction, PCEA: *Pouteria campechiana*'s ethyl acetate fraction, PCB: *Pouteria campechiana*'s butanolic fraction, VC: Vitamin C.

3.1.3. Presentation of the Score of Antioxidant Activity of the Different Fruits' Fractions

Two antioxidant tests were performed in order to choose the fruits' fractions to be used for *In vivo* tests. Overall, PCH showed the highest antioxidant activity for *P. campechiana's* fruit; and LMEA presented the highest activity for *L. microcarpa's* fruit. In this regard, LMEA and PCH were chosen to continue the study (Table 3).

Table 3. *Overview of antioxidant activities of the different fruits' fractions.*

	DPPH assay	FRAP assay
LMH	++	++++
LMEA	++++	+++++
LMB	+++	+
PCH	+++++	++++++
PCEA	++++++	+++
PCB	+	++

LMH: *Lannea microcarpa*'s hexanic fraction, LMEA: *Lannea microcarpa*'s ethyl acetate fraction, LMB: *Lannea microcarpa*'s butanolic fraction, PCH: *Pouteria campechiana*'s hexanic fraction, PCEA: *Pouteria campechiana*'s ethyl acetate fraction, PCB: *Pouteria campechiana*'s butanolic fraction.

3.2. Compounds Revealed by Gas Chromatography-mass Spectrometry

The chemical analysis of LMEA revealed 49 compounds. Among the major compounds contained in the fraction, were phenolic compounds like catechol, syringol, 2,4-Di-tert-butylphenol (2,4-DTBP), (Z)-3-(Heptadec-10-en-1-yl)phenol and Phenol, 3-(10Z)-10-nonadecen-1-yl; and the phytosterol gamma-sitosterol (Table 4).

PCH fraction revealed 59 compounds and the major compounds contained in the fraction included unsaturated fatty acids such as palmitoleic acid, cis-Vaccenic acid, oleic acid and linolenic acid; and triterpenoids like chondrillasterol, loliolide, α-amyrin and β-amyrin (Table 5).

Table 4. *Compounds of LMEA fraction revealed under GC-MS.*

No	Compound Name	Retention time (min)	Molecular weight	Area (%)
1	2-(Cyclohexylmethylidene)hydrazine-1-carbothioamide	4.0221	185.1	0.34
2	9-[2-Deoxy-.beta.-d-ribohexopyranosyl]purin-6(1H)-one	4.3426	282.1	0.40
3	Cyclododecane	4.7717	168.2	2.20
4	Catechol	4.8862	110	3.37
5	4-Vinylphenol	5.2123	120.1	0.98
6	1,2-Benzenediol, 3-methyl-	6.3453	124.1	0.96
7	Hydrocinnamic acid	6.9976	150.1	2.55
8	Phenol, 2,6-dimethoxy-	7.3409	154.1	3.35
9	1-Tetradecene	7.8616	196.2	3.42
10	beta.-D-Glucopyranose	9.0690	180.1	0.31
11	6-Benzyloxy-2,6-dimethyl-octa-2,7-dien-1-ol	9.2349	260.2	0.26
12	2,4-Di-tert-butylphenol	9.5611	206.2	2.95
13	Geranyl isovalerate	9.9216	238.2	0.12
14	Cetene	10.5624	224.3	3.96
15	Ethyl.alpha.-d-glucopyranoside	10.8027	208.1	1.57
16	Tetracyclo[4.4.1.1(7,10).0(2,5)]dodec-3-en-11-ol	11.0946	176.1	0.13
17	Malonodinitrile, 2-(5-dimethylaminopenta-2,4-dienylideno)-	11.2090	173.1	0.09
18	2,2-Diphenylacetamide, N-[2-(1H-indol-3-yl)-1-methylethyl]-	11.4265	368.2	0.21
19	Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)- 6-(1-methylethyl)-, [1R (1.alpha.,2.alpha.,3.beta.,6.alpha.)]-	12.1360	222.2	0.20
20	Tetradecanoic acid	12.5022	228.2	0.30
21	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a- tetrahydrobenzofuran-2(4H)-one	12.7368	196.1	0.27
22	1-Octadecene	12.8913	252.3	3.68
23	2,6-Dihydroxybenzaldehyde, carbamoylhydrazone	12.9600	195.1	0.16
24	E-10-Dodecen-1-ol propionate	13.2175	240.2	0.16
25	9-Eicosyne	13.4406	278.3	0.20
26	Phthalic acid, butyl 5-ethyl-1,3-dioxan-5-yl ester	13.8240	350.2	0.55
27	E-6-Octadecen-1-ol acetate	13.9499	310.3	0.16
28	Hexadecanoic acid, methyl ester	14.4649	270.3	0.71
29	n-Hexadecanoic acid	14.8711	256.2	1.82
30	1,4-Dibutyl benzene-1,4-dicarboxylate	14.9741	278.2	3.10
31	Hexadecanoic acid, ethyl ester	15.3003	284.3	4.81
32	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	16.6392	306.3	0.34

