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Acetylcholinesterase inhibitory, anti-inflammatory and antioxidant properties of some Cameroonian medicinal plants used to treat some neurological disorders

Viviane L.N. Ngoungoure[1], Emmanuel Mfotie Njoya[2], Simon Ngamli Fewou[3], Armand Fils Ella[1], Lyndy J. McGaw[2], and Paul F. Moundipa[1].

Abstract

Background: Besides the principal hallmark of Alzheimer's disease (AD) and Parkinson's disease (PD) which is accumulation of mis-folding protein in the brain; cholinergic deficit, neuro-inflammation and oxidative stress are also implicated in the pathogenesis of the above-mentioned diseases. In the present study, different plant parts were collected in an ethnopharmacological survey carried out in the Noun Division (West Region, Cameroon).

Methods: A total of 29 methylene chloride-methanol (1:1; v/v) extracts were prepared and screened for their acetylcholinesterase inhibitory activity, anti-inflammatory activity and antioxidant activity. Total phenolic and flavonoid contents were also quantified.

Results: The extract of *Crinum purpurascens* had strong activity against acetylcholinesterase with IC50 value of 99.40 µg/mL. Most of the extracts tested inhibited nitric oxide (NO) production in a dose dependent manner on LPS-stimulated RAW 264.7 macrophage cells. The extract from *C. purpurascens* had strong activity against NO production (IC50 of 12.8 µg/mL). The extract of *Annona muricata* seeds had the highest 15-lipoxygenase inhibitory activity with IC50 of 30.43 µg/mL. In addition, most of the extracts had good antioxidant potential, particularly the extracts of *Annona senegalensis*, *Dacryodes edulis* and *Cola acuminata*. High levels of total phenolic content were found in A. senegalensis and C. acuminata.

Conclusion: The traditional use of these medicinal plants for the treatment of neurological disorders was established through this result. Further experiments for the discovery of the components in the most active extract (*C. purpurascens*) are underway.

Keywords: Medicinal plants; Bamoun; anticholinesterase; inflammation; antioxidant; neurological disorders.

*Correspondence: Tel: (+237) 677310383. E-mail: pmoundipa@hotmail.com; ORCID: https://orcid.org/0000-0002-2694-5569 (Paul F. Moundipa)
Other authors ORCID: https://orcid.org/0000-0003-1163-7202 (Emmanuel Mfotie Njoya); https://orcid.org/0000-0001-8447-0613 (Lyndy J. McGaw)
[1]Laboratory of Pharmacology and Toxicology, Department of Biochemistry, Faculty of Science, University of Yaoundé I, 812 Yaoundé, Cameroon; [2]Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, Pretoria 0110, South Africa; [3]Faculty of Health Sciences, Université des Montagnes, Bangangté, Cameroon.

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Background

Medicinal plants have been used for the development of new drugs and continue to play an invaluable role in the progress of drug discovery. Several medicinal plants are of particular interest in several parts of Africa and therefore they are strongly qualified for drug development [1]. In Africa, medicinal plants are used in traditional practices to treat neurological diseases [2, 3]. Neurodegenerative disease, such as Alzheimer's disease (AD) or Parkinson's disease (PD), is an umbrella term for a range of conditions primarily affecting neurons in the human brain. Besides the principal hallmark of these diseases which is the accumulation of protein in some regions of the brain; cholinergic deficit, neuro-inflammation and oxidative stress are also implicated in their pathogenesis [4, 5]. In addition to AD, acetylcholine esterase (AChE) inhibition is also considered a promising therapeutic strategy for other types of dementia and Parkinson's disease [6]. Current medications for the treatment of AD or PD include AChE inhibitors for mild to moderate cases [6-9]. These acetylcholinesterase inhibitors are donepezil, rivastigmine and galanthamine. Unfortunately, these drugs have been reported to have adverse effects including gastrointestinal disturbances, hepatotoxicity, nausea, vomiting, diarrhea, dizziness, syncope and bradycardia [10, 11]. In order to prevent or to limit the progression of disease, most pharmacological research has focused on acetylcholinesterase (AChE) inhibitors with fewer side effects to alleviate cholinergic deficit and improve neurotransmission [12].

Emerging evidence indicates the role of inflammation in the pathogenesis of neurodegenerative diseases including AD and PD. Neuro-inflammatory processes may contribute to the cascade of events leading to the progressive neuronal damage observed in ageing and age-related cognitive disorders such as PD and AD [13]. During inflammation, many pro-inflammatory mediators including nitric oxide (NO) production and the activation of enzymes such as lipoxygenases (LOX) are increased. In neurodegenerative diseases, a high level of NO is released from nerve cells and this causes extensive damage to surrounding tissues, including neurons. In addition, increased activities of lipoxygenases under pathological situations produce eicosanoids that elicit cytotoxic inflammatory responses. Oxidative stress plays a crucial role in the pathogenesis of many ailments including neurodegenerative diseases such as AD and PD [14-16]. The production of free radicals during metabolism in cells can be deleterious when there is some imbalance between the production and elimination. It has been reported that during aging or brain injury, this production becomes very high and contributes to the modification of biological molecules such as nucleic acids, proteins, lipids and carbohydrates [17]. Therefore, medicinal plants with high phenolic contents and antioxidant activities may have great relevance in the prevention and therapy of neurodegenerative diseases such as AD and PD [18-20]. Taking into account these different structural and pathological conditions that characterize neurodegenerative diseases; a variety of targets and more efficient methods are required for their treatment.

In Cameroon, there is a rich tradition in the use of herbal medicine for the treatment of several ailments [21]. The present investigation was conducted to study *in vitro* anticholinesterase, anti-inflammatory and antioxidant activities of some medicinal plants, which can lead to the discovery of new and promising sources of potential drugs against neurodegenerative diseases.