No	Compound Name	Retention time (min)	Molecular weight	Area (%)
33	Phytol	16.8910	296.3	0.22
34	cis-9-Tetradecenoic acid, isobutyl ester	17.2629	282.3	0.41
35	Linoleic acid ethyl ester	17.5605	308.3	0.98
36	9,12-Octadecadienoyl chloride, (Z,Z)-	17.6463	298.2	0.85
37	Methyl 7,9-tridecadienyl ether	17.9725	210.2	1.87
38	Butyl citrate	19.0139	360.2	0.24
39	3-Chloropropionic acid, heptadecyl ester	20.8049	346.3	0.80
40	4-(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-butan-2-one	21.9150	208.2	0.19
41	Glycerol 1-palmitate	22.4299	330.3	0.40
42	Bis (2-ethylhexyl) phthalate	23.0365	390.3	8.40
43	E-2-Methyl-3-tetradecen-1-ol acetate	23.6602	268.2	0.44
44	(Z)-3-(Heptadec-10-en-1-yl)phenol	25.0621	330.3	2.00
45	Phenol, 3-(10Z)-10-nonadecen-1-yl-	27.9059	358.3	5.11
46	Stigmasta-3,5-diene	30.6125	396.4	1.42
47	Campesterol	32.9757	400.4	1.77
48	Stigmasterol	33.6509	412.4	1.89
49	.gamma.-Sitosterol	34.9440	414.4	29.39

Table 5. *Compounds of PCH revealed under GC-MS.*

No	Compound Name	Retention time (min)	Molecular weight	Area (%)
1	trans-2,3-Epoxydecane	7.0262	156.2	0.07
2	n-Decanoic acid	7.4153	172.1	0.11
3	d-Mannitol, 1,4-anhydro-	8.2793	164.1	0.22
4	Cyclododecane	9.0232	168.2	0.12
5	Dodecanoic acid	10.1676	200.2	0.26
6	Cetene	10.5624	224.3	0.05
7	1-Tetradecanol	11.5809	214.2	0.06
8	13-Tetradecenal	11.8556	210.2	0.18
9	Heptadecanal	12.0558	254.3	0.10
10	Tetradecanoic acid	12.5594	228.2	1.63
11	Loliolide	12.7310	196.1	0.14
12	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	13.8297	278.2	0.07
13	Hexadecanoic acid, methyl este	14.4591	270.3	0.32
14	Palmitoleic acid	14.6823	254.2	2.22
15	n-Hexadecanoic acid	15.0199	256.2	8.84
16	Hexadecanoic acid, ethyl ester	15.3117	284.3	0.58
17	Isopropyl palmitate	15.7065	298.3	0.05

No	Compound Name	Retention time (min)	Molecular weight	Area (%)
18	8,11-Octadecadienoic acid, methyl ester	16.6506	294.3	0.06
19	9-Octadecenoic acid, methyl ester, (E)-	16.7307	296.3	0.37
20	cis-Vaccenic acid	17.4002	282.3	14.70
21	Ethyl Oleate	17.6520	310.3	1.36
22	E-15-Heptadecenal	17.9839	252.2	0.26
23	Heptadecane, 9-hexyl-	19.4544	324.4	0.27
24	Oxirane, heptadecyl-	19.8664	282.3	0.21
25	i-Propyl 9-octadecenoate (Z)	19.9236	324.3	0.09
26	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	19.9694	292.2	0.16
27	1-Hexadecanol, 2-methyl-	20.8106	256.3	0.20
28	Tetracosane	20.8792	338.4	0.07
29	1,21-Docosadiene	21.3141	306.3	0.16
30	9-Octadecenoic acid (Z)-, oxiranylmethyl ester	21.9950	338.3	0.09
31	7Z,10Z,13Z,16Z,19Z-Docosapentaenoic acid	22.0408	330.3	0.17
32	cis-9-Hexadecenoic acid, heptyl ester	22.1839	352.3	0.31
33	Pentacosane	22.3097	352.4	0.32
34	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	22.4642	330.3	1.93
35	Oxirane, hexadecyl-	22.7732	268.3	0.44
36	2,6,10-trimethylundecanoic Acid, 2,2,2- trifluoroethyl ester	22.9277	310.2	0.10
37	4-Chlorobutyric acid, eicosyl ester	23.6487	402.3	0.15
38	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-	24.0550	170.1	0.47
39	17-Pentatriacontene	24.2037	490.5	0.11
40	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	25.0392	356.3	2.01
41	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	25.1135	352.3	2.63
42	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	25.2509	356.3	0.29
43	Octadecanoic acid, 2,3-dihydroxypropyl ester	25.3539	358.3	0.36
44	Octacosanol	26.4296	410.4	0.09
45	1-Benzoxirene, 5a-[3-oxo-1-butenyl]perhydro-2- hydroxy-1a,5,5-trimethyl-, acetate	26.4926	266.2	0.16
46	4-(2-Methyl-cyclohex-1-enyl)-but-3-en-2-one	26.6528	164.1	0.31
47	Squalene	26.9160	410.4	1.78
48	Nonacosane	27.8258	408.5	1.42
49	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7- oxabicyclo[4.1.0]hept-1-yl)-	29.2735	224.1	0.17
50	Loliolide acetate	30.5781	238.1	4.99
51	Hentriacontane	30.6639	436.5	0.30
52	Vitamin E	31.3162	430.4	1.06
53	Chondrillasterol	34. 9211	412.4	3.65
54	.beta.-Amyrin	35.7108	426.4	1.94

No	Compound Name	Retention time (min)	Molecular weight	Area (%)
55	Stigmast-7-en-3-ol, (3.beta.,5.alpha.)-	36.3345	414.4	1.08
56	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	36.8151	426.4	0.37
57	12-Oleanen-3-yl acetate, (3.alpha.)-	38.8579	468.4	37.64
58	Lup-20(29)-en-3-ol, acetate, (3.beta.)-	39.9966	468.4	1.83
59	Lanosta-8,24-dien-3-ol, acetate, (3.beta.)-	40.1511	468.4	0.90

3.3. Different Responses to the Formulation of the Nutraceutical

3.3.1. Responses of Antioxidant Activities

(i). DPPH Scavenging Test

Among all the mixtures, the mixture LMEA/PCH 3 (62.5/37.5) gave the best response characterized by the lowest IC 50 (20 ± 2.754 µg/ml) (Figure 3).

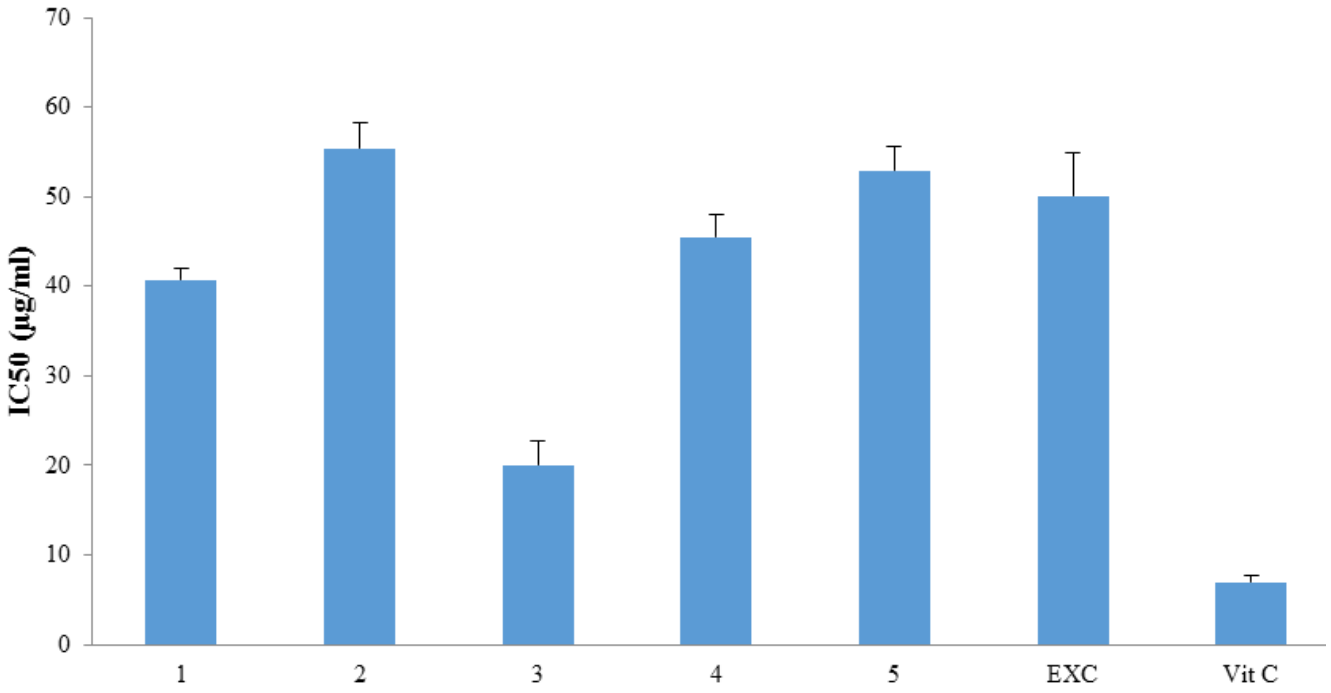

Figure 3. *Capacity of the different mixtures to scavenge DPPH radical.*

1: LMEA/PCH (47.5/52.5), 2: LMEA/PCH (55/45), 3: LMEA/PCH (62.5/37.5), 4: LMEA/PCH (40/60), 5: LMEA/PCH (70/30), EXC: excipient, VIT C: Vitamin C, LMEA: *Lannea microcarpa*'s ethyl acetate fraction, PCH: *Pouteria campechiana*'s hexanic fraction.

(ii). Ferric Reducing Antioxidant Power (Frap) Assay

The mixture LMEA/PCH 3 gave the highest response among the mixtures shown by the highest capacity to reduce the ferric ion (0.715 ±0.028 µM Fe^{2+}). This activity was close to that of vitamin C used as standard (0.788 ±0.023 µM Fe^{2+}) (Figure 4).