Methods

Survey and plant collection

The ethnopharmacological survey was carried out in the Noun Division, located in the West region of Cameroon. A semi-structured questionnaire was developed to investigate what types of medicinal plants were used by the practitioners to improve memory loss or for the treatment of neurological disorders like epilepsy, convulsion, insomnia, schizophrenia, depression, migraine, headaches, agitation etc. During the interviews, each practitioner was interviewed at 3 stages about vernacular name, life form, the current diseases, plant species used for the treatment, their medicinal effects, the plant part used, mode of preparation (e.g., infusion and decoction), route of administration (oral or external), administration form (e.g., juice, fruit, salad or jam, poultice, paste, inhalation, chewing and flavoring). Every herbalist was asked to list the main diseases he treated, and the plants used in the treatment. He was also asked to provide samples of the plant species from his own collection as well as from the field; and if possible, plant photographs. Two voucher specimens per medicinal plant were collected in the field, pressed and then dried prior for identification at the Cameroon National Herbarium (HNC). All plant specimens were collected in both flowering and fruiting conditions. Samples of medicinal plants were collected for scientific identification and herbarium preparation following standard procedures.

Extraction

This survey allowed us to identify twenty-six medicinal plants (Table 1) used to improve memory loss or to treat some neurological disorders such as epilepsy, headaches, depression, anxiety, migraine, convulsions and schizophrenia. Among the 26 medicinal plants identified some of them are used individually or as the mixture of two plants. Different parts of plants (leaves, barks, bulbs, rhizomes, roots, flowers and aerial parts) were collected in the locality of Foumbot (Noun Division, West Region, Cameroon) and they were washed with distilled water and dried at room temperature for several weeks. The dried plant materials were powdered using a grinder. The powder obtained was kept at 4°C until the preparation of extracts. A preliminary extraction test with different solvents: ethanol; methanol and the mixture methylene chloride/methanol on Thin Layer Chromatography (TLC) (data not shown) allowed us to select the mixture methylene chloride/methanol as the best solvents for the extraction. Hundred grams of powdered plant materials were soaked in 500 mL of solvent methylene chloride/methanol (1:1; v/v) for 48 hours. The final extracts were passed through Whatman N°1 filter paper and the filtrates obtained were concentrated under vacuum at low pressure on a rotary evaporator (RV10 Basic, IKA). The crude extracts obtained were stored at 4°C until further use.

Chemicals

AChE (type VI-S from electric eel), acetylthiocholine iodide (ATCI), 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB), 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium persulfate, ferrozine, ferrous sulfate, potassium ferricyanide, trichloroacetic acid, ferric chloride, sodium nitroprusside, L-ascorbic acid, Folin-Ciocalteu reagent, gallic acid, 6-hydroxy-2,5,7,8-tetrametylchroman-2-carboxylic acid (Trolox®), aluminium chloride, quercetin and 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were

purchased from Sigma-Aldrich (St. Louis, MO, USA). The 15-lipoxygenase from *Glycine max* purchased from Sigma (Germany) and Tris (hydroxymethyl) aminomethane from Sigma (Switzerland). Foetal calf serum (FCS), penicillin/streptomycin/fungizone (PSF) and Dulbecco's modified Eagle's medium (DMEM) were obtained from Highveld Biological Products (South Africa). Phosphate buffered saline (PBS) and trypsin were purchased from Whitehead Scientific (South Africa).

Acetylcholinesterase inhibitory activity

The acetylcholinesterase inhibition assay was determined by the Ellman colorimetric method [22] as described by Adewusi et al. [23] with slight modifications. Three buffers were prepared for the assay: Buffer A (50 mM Tris- HCl, pH 8), Buffer B (50 mM, pH 8, containing 0.1% bovine serum albumin) and Buffer C (50 mM Tris-HCl, pH 8, containing 0.1M NaCl and 0.02M $MgCl_2.6H_2O$). Briefly, 50 µL of plant extracts dissolved in DMSO, 100 µL of 15mM ATCI in water, 500 µL of 3mM DTNB in Buffer C, 250 µL of Buffer B were mixed in a spectrophotometer quartz cuvette. Absorbance was measured at 405 nm every 15s, three times consecutively on a spectrophotometer (Genesys, Hach). Thereafter, 100 µL of AChE (0.28U/mL) were added to the cuvette and the absorbance measured during 5 minutes every 15s. Galanthamine served as positive control. Any increase in absorbance due to the spontaneous hydrolysis of the substrate was corrected by subtracting the absorbance before adding the enzyme from the absorbance after adding the enzyme. The percentage inhibition was calculated using the following formula (1):

$$\%\ \text{Inhibition} = 100 - \left|\frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right| \times 100$$

Formula (1)

Anti-inflammatory assays of extracts

Nitric oxide inhibition assay on LPS-activated RAW 264.7 macrophages

Cell culture and treatment with extracts

The RAW 264.7 macrophage cell lines obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) were cultured at 37°C under 5% CO_2 in 75 cm^2 plastic culture flasks in DMEM containing 4.5 g/L and 4 mM L -glutamine supplemented with 10% FCS and 1% PSF solution. One hundred microliters of a cell suspension (2×10^6 cells per mL) was seeded in 96 well-microtitre plates and the cells were incubated overnight at 37°C with 5% CO_2 to allow attachment at the bottom of the plates. Then, the cells were activated by incubation in medium containing LPS (final concentration: 1 µg/mL) alone (negative control) or LPS with different concentrations (100, 50, 25 and 12.5µg/mL) of the extracts dissolved in DMSO and further diluted in the culture medium. Quercetin, a standard NO inhibitor, served as a positive control [24].