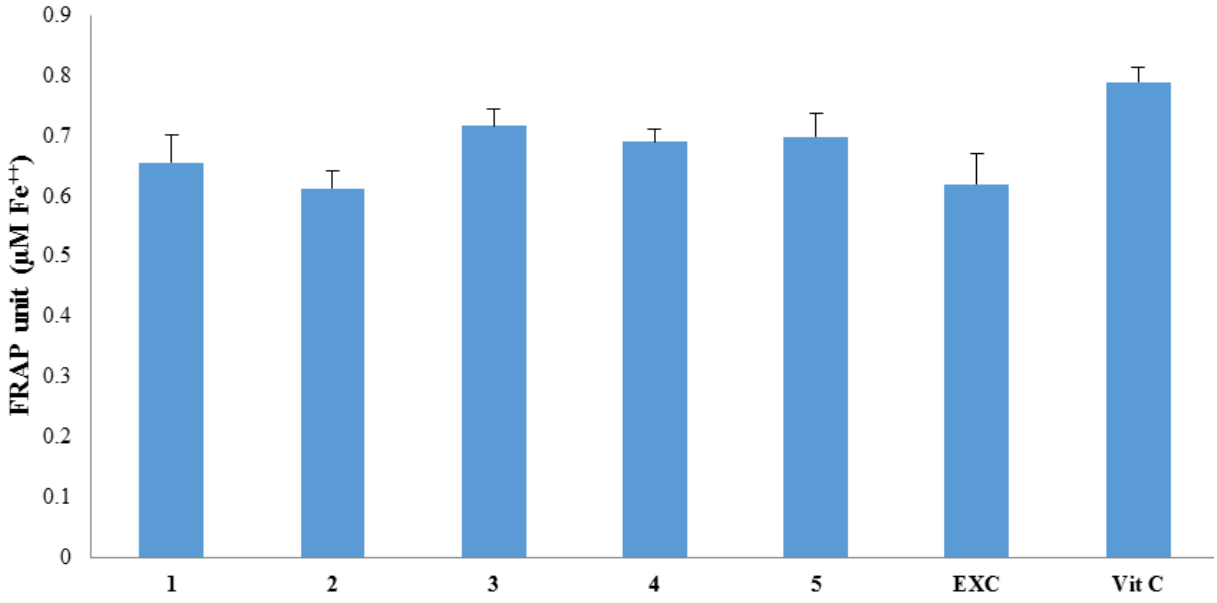

Figure 4. *Capacity of plants' extracts to reduce ferric ion.*

1: LMEA/PCH (47.5/52.5), 2: LMEA/PCH (55/45), 3: LMEA/PCH (62.5/37.5), 4: LMEA/PCH (40/60), 5: LMEA/PCH (70/30), EXC: excipient, Vit C: Vitamin C, LMEA: *Lannea microcarpa*'s ethyl acetate fraction, PCH: *Pouteria campechiana*'s hexanic fraction.

3.3.2. Presentation of the Score of Antioxidant Activity of the Different Mixtures

Two antioxidant tests were performed in order to choose the best mixture to make the nutraceutical for *In vivo* tests. Overall, the mixture 3 gave the best response by displaying the highest antioxidant activity (Table 6).

Table 6. *Overview of antioxidant activities of the different mixtures.*

Mixtures	DPPH assay	FRAP assay
1	++++	++
2	+	+
3	+++++	+++++
4	+++	+++
5	++	++++

1: LMEA/PCH (47.5/52.5), 2: LMEA/PCH (55/45), 3: LMEA/PCH (62.5/37.5), 4: LMEA/PCH (40/60), 5: LMEA/PCH (70/30), LMEA: *Lannea microcarpa*'s ethyl acetate fraction, PCH: *Pouteria campechiana*'s hexanic fraction.

3.4. Effect of the Treatment on the Animals' Behaviour

3.4.1. In the Morris Water Maze

The time to find the platform varied from one group to another. Among the groups treated with the fruits, the lowest escape latency was observed in the group that was administered the nutraceutical (trial 1: 14 ±1.87 s, trial 2: 13.4 ±3.91 s, trial 3: 13.2 ±2.58 s) (Table 7).

Table 7. *Effect of the treatment on the escape latency in the MWM.*

	TRIAL 1 (s)	TRIAL 2 (s)	TRIAL 3 (s)
CG	57.6 ±4.66	19.2 ±7.72	21.6 ±1.51
DPZ	59.25 ±5.25	18 ±7.07	32.25 ±8.99
NC	29.8 ±4.96	35.4 ±4.72	57.4 ±4.61
LMF	52 ±8.83	60.2 ±1.78	25.8 ±6.9
PCF	11.75 ±4.03	26.5 ±5.74	22.25 ±4.57
LM/PC	14 ±1.87	13.4 ±3.91	13.2 ±2.58
EXC	40.66 ±5.13	35.66 ±6.65	55 ±4.58

CG: normal control that received only a saline water (0.9%); DPZ: group that received the standard drug donepezil, NC: negative group that was administered only $AlCl_3$; LMF: group that received *L. microcarpa*'s fruit fraction at 50 mg/kg bw, PCF: group that received *P. campechiana*'s fruit fraction at 50 mg/kg bw, LM/PC: group that received the nutraceutical at 50 mg/kg bw, EXC: group that received the excipient (paraffin oil) at 5 ml/kg.

The percent time spent in the quadrant containing the platform was also evaluated. The findings showed that among the groups treated with fruits, the group treated with the nutraceutical showed the highest percent time (42.85%).

However the group treated with the excipient presented the lowest (4.84%) (Table 8).

Table 8. *Effect of the treatment on the time spent in the quadrant containing the platform in the MWM.*

	TRIAL 1 (s)	TRIAL 2 (s)	TRIAL 3 (s)
CG	7.29	29.16	26.85
DPZ	10.12	47.22	29.45
NC	22.81	19.20	8.01
LMF	8.84	3.65	30.23
PCF	21.27	26.41	15.73
LM/PC	42.85	41.79	36.36
EXC	16.39	19.62	4.84

CG: normal control that received only a saline water (0.9%); DPZ: group that received the standard drug donepezil, NC: negative group that was administered only $AlCl_3$; LMF: group that received *L. microcarpa*'s fruit fraction at 50 mg/kg bw, PCF: group that received *P. campechiana*'s fruit fraction at 50 mg/kg bw, LM/PC: group that received the nutraceutical at 50 mg/kg bw, EXC: group that received the excipient (paraffin oil) at 5 ml/kg.

3.4.2. In the Open Field

The general trend showed that the group that received DPZ spent the highest time in the central square of the open field (10 ± 2.49 s); the normal control group presented the highest tendency to escape the field (1.25 ± 0.5); and the non-treated group presented the highest number of floor crossings (20.25 ± 3.86) compared to the other groups (Table 9).

Table 9. *Effect of the treatment on the locomotory activity in the Open field.*

	TSCS	NTE	NFC
CG	1.25	9	14.25
DPZ	10	4.25	16
NC	1.75	5	20.25
LMF	3.25	8.25	8
PCF	4.75	4.25	8.25
LM/PC	3.75	8.25	11.75
EXC	4	6.75	11.75

TSCS: time spent in the central square, NTE: number of trials of escaping the field, NFC: number of floor crossings, CG: normal control that received only a saline water (0.9%); DPZ: group that received the standard drug donepezil, NC: negative group that was administered only $AlCl_3$; LMF: group that received *L. microcarpa*'s fruit fraction at 50 mg/kg bw, PCF: group that received *P. campechiana*'s fruit fraction at 50 mg/kg bw, LM/PC: group that received the nutraceutical at 50 mg/kg bw, EXC: group that received the excipient (paraffin oil) at 5 ml/kg.

3.5. Effect of Treatment on Biochemical Parameters

The non-treated group showed the lowest catalase activity (6.37 ± 0.52 µM H_2O_2/min/mg of protein) and the group that was administered PCH the highest activity (8.63 ± 1.00 µM H_2O_2/min/mg of protein). However, the group that received the nutraceutical showed an activity close to that of PCH compared to the other groups. The group treated with the nutraceutical, LMF and PCF had the lowest MDA level ((1.96 ± 0.09) × 10^{-6} µM), and the non-treated group showed the highest MDA level ((4.07 ± 0.22) × 10^{-6} µM). The groups treated with the excipient, LMEA and the non-treated group showed the lowest GSH concentration (1.47 ± 0.00 mol/mg proteins), whereas the group treated with the nutraceutical had the highest GSH level (2.20 ± 0.00 mol/mg proteins). The nutraceutical reduced the level of NO (9.55 ± 0.29 µM) compared to all the other groups. The group that was given the standard drug DPZ had the lowest AChE activity ((2.10 ± 0.12) × 10^{-6} µM/min/mg protein) and the group that received the excipient and the non-treated group the highest activity ((8.55 ± 0.73) × 10^{-6} µM/min/mg protein). GABA concentration significantly varied between the different groups. The normal control and the non-treated groups showed the lowest GABA content (28.21 ± 0.45 µg/ml) and the group treated LMEA the highest content (37.57 ± 1.89 µg/ml) (Table 10).

Table 10. *Effect of the treatment on biochemical parameters.*

BRAIN	AChE (10^{-6} µM/min/mg protein)	GABA (µg/ml)	Catalase (µMH_2O_2/min/mg proteins)	MDA (µM)	Glutathione (mol/mg proteins)	NO (µM)
CG	4.00 ± 0.15^{b}	28.21 ± 0.45^{a}	8.12 ± 1.06^{cd}	2.02 ± 0.02^{a}	1.96 ± 0.21^{c}	12.25 ± 0.11^{d}
DPZ	2.10 ± 0.12^{a}	29.85 ± 1.57^{b}	6.29 ± 0.74^{a}	2.04 ± 0.08^{a}	1.71 ± 0.21^{b}	12.36 ± 0.09^{e}

BRAIN	AChE (10^{-6} µM/min/mg protein)	GABA (µg/ml)	Catalase (µMH_2O_2/min/mg proteins)	MDA (µM)	Glutathione (mol/mg proteins)	NO (µM)
NC	8.07 ±1.96^{de}	28.25 ±1.20^{a}	6.37 ±0.52^{a}	4.07 ±0.22^{c}	1.47 ±0.00^{a}	12.06 ±0.33^{d}
LMF	5.30 ±0.07^{c}	37.57 ±1.89^{e}	7.58 ±0.52^{bc}	1.96 ±0.09^{a}	1.47 ±0.00^{a}	11.26 ±0.34^{c}
PCF	4.49 ±0.25^{bc}	31.58 ±0.04^{c}	8.63 ±1.00^{d}	1.97 ±0.15^{a}	1.54 ±0.12^{ab}	11.06 ±0.72^{c}
LM/PC	4.51 ±0.26^{bc}	36.23 ±1.83^{d}	7.81 ±0.31^{bc}	1.96 ±0.09^{a}	2.20 ±0.00^{d}	9.55 ±0.29^{a}
EXC	8.55 ±0.73^{e}	29.79 ±2.05^{ab}	7.08 ±0.58^{ab}	2.50 ±0.11^{b}	1.37 ±0.08^{a}	10.17 ±0.11^{b}

CG: normal control that received only a saline water (0.9%); DPZ: group that received the standard drug donepezil, NC: negative group that was administered only $AlCl_3$; LMF: group that received *L. microcarpa*'s fruit fraction at 50 mg/kg bw, PCF: group that received *P. campechiana*'s fruit fraction at 50 mg/kg bw, LM/PC: group that received the nutraceutical at 50 mg/kg bw, EXC: group that received the excipient (paraffin oil) at 5ml/kg.