Measurement of nitrite

Nitric oxide released from macrophages was determined by measuring the nitrite concentration in cell culture supernatant using the Griess reagent. After 24 hours of incubation, 100 µL of culture supernatant from each well was transferred into new 96-well microtitre plate and an equal volume of Griess reagent was added. The mixture was left in the dark at room temperature (25°C) for 15 minutes and the absorbance was measured at 550 nm on a microplate reader (Synergy Multi-Mode Reader, BioTek). The concentrations of nitrite were determined by using the linear regression analysis from a sodium nitrite standard curve. Percentage inhibition was then calculated based on the ability of extracts plants to inhibit nitric oxide formation by cells compared with the negative control (LPS-treated cells without extracts), which was considered as 0% inhibition (see formula 1).

Cell viability

To determine whether the observed nitric oxide inhibition was not due to cytotoxicity of the tested sample, a cytotoxicity assay was also performed on the culture as previously described by Mosmann [25] with slight modifications. After removal of media, the cells were topped up with 200 µL of fresh DMEM and 30 µL of 5 mg/mL MTT solution. The cells were further incubated at 37°C in 5% CO_2 for 4 hours. The medium was carefully removed using a suction pump (Integra, USA), without disturbing the MTT formazan crystals. The formed formazan salts were dissolved in 50 µL of DMSO. The absorbance was read at 570 nm on a microplate reader (Synergy Multi-Mode Reader, BioTek). The percentage cell viability was calculated with the control (LPS-treated cells without extracts) taken as 100% viability.

Soybean lipoxygenase inhibition assay

The assay was performed according to procedure described by Dzoyem and Eloff [26]. The assay is based on the formation of the complex Fe^{3+}/xylenol orange with absorption at 560 nm. The 15-lipoxygenase from *Glycine max* was incubated with extracts or positive control (quercetin) at room temperature (25°C) for 5 minutes. Then, linoleic acid (final concentration, 140 µM) in Tris-HCl buffer (50 mM, pH 7.4) was added and the mixture was further incubated at room temperature (25°C) for 20 minutes in the dark. The assay was terminated by adding 100 µL of FOX reagent [sulfuric acid (30 mM), xylenol orange (100 µM), iron (II) sulfate (100 µM), methanol/water (9:1)]. The lipoxygenase inhibitory activity was determined by calculating the percentage of the inhibition of hydro-peroxide production from the changes in absorbance values at 560 nm after 30 minutes at 25°C.

$$\%\ \text{Inhibition} = \left[\frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}}\right] \times 100$$

Formula (2)

Antioxidant assays

DPPH radical scavenging assay

The capacity of the extracts to reduce the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was assessed using the method described by Magama et al. [27] with slight modifications. Our reaction mixture consisted of 3.1 mL of a solution of DPPH in methanol (40 µg/mL) and 50 µL of extract. The mixture was kept in dark for 30 minutes at room temperature and the absorbance was recorded spectrophotometrically at 517 nm. The normal purple colour of DPPH will turn into yellow when its single electron is paired with a hydrogen atom coming from a potential antioxidant. Ascorbic acid was used as the positive control. Percentage inhibition was calculated using the formula (2). The IC_{50} values were determined by plotting the percentage of DPPH radical

inhibitory activity at different concentrations of the extracts (0.1; 1; 10 and 100 µg/mL).

Reducing ability assay

The reducing ability of the extracts was assayed as described by Moyo et al. [28]. Fifty microliters of extract (100 µg/mL) was added to 1.1 mL of phosphate buffer (0.2 mM, pH 6.6) and 1 mL of potassium ferrocyanate [K_3Fe (CN) $_6$] (0.25%). After incubation at 50°C for 20 minutes, 1 mL of trichloroacetic acid (10%) was added to the mixture before centrifugation at 3000 rpm for 10 minutes. The supernatant (1 mL) was collected and mixed with 1 mL of distilled water and 0.2 mL of ferric chloride (0.02%). The absorbance was measured spectrophotometrically at 700 nm. The reducing ability was measured by reading the absorbance of the reaction mixture and a higher absorbance indicated increased reducing power. Ascorbic acid was used as positive control.

Total antioxidant assay

The antioxidant potential of the extracts was assessed by the phosphomolybdenum reduction assay with slight modifications. Fifty microliters of extracts (100 µg/mL) were mixed with 1 mL of sulfuric acid (0.6 M H_2SO_4), 1.05 mL of sodium phosphate 28 mM and 1.05 mL of ammonium molybdate (4 mM). The tubes were incubated at 95°C for 90 minutes and the mixture was cooled at room temperature. The absorbance of the mixture was measured spectrophotometrically at 695 nm. For standard, the appropriate solution of ascorbic acid was used and calibration curve (y= 0.0033x) was obtained using the concentrations ranging from 0 to 100 µg/mL of ascorbic acid. The antioxidant capacity is expressed as ascorbic acid equivalents (mg AAE/g of extract).

Inhibition of lipid peroxidation using Thiobarbituric Acid Reactive Substances (TBARS)

The inhibition of lipid peroxidation activity was determined according to the method described by Asaduzzaman et al. [29] with slight modifications. Brains were obtained from rats (Wistar albino), dissected and homogenized in ice-cold KCl (1.15%) to produce a brain tissue homogenate of 10% which was centrifuged at 1300 g for 10 minutes at 4°C. An aliquot (1 mL) of the supernatant was incubated with the sample in the presence of $FeCl_2$ (0.5 mM; 0.05 mL) and H_2O_2 (0.5 mM; 0.05 mL) at 37°C for 1 hour. The reaction was stopped by the addition of trichloroacetic acid (15% w/v, 1 mL), followed by thiobarbituric acid (TBA, 0.67%, w/v, 1 mL), and the mixture was then heated at 100°C for 15 minutes. After centrifugation at 3000 rpm for 5 minutes to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by reading its absorbance spectrophotometrically at 532 nm. Trolox was used as positive control. The inhibition ratio (%) was calculated using the formula (2).