3.6. Histopathological Analysis

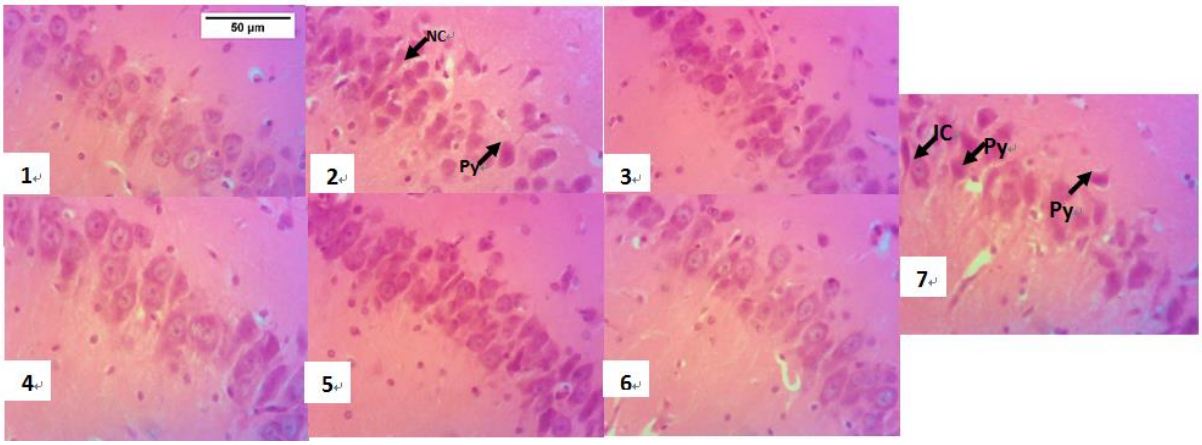

Figure 5. *Microphotographs of the Ammon's Horn 2 (X250) of the hippocampus stained with hematoxylin-eosin (HE).*

1 = Normal control group; 2 = Non-treated group; 3 = Positive control; 4 = Group treated with the fruit powder; 5 = Group treated with LMEA fraction; 6 = Group treated with PCH fraction; 7 = Group treated with the excipient; PC = Pyramidal cells; Py = Pyknosis; NC = Neuron cytolysis; IC = Impaired cell.

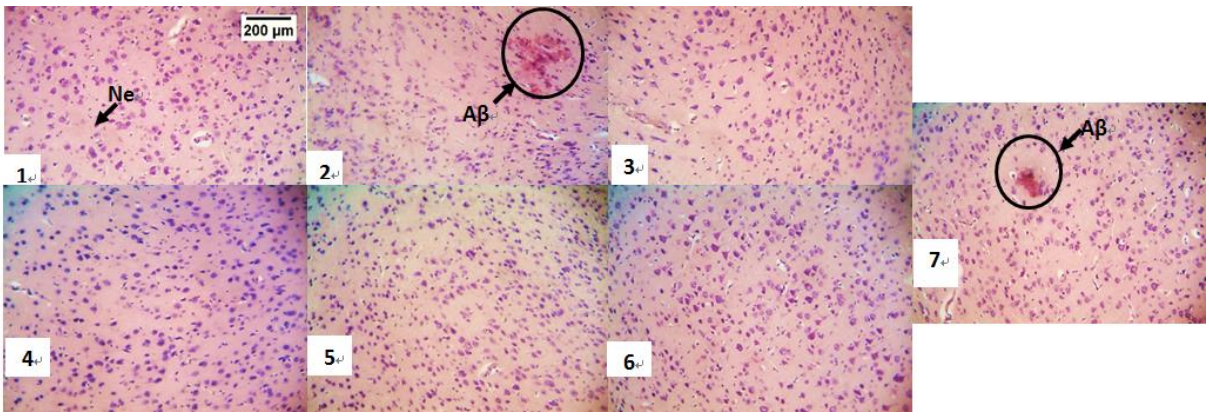

Figure 6. *Microphotographs of the brain (X100) stained with red congo.*

1 = Normal control group; 2 = Non-treated group; 3 = Positive control; 4 = Group treated with the fruit powder; 5 = Group treated with LMEA fraction; 6 = Group treated with PCH fraction; 7 = Group treated with the excipient; Ne = Neuron; Aβ = Amyloid β plaque.

4. Discussion

Catechol, 2,4-Di-tert-butylphenol (2,4-DTBP) present in LMEA are phenolic compounds generally found in vegetables and fruits, which have remarkable antioxidant properties [24, 25]. LMEA also revealed sitosterol which is a phytosterol with neuroprotective activity [26]. The fruits of the genus *Lannea* have previously been reported to harbor bioactive compounds such as PUFAs, phytposterols, prenylated flavonoids, and flavonoids [27, 28] which tie with some compounds detected in LMEA. GC-MS also revealed the presence of PUFAs and phytosterols in PCH. β-Amyrin is a triterpenoid with antioxidant and neuroprotective activities [29, 30]. This confers PCF a lot of interesting properties that can be investigated in many biological mechanisms including antioxidant mechanisms to maintain the redox balance and cognitive processes.