Determination of the phytochemical content of the extracts

Determination of total phenolic content (TPC)

Total phenolic content was performed according to the Folin–Ciocalteu method [30] with some modifications. Briefly, 0.05 mL of sample (100 µg/mL), 2.4 mL of distilled water and 0.2 mL of Folin–Ciocalteu's reagent (2N) were introduced in a test tube, and then 0.5 mL of Na_2CO_3 (20%) was added. The reaction mixture was incubated in dark at room temperature for 1 hour and the absorbance of the mixture was read on a spectrophotometer at 765 nm. The sample was tested in triplicate and a calibration curve (y=0.0069x) with six data points for gallic acid with the concentration ranging from 0 to 50 µg/mL was obtained. The results were projected to the gallic acid calibration curve and the TPC of the samples was expressed as mg of gallic acid equivalents per gram of extract (mg GAE/g extract).

Determination of total flavonoid content (TFC)

Aluminium chloride colorimetric method was used to determine the total flavonoid content of the extracts. Briefly, 0.5 mL of 2% $AlCl_3$ was added to 0.5 mL of sample and after 30 minutes of incubation at room temperature, the absorbance was measured spectrophotometrically at 430 nm (the final concentration was 100 µg/mL). The calibration curve (y=0.0242x) was obtained by using a solution of quercetin dissolved in methanol with the concentration ranging from 0 to 50 µg/mL. The TFC of samples was expressed as mg quercetin equivalent (QE) / gram of extract.

Statistical analysis

Data are presented as the mean ± standard deviation (SD) of triplicate tests. IC_{50} values of samples were calculated by plotting the linear regression curve of the percentage of inhibition against the logarithm of the concentrations using GraphPad Prism Software 3.0. Results were analysed statistically using the same software and they were identified as significantly different when $p<0.05$.

Results

Acetylcholinesterase inhibitory activity

All the extracts were tested in a preliminary assay at a single concentration of 200 µg/mL compared to galanthamine (positive control) at 100 µg/mL. The inhibitory activities of extracts are given in Table 2. It was observed from this study that five crude extracts exhibited considerable acetylcholinesterase inhibitory activity (greater than 50% inhibition). The 50% inhibitory concentrations (IC_{50}) values were as follow: *Crinum purpurascens* (99.40 ± 1.28µg/mL); *Emilia coccinae* (150.40 ± 1.43µg/mL); *Erythrina senegalensis* (166.20 ± 2.98 µg/mL); *Ageratum conyzoides* (176.2 ± 0.06 µg/mL) and *Piper umbellatum* (180.60 ± 1.64 µg/mL). These plants were less active compared to galanthamine (IC_{50} of 24.65 ± 2.12 µg/mL).

Anti-inflammatory activities

The NO production inhibitory activity of extracts was evaluated on LPS-activated RAW 264.7 macrophage cells. This assay was validated by using LPS-activated cells without treatment with the extracts as negative control, LPS-activated cells and treated with quercetin (a standard inhibitor of NO). Most of the extracts showed dose dependent inhibition of NO production at the concentration of 12.5, 25, 50 and 100 µg/mL (data not shown). At the highest concentration (100 µg/mL), most of the extracts evaluated had percentage inhibition greater than 50% (Table 3). In addition, the percentage of viability at 100 µg/mL varied considerably between 48% to 126%. The IC_{50} values of different extracts were calculated and are presented in Table 3.

The 15-lipoxygenase inhibitory activity was measured using the 96-well microplate-based ferric oxidation of xylenol

orange (FOX) assay. The results summarized in Table 2 indicate that, at 100 µg/mL, the extract of *Annona muricata* seeds had the highest anti-15-lipoxygenase activity with 93.72% inhibition. The extracts of *Cola acuminata, Annona senegalensis* bark, *Erythrina senegalensis* and *Anthocleista sweinfurthii* were not active at tested concentrations. The IC_{50} values ranged between 30.43 µg/mL (*Annona muricata* seeds) and 93.60 µg/mL (*Acanthus montanus*) and the IC_{50} value of less active extracts (less than 50% inhibition) were considered as greater than 100 µg/mL.

Antioxidant activities

The free radical scavenging activity of different extracts, along with the standard reference ascorbic acid was carried out, and the concentration of the extract necessary to reduce the initial concentration of DPPH by 50% (IC_{50}) was determined for the extract which showed the percentage of inhibition higher than 50% (see Table 4). At 100 µg/ml, fifteen (15) crude extracts exhibited more than 50% DPPH scavenging activity. The results are summarized in Table 4. A lower value of IC_{50} indicates higher antioxidant activity. The best free radical scavenging activity was obtained with the barks of *A. senegalensis* extract (IC_{50} of 7.40 µg/mL) which was better than ascorbic acid used as positive control (IC_{50} of 8.41 µg/mL). Other extracts from *E. senegalensis* (IC_{50} of 12.20 µg/mL), *A. sweinfurthii* (IC_{50} of 11.03 µg/mL) and *C. acuminata* (IC_{50} of 22.35 µg/mL) had also good DPPH scavenging potential.

In the reducing power assay, the presence of reductants (antioxidants) in the extracts would result in the reduction of Fe^{+3}/ferriccyanide complex to the ferrous form by donating an electron. A higher absorbance indicates a higher reducing power. The greater reducing power was obtained with the extract of *C. acuminata* (0.62 ± 0.032).

The total antioxidant activity of the crude extracts varied between 149.39 to 385.15mg AAE/g extract (See Table 4). The extract of *Dacryodes edulis* had the highest total antioxidant capacity with 385.15 mg AAE/g of extract. A higher absorbance means higher antioxidant capacity.

In the lipid peroxidation inhibition activity, the activity of plant extracts against non-enzymatic lipid peroxidation in rat brain homogenate indicated that at 100 µg/mL, nine crude extracts exhibited more than 50% inhibitory activity in the brain homogenate. The best IC_{50} value was obtained with *Alchornea laxiflora* (3.20 µg/mL), *D. Edulis* (6.60 µg/mL), *C. acuminata* (9.50 µg/mL), *Ocimum gratissimum* (11.20 µg/mL) and *A. senegalensis* (29.20 µg/mL) compared to the positive control Trolox (4.35 µg/mL).

Phytochemical content of the extracts

The total phenolic content (TPC) obtained as mg GAE/g of extract varied considerably between plant extracts as shown in Table 5. The extract of *A. senegalensis* had the highest TPC (100 mg GAE/g of extract), followed by *C. acuminata* (93,18 mg GAE/g of extract), *A. laxiflora* (92.12 mg GAE/ g of extract), *A. sweinfurthii* (91.81 mg GAE/g of extract), *D. edulis* (85.45 mg GAE/ g of extract). The total flavonoid content (TFC) of plant extracts ranged between 0.111 to 6.43 mg QE/g of extract (Table 5). Very low correlation was found between the antioxidant activities and the total phenolic and flavonoid contents (data not shown).

Discussion

Many medicinal plants used for the treatment of cognitive dysfunction have been explored for the development of new drugs against neurodegenerative diseases and these plants contain various bioactive compounds [31, 32]. In Cameroon, many plants used to treat various ailments displayed good activities *in vitro* (antimalarial, antimicrobial, antiparasitic, anti-proliferative, anti-inflammatory, antidiabetic, etc.). Most biologically active compounds comprise terpenoids, phenolics, and alkaloids [21]. The ethnopharmacological survey carried out in the Noun Division allowed us to identify twenty-six plants used to improve memory loss or to treat some neurological disorders such as epilepsy, headaches, depression, anxiety, migraine, convulsions and schizophrenia. The anticholinesterase, anti-inflammatory and antioxidant activities of these plants were determined. All the plant extracts used in this study inhibited acetylcholinesterase, but five of them had very good inhibitory activity including the extracts of *C. purpurascens* (99.40 ± 1.28 µg/ml), *E. coccinea* (150.40 ± 1.43µg/ml), *E. senegalensis* (166.20 ± 2.98 µg/ml), *A. conyzoides* (176.2 ± 0.06 µg/ml) and *P. umbellatum* (180.60±1.64 µg/ml). The best inhibitory activity was shown by the extract of *C. purpurascens* belonging to the family Amaryllidaceae. Some studies report the potential of plants from the Amaryllidaceae family as acetylcholinesterase inhibitors (AchEIs), specially *Crinum* species [33-36]. It is known that plants from the Amaryllidaceae family contain various compounds which are inhibitors of the enzyme acetylcholinesterase [37, 38]. Similarly, this study identified the *C. purpurascens* (Amaryllidaceae) crude extract as containing AchEIs. The activity shown by four other plant extracts , namely *E. coccinea*, *E. senegalensis, A. conyzoides* and *P. umbellatum* may be explained by the presence of some bioactive compounds in the extracts [39]; and other studies have reported the neuropharmacological activities of some of these plants [40-44].

In the central nervous system, NO plays important roles in processes such as neurotransmitter release, neurotransmitter reuptake, neurodevelopment, regeneration, synaptic plasticity, and regulation of gene expression; but excessive production leads to neural injury. Nitric oxide (NO) has been linked to neurodegenerative disorders due to the increased expression of several enzymes relevant in neurodegeneration [45]. The inducible forms of nitric oxide synthase are the most important pro-inflammatory enzymes responsible for increasing the levels of NO [46]. Therefore, inhibition of NO production in LPS-stimulated RAW 264.7 cells is one of the possible ways to screen various anti-inflammatory drugs [47]. The anti-inflammatory activity of the crude extracts was carried out by assessing inhibition of NO production by RAW 264.7 cells as well as viability testing using the MTT assay. Most of the extracts at 100 µg/mL had no cytotoxic effect, indicating that the inhibitory effect of plant extracts on RAW 264.7 cells is not due to their cytotoxicity. The NO production induced by LPS was inhibited by most of the crude extracts, particularly the extract of *C. purpurascens*. The inhibitory activity of NO production by medicinal plants may arise from inhibition of the expression of nitric oxide synthase. Many natural compounds from medicinal plants have been known as inhibitors of the expression of iNOS in LPS-activated macrophages [48]. Lipoxygenases (LOX) form a family of lipid-peroxidizing enzymes, which play regulatory roles in the inflammatory process and neurodegenerative diseases. Theses enzymes are widely expressed in the central nervous system [49]. The 15-LOX enzyme converts arachidonic acid into its corresponding lipid peroxides, which are further converted, in the

presence of glutathione, to different inflammatory mediators. It has been shown that lipoxygenase inhibitors are able to rescue both neuronal as well as oligodendrocyte cells from cell death induced by oxidative stress [50]. Therefore, the inhibition of 15-LOX may be an emerging drug target for the treatment of neurodegenerative diseases. The extract of *Annona muricata* seeds (IC_{50} of 30.43µg/mL) had the best 15-LOX inhibitory activity and can therefore be considered as containing potential LOX inhibitors.