As reported by [31], the antioxidant activity of scavenging DPPH radical is considered high (IC50 < 20 µg/ml), moderate (20 < IC50 < 75 µg/ml) or low (IC50 > 75 µg/ml). This therefore means that PCEA which gave the highest activity upon DPPH scavenging test had a moderate activity, and LMEA a low activity. However, it was observed that mixing the fractions LMEA and PCH at a proportion of 62.5/37.5 gave a more important activity than that of each fraction tested singly. The mixture LMEA/PCH 62.5/37.5 gave the highest activity to scavenge DPPH radical and that activity was high according to [31]. Likewise, the activity to reduce ferric ion increased when the fractions were mixed and the highest activity was shown by the mixture LMEA/PCH (62.5: 37.5). This could be explained by the presence of triterpenoids, PUFAs and phenolic compounds in the mixture as well as the synergistic effect they could have. Phenolic compounds possess hydroxyl groups which are involved in protons and electrons transfer to reduce oxidant compounds or free radicals [32].

The spatial and learning memories were assessed in the Morris water maze. The group treated with the nutraceutical displayed the lowest escape latency (EL) and the highest time in the quadrant containing the platform. In the MWM, when searching the platform, the animal uses some signals like cues in the water maze that help it to recognize the quadrant that contains the platform. This memory develops with time as a long-term memory as the animal is constantly trained, and informs on the state of the Ammon's Horn 2 involved in the memory process [33]. The more the animal stays in the quadrant containing the platform, the more the spatial memory develops. Loliolide a triterpenoid contained in the nutraceutical has been reported to enhance neuroprotection by maintaining mitochondrial integrity, reducing cell apoptosis and inhibiting NF-Kb pathway [34].

The open field test is used to test brain regions to know whether they function well or not [35]. The group treated with the nutraceutical was more or less anxious regarding the highest tendency of escaping the open field they showed. An elevated number of floor crossings was also noted in the non-treated group. However, Anxiety becomes more pronounced in old rats and administering them Al may augment the level of anxiety [36].

Catalase is a widely distributed antioxidant enzyme present in all aerobic organisms for antioxidant defense mechanism. The nutraceutical highly increased catalase activity. Catechol and phytosterols contained in the nutraceutical especially gamma-sitosterol and campesterol, might have acted using their conjugated bonds and hydroxyl groups to enhance catalase production in the brain [37]. Catalase contains four iron-containing heme groups that allow it to react with the hydrogen peroxide. Deficiency in catalase is postulated to be related to redox stress. Curcumin a polyphenolic compound triggered the increase of catalase activity to reduce the incidence of oxidative stress in breast cancer [38].

The brain is a vital organ made up of PUFAs. A significant increase in MDA in the non-treated group could be due to the reaction of Al with fatty acids. Al may promote ROS production through Fenton reactions, where Al converts anion superoxide ($O^{2-\bullet}$) and hydrogen peroxide (H_2O_2) to hydroxyl radical ($HO^{\bullet}$) species, destabilizing conjugated bonds; leading to lipid peroxidation [39] with release of final oxidative products such as carbonyls, peroxynitrites, and MDA within neurons [40]. However, treating animals with the nutraceutical has significantly lowered MDA production, showing that the nutraceutical may contain bioactive compounds that prevent lipid peroxidation in living tissues. Oxidative stress is considered as one of the major causes of neurodegenerative diseases, by generating lipid peroxides. In AD dementia, these final products of lipid peroxidation enhance the activation of amyloid precursor protein (APP) with as consequence an overproduction of amyloid-β protein; the extracellular hallmarker involved in AD's pathogenesis [41]. Likewise, [42] reported a decrease in MDA when assessing *Anacardium Occidentale*'s fruit extract on the brain of a model of AD's rat. An increase of lipid peroxidation was also highlighted in patients with temporal progression of chronic spinal cord injury [43]. The nutraceutical formulated in this study contains a lot of bioactive components including phenols and triterpenoids. They both carry hydroxyl groups and conjugated bonds, which render them excellent electron donors to balance redox reactions, thus preventing lipid peroxidation. One might opine that stigmasterol, a phytosterol that easily crosses the BBB, could have triggered antioxidant mechanisms in the brain to hinder MDA production.

The conversion of H_2O_2 to water requires a peroxidase which works with glutathione. Reduced glutathione (GSH) serves as an antioxidant in living organisms. The group treated with the nutraceutical showed an increase in GSH. Catechol is a phenolic compound with antioxidant properties. It promotes antioxidant defense mechanisms through genes' expression of antioxidant enzymes like catalase and glutathione peroxidase. This latter needs GSH to perform its antioxidant activity [44]. The high GSH concentration in the group that was given the nutraceutical shows that the

nutraceutical certainly contains compounds that can enhance GSH production to counteract ROS and RNS formation.