Several studies have reported the implication of oxidative stress in the pathogenesis of AD and PD. The brain is very sensitive to free radicals which lead to oxidation of protein, lipid peroxidation and oxidation of some macromolecules found in the brain [51, 52]. An appropriate way to delay oxidative stress in the brain is the use of antioxidants presents in fruits, vegetables and plants. Many methods are used to evaluate antioxidant activities of plants. In this study, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, lipid peroxidation (MDA), total antioxidant and ferrous ion-chelating power tests were used to evaluate the antioxidant activities of the crude extracts. The DPPH free radical scavenging activity is a widely used model for evaluating the free radical scavenging ability of various compounds [28]. The decreased of the absorbance in this assay is a result of a colour change from purple to yellow, as radicals are scavenged by antioxidants through the donation of hydrogen to form the stable DPPH molecule. Substances that are able to perform this reaction can be considered as antioxidants or radical scavengers [53]. The reducing power of a compound is a supporting feature for its antioxidant capacity. In the reducing power assay, antioxidant compounds convert the oxidative form of iron (Fe^{+3}) in ferric chloride to ferrous (Fe^{+2}). Some authors have reported that the reducing properties of antioxidants are generally associated with the presence of reductants. The extract of *C. acuminata* showed the highest reducing power, therefore suggesting the presence of reductants within this extract. Many studies carried out on *C. acuminata* concern the antioxidant activity of the seeds, commonly called kola nuts [54-56]. To the best of our knowledge, this is the first report of the antioxidant activity of the bark extract of *C. acuminata*. The phosphomolybdenum method was used to determine the total antioxidant activity of the different extracts, and the extract of *D. edulis* had the highest antioxidant capacity (385.15 mg AAE/g of extract). This high antioxidant capacity can be attributed to the presence of phenols (85.45±7.71 mg GAE/g extract) in the extract. Lipid peroxidation is the mechanism by which lipids are attacked by reactive oxygen species (ROS) that have sufficient reactivity to abstract a hydrogen atom from a methylene carbon in their side chain [51]. Lipid peroxidation has been reported to be elevated in the brain of patients suffering from AD [52]. During lipid peroxidation, low molecular weight end products, generally malonaldehyde, are formed by oxidation of poly-unsaturated fatty acids that may react with two molecules of thiobarbituric acid to give a pinkish red chromogen. The lipid peroxidation is induced by H_2O_2/ Fe^{2+}. The extract of *A. laxiflora* (3.20 µg/mL) was the most active compared to the standard trolox (4.35 µg/mL) and this activity may be due to its high content of phenolic compounds (92.12 mg GAE/g extract).

Phenolic compounds, which include flavonoids, are commonly found in the plant kingdom, and they have been reported to have multiple biological effects, including antioxidant activity. Phenolic compounds are known as powerful chain-breaking antioxidants and they are very important plant constituents because of their scavenging ability, which is due to their hydroxyl groups [28]. Several studies have evaluated the relationship between the antioxidant activity of plant products and their phenolic content. Flavonoids are a group of polyphenolic compounds, which exhibit various biological effects relevant in neurodegenerative diseases [57, 58]. High total phenolic and flavonoid contents lead to better antioxidant activity. Indeed, data presented in Table 5 shows that the type and quantity of phytochemicals found varies among different plant extracts. Unfortunately, very low correlation was observed between antioxidant activities and phenolic or flavonoid content and this may be due to the diversity of the plants used that belong to different families or species.0o

Table 1. Medicinal plant used for the treatment of neurological diseases as identified by the ethnopharmacological survey.

Family	Scientific name Voucher Specimen Number	Vernacular name in Bamoun*	Part(s) used	Mode of preparation	Disease treated
Acanthaceae	*Acanthus montanus* (Nees) T.Anderson 23603/SRF/Cam.	Fozem	Leaves	Decoction	Depression
Agavaceae	*Draceana deisteliana* Engl 27673/YA	Nkunkù	Leaves	Decoction	Convulsion
Amarylidaceae	*Crinum purpurascens* Herb. 40058/HNC	NduéKé	Bulbs	Decoction	Epilepsy
Annonaceae	*Annona muricata* L. 32879/HNC	Chawa-chawa	Leaves, seeds	Decoction	Depression/badly nerves
	Annona senegalensis Pers. 7783 YA	Kuopshe-kuopshe	Leaves, barks	Decoction	Convulsion/Epilepsy
Apocynaceae	*Voacanga africana* Stapf 47215 YA	Pè pepkèn	Barks	Decoction	Schizophrenia
	Dacryodes edulis (G.Don) H.J.Lam 18258/HNC	Youom	Barks	Decoction	Depression/Epilepsy
Araliaceae	*Polyscias fulva* Hiern 2990 YA	Pugue (Pûngùe)	Barks	Decoction	Anxiety/Depression
Asteraceae	*Ageratum conyzoides* L. 20074/ SRF/ Cam	Menjotefu	Aerial part	Maceration	Epilepsy/Depression
	Emilia coccinea (Sims) G. Don 29441/HNC	Shin Mû	Leaves	Decoction	Headache
	Vernonia guineensis benth 11133/SRF/Cam	Mgbùkwet	Rhizomes	Decoction	Epilepsy /memory loss
	Spilanthes africana DC. 33075/HNC	Mba kouom	Flowers	Decoction	Headache
	Bidens pilosa L. 41693/HNC	Fuwen	Whole plant	Decoction	Epilepsy/Depression
Cesalpilaceae	*Senna alata* L. 29494/HNC	Sùé nâsa	Leaves	Decoction	Convulsion/agitation
Clusiaceae	*Allanblackia florinbunda* Oliv. 1380/HNC	/	Leaves	Decoction	Epilepsy
Euphorbiaceae	*Euphorbia hirta* L. 14288/SRF/Cam	Bùmon	Aerial part	Decoction	Depression
	Croton macrosthachyus Hochst et Del 17909/SRF/Cam	Kùteshâ	Roots Barks	Decoction	Epilepsy
	Alchornea laxiflora (Benth.) Pax & K.Hoffm. 2093 YA	Meshé	Leaves	Decoction	Anxiety/Depression
Fabaceae	*Erythrina senegalensis* DC. 35259 YA	Megham	Leaves, barks	Decoction	Epilepsy
	Piliostigma thonningii (Schum.) Milne-Redh. 2689 YA	Pien	Leaves, barks	Decoction	Depression
Labiaceae	*Ocimum gratissimum* L. 42852 YA	Sem mvù	Leaves	Decoction	Depression
Loganiaceae	*Anthocleista schweinfurthii* Gilg 2281 YA	Yü'rum	Leaves	Decoction	Convulsion/agitation
Moraceae	*Fiscus exasperata* Vahl 5123/SRF/Cam	Ghùghu	Leaves, barks	Decoction	Epilepsy
Piperaceae	*Piper umbellatum L.* 20934/SRF/Cam	Boupouete	Leaves	Decoction	Anxiety/depression
Sterculiaceae	*Cola acuminata* (P. Beauv.) Schott & Endl. 1729/SRF/Cam	Shin pè'	Barks	Decoction	Schizophrenia/ memory loss
Solanaceae	*Nicotiana tabacum* L. 18637/SRF/Cam	Dapâ	Leaves	Decoction	Headache /migraine