In this study, it could be stated that the reduction of nitric oxide (NO) in the group that received the nutraceutical is brought about by bioactive compounds contained in the nutraceutical. Campesterol is a phytosterol that possesses the capacity to scavenge free radicals, hence to reduce the production of NO in the brain. It may activate many signaling pathways involved in NO production. It is strongly believed that campesterol reduces S-nitrosylation status of cytoskeletal proteins α- and β-spectrin which is a post-translational process involved in NO genesis [45].

Acetylcholine (Ach) is an important neurotransmitter involved in memory process. The lower activity of AChE observed in the group that was administered the nutraceutical compared to the non-treated group, showed that the nutraceutical obviously lowered AChE activity to prolong nerve impulse transmission. This anti-cholinesterasic activity might be attributed to stigmasterol and sitosterol. Stigmasterol has been reported to counteract neurodegeneration by promoting neurogenesis and cholinergic transmission. This could be done by the activation of SIRT 1 gene to promote antioxidant defense mechanisms and impulse transmission as well [5]. Besides, catechol is a phenolic compound that has been reported a powerful antioxidant by its conjugated bonds and its hydroxyl functional group. Like other phenolic compounds such as catechin, gallic acid and reverastrol, catechol may hamper production of ROS and RNS in the brain to enable acetylcholine synthesis and cholinergic transmission [4].

GABA is the most abundant inhibitory neurotransmitter in the central nervous system that acts by activating ion channels during action potential propagation through the synaptic cleft [46]. Highest level of GABA in the group that was administered the nutraceutical could be due to bioactive compounds that could have triggered GABA synthesis. Subsequently to this, GABA might have brought about inhibition of the membrane excitability. This mechanism maintains K^+ channels open to enable efflux of K^+, chloride channels open to enable influx of chloride ions during depolarization or hyperpolarisation, and Ca^{++} channels close to keep the membrane non excitable during the refractory period [47]. However, the lowest GABA level shown by the non-treated group could probably be due to the neurotoxic effect of Al which triggers senile plaques formation in the brain. Many studies reported reduction of GABA in the brain of AD's patients [48].

Features such as brain cell pyknosis, neuron loss, neuron cytolysis, and impaired cell displayed by the microphotograph of the brain Ammon's horn 2 of the group non-treated and that received the excipient, showed that exposing animals to a heavy metal like Al triggers OS that brings about brain cell damage and death [40]. ROS target molecules such as proteins, lipids and nucleic acids which are denatured, leading to cell rupture or impairment. Besides, in those groups, antioxidant enzymes production was low to hinder the deleterious effect of ROS. Nevertheless, treating rodents with the nutraceutical increased levels of antioxidant enzymes and compounds which counteracted ROS production to protect the brain against damage. The Ammon's horn 2 is a region of the hippocampus that possesses anatomical connectivity and signaling molecules involved in cognition, particularly in the spatial, learning, short and long term memory formation [49]. Senile amyloid β plaques displayed by the red congo stain in the negative control and the group treated with the excipient could be due to the amyloidogenic efect of Al that once accumulated in the brain cortex, may denature proteins and enzymes in the brain to promote the activity of amyloid precursor protein (APP), leading to amyloid β protein synthesis and its accumulation in the extracellular space. Al may also disrupt amyloid β protein metabolism by inhibiting its catabolism, thus enhancing its assemblage into senile amyloid β plaques [50]. This activity could be related to triterpenoids. Previously published works have shown the anti-amyloidogenic property of terpenoids [51].

5. Conclusion

In summary, the phytochemical composition of *Lannea microcarpa*'s ethyl acetate and *Pouteria campechiana*'s hexanic fractions that made up the nutraceutical revealed the presence of phenolic compounds, phytosterols (stigmasterol, γ-sitosterol and campesterol), triterpenoids (loliolide, β-amyin), and unsaturated fatty acids; that can be exploited as antioxidant enhancers. Treating animals with the nutraceutical improved spatial and learning memories, augmented antioxidant defense mechanisms by an increase of catalase, superoxide dismutase, reduced glutathione and reduction of malondialdehyde and nitric oxide production, lowered acetylcholinesterase activity, and Alzheimer's disease hallmarkers in the hippocampus. The anti-oxidant, anti-amyloidogenic and anti-cholinesterasic actvities the nutraceutical showed place *Lannea microcarpa*'s and *Pouteria campechiana*'s fruits as potential sources to exploit for search of therapeutics against Alzheimer's disease.

Abbreviations

Ach	Acetylthiocholine
AChE	Acetylcholinesterase
AChEIs	Acetylcholinesterase Inhibitors
AD	Alzheimer's Disease
AH	Ammon's Horn
APP	Amyloid Precursor Protein
BBB	Blood Brain Barrier
DPZ	Donepezil
EL	Escape latency
FRAP	Ferric Reducing Antioxidant Power
GABA	gamma-aminobutyric Acid
GC-MS	Gas Chromatography-Mass Spectrometry
GSH	Reduced Glutathione
HE	Hematoxylin/Eosin

LMEA	*Lannea microcarpa*'s Ethyl Acetate Fraction
MDA	Malondialdehyde
MWM	Morris Water Maze
OFT	Open Field Test
OS	Oxidative stress
PCH	*Pouteria campechiana*'s Hexanic Fraction
PUFAs	Polyunsaturated Fatty Acids
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RT	Retention Time
TBA	Thiobarbuturic Acid

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Author Contributions

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Data will be available by authors on request.

Conflicts of Interest

All contributors declare that they have no competing interests in this study.

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