* Bamoun is the traditional language spoken in the study area. SRF/Cam*:* Société des Réserves forestières du Cameroun. HNC: Cameroon National Herbarium; YA:Yaounde Authenfication

Table 2. Acetylcholinesterase (AChE) and 15-lipoxygenase (15-LOX) inhibitory potential of some Cameroonian medicinal plants used traditionally to treat neurological disorders

Medicinal plant	Plant part used	AChE % Inhibition (at 200 µg/ml)	AChE IC_{50} (µg/ml)	15-LOX % Inhibition (at 100 µg/mL)	15-LOX IC_{50} (µg/mL)
Acanthus montanus	Leaves	34.29±3.26	> 200	56.91±6.24	96.60±4.40
Ageratum conyzoides	aerial part	60.22±4.75	**176.2±0.06**	76.36±3.23	58.97±0.69
Alchornea laxiflora	Leaves	36.02±0.81	> 200	54.58±2.39	90.42±0.42
Voacanga africana	Bark	7.49±2.03	> 200	38.11±7.66	> 100
Annona muricata	Leaves	45.82±0.818	> 200	86.19±3.05	37.12±2.23
Bidens pilosa	Whole plant	34.29±1.63	> 200	85.82±1.71	41.63±2.10
Dacryodes edulis	bark	46.10±2.85	> 200	39.34±4.54	> 100
Cola acuminata	bark	25.93±0.40	> 200	-75.87±28.77	NA
Crinum purpurascens	bulbs	66.38± 1.58	**99.40±1.28**	69.34±7.21	67.94±5.56
Croton macrosthachyus	root bark	40.05±1.63	> 200	42.22±2.32	> 100
Emilia coccinea	leaves	70.86±0.79	**150.40±1.43**	45.71±6.57	> 100
Euphorbia hirta	aerial part	28.53±0.81	> 200	83.87±4.61	38.10±2.45
Ficus exasperata	leaves	30.54±1.22	> 200	93.16±12.08	35.44±1.55
Nicotiana tabacum	leaves	42.93±3.26	> 200	93.63±2.00	36.51±0.29
Piliostigma thonningii	leaves	35.44±0.00	> 200	85.17±4.84	36.07±5.48
Piliostigma thonningii	bark	21.61±1.63	> 200	9.95±4.16	> 100
Piper umbellatum	leaves	59.38±0.39	**180.60±1.64**	70.73±8.89	45.88±5.63
Polyscias fulva	bark	45.24±0.81	> 200	17.94±0.66	> 100
Senna alata	leaves	39.76±4.48	> 200	32.70±3.80	> 100
Spilanthes africana	flowers	38.61±1.22	> 200	44.67±5.15	> 100
Vernonia guineensis	rhizomes	8.93±1.63	> 200	13.51±5.91	> 100
Annona muricata	seeds	10.37±2.85	> 200	93.72±0.39	**30.43±1.40**
Allanblackia floribunda	leaves	37.17±3.26	> 200	19.90±3.36	> 100
Annona senegalensis	bark	8.79±0.51	> 200	-43.90±12.69	NA
Annona senegalensis	leaves	35.89±2.59	> 200	77.54±0.42	39.02±11.54
Draceana deisteliana	leaves	28.93±1.03	> 200	85.86±1.65	40.22±2.56
Ocimum gratissimum	leaves	36.26±2.07	> 200	86.00±1.35	35.22±1.88
Erythrina senegalensis	bark	67.39±1.55	**166.20±2.98**	-16.48±14.35	NA
Anthocleista sweinfurthii	bark	41.75±0.51	> 200	-74.46±3.70	NA
Galanthamine		92.99±0.39	24.65±2.12***	ND	ND
Quercetin		ND	ND	93.07±8.39	16.14±1.15*

Results are represented as mean ± standard deviation, n = 3. ND: not determined, NA: Not active.
Significant difference * ($p<0.05$); ***($p<0.001$) compare to positive control.

Table 3. Nitric oxide inhibitory activities of plant extracts on LPS-activated RAW 264.7 macrophages and their percentage of viability after 24 hours of incubation.

Medicinal plant	Plant part used	NO (µM)	% NO inhibition (at 100 µg/mL)	% viability (at 100 µg/mL)	NO IC_{50} (µg/mL)
Acanthus. Montanus	leaves	0.65±0.11	94.35±1.01	74.60±16.29	22.73±5.19
Ageratum conyzoides	aerial part	2.91± 0.22	84.71±1.16	95.96±1.04	35.14±0.31
Alchornea laxiflora	leaves	6.07±0.31	68.10±1.64	75.39±2.43	66.57±4.01
Voacanga Africana	bark	13.76±0.05	27.70±0.27	119.55±1.44	> 100
Annona muricata	leaves	4.44±0.11	76.67±0.61	67.63±2.29	51.59±2.46
Bidens pilosa	whole plant	5.55±0.15	70.81±0.82	91.93±3.74	51.05±0.82
Dacryodes edulis	bark	5.47±0.02	72.51±0.13	103.13±5.20	49.16±2.39
Cola acuminata	bark	17.06±0.05	14.35±0.26	124.46±5.58	> 100
Crinum purpurascens	bulbs	0.73±0.18	**96.31±0.91**	48.10±0.08	**12.08±1.68**
Croton macrosthachyus	root bark	1.48±0.02	92.52±0.13	96.20±1.30	24.85±0.99
Emilia coccinea	Leaves	8.15±0.22	56.51±1.18	83.78±9.22	99.41±5.09
Euphorbia hirta	aerial part	8.18±0.31	56.36±1.66	62.96±0.44	88.98±9.52
Ficus exasperata	Leaves	9.83±0.14	47.56±0.76	102.96±0.85	> 100
Nicotiana tabacum	Leaves	7.85±0.31	58.14±1.66	95.82±2.52	95.01±12.14
Piliostigma thonningii	Leaves	5.03±0.30	73.14±1.59	92.72±2.01	51.98±1.40
Piliostigma thonningii	Bark	10.80±0,22	44.41±1.14	111.95±9.03	> 100
Piper umbellatum	Leaves	4.87±0.13	74.92±0.67	84.51±1.74	46.19±1.12
Polyscias fulva	Bark	9.60±0.24	50.58±1.27	115.77±3.43	111.77±10.85
Senna alata	leaves	6.97±0.09	64.82±0.46	124.36±10.42	64.93±1.42
Spilanthes Africana	flowers	8.39±0.06	57.65±0.32	108.84±1.54	78.03±8.89
Vernonia guineensis	rhizomes	9.97±0.28	49.69±1.44	107.36±4.66	> 100
Annona muricata	seeds	3.10±0.52	84.31±2.63	106.17±7.73	40.25±2.49
Allanblackia floribunda	leaves	1.05±0.01	90.82±0.11	71.62±4.03	37.55±1.80
Annona senegalensis	bark	8.46±0.22	57.63±0.61	117.15±16.82	86.77±7.54
Annona senegalensis	leaves	0.85±0.03	92.59±0.33	70.14±7.30	33.83±2.34
Draceana deisteliana	leaves	10.81±0.14	46.34±0.71	95.94±4.46	> 100
Ocimum gratissimum	leaves	10.54±0.52	47.67±2.58	126.04±1.52	> 100
Erythrina senegalensis	bark	1.57±0.13	92.17±0.64	55.34±0.97	33.58±1.08
Anthocleista sweinfurthii	bark	6.36±0.05	44.77±0.45	86.79±3.64	> 100
Quercetin		0.47±0.00	97.66±0.00	67.85±3.64	6.59±0.87**

Results are represented as means ± standard deviation, n = 3. Significant difference *($p<0.01$); compare to quercetin (positive control).

Table 4. Antioxidant activities and inhibitory concentrations (IC_{50}) values of plant extracts

Medicinal plant	Plant part used	DPPH radical scavenging activity IC_{50} (µg/mL)	Total antioxidant capacity at 100µg/ml (mg AAE/g)	Reducing power ability (Absorbance at 700 nm	Lipid Peroxidation IC_{50} (µg/mL)
Acanthus montanus	leaves	> 100	198.18±0.42	0.134±0.024	> 100
Ageratum conyzoides	Aerial part	> 100	200.60± 5.99	0.149±0.002	> 100
Alchornea laxiflora	leaves	38.02±1.74***	296.96±2.99	0.501±0.007	**3.20±0.10ns**
Voacanga Africana	bark	71.10±2.57***	165.75±6.21	0.436±0.014	> 100
Annona muricata	leaves	91.04±4.55***	254.54±2.57	0.276±0.014	> 100
Bidens pilosa	whole plant	49.49±2.76***	344.84±0.85	0.454±0.002	> 100
Dacryodes edulis	bark	36.57±2.87***	**385.15±3.64**	**0.516±0.002**	**6.60±2.77ns**
Cola acuminata	bark	22.35±0.27***	289.09±2.57	**0.620±0.037**	**9.50±0.04****
Crinum purpurascens	bulbs	> 100	191.51±1.28	0.200±0.009	39.60± 1.97***
Croton macrosthachyus	Root bark	> 100	257.57±4.71	0.169±0.015	59.80±3.81***
Emilia coccinea	Leaves	> 100	203.63±1.28	0.22±0.009	> 100
Euphorbia hirta	Aerial part	> 100	268.48±4.28	0.273±0.096	> 100
Ficus exasperata	Leaves	> 100	292.72±5.57	0.183±0.009	> 100
Nicotiana tabacum	Leaves	> 100	249.39±2.78	0.342±0.007	> 100
Piliostigma thonningii	Leaves	> 100	253.33±0.85	0.354±0.00	> 100
Piliostigma thonningii	Bark	40.05±3.48***	350.60±3.64	0.571±0.032	68.10±2.40***
Piper umbellatum	Leaves	63.30±2.13***	335.75±5.99	0.391±0.118	53.50±3.32***
Polyscias fulva	Bark	56.74±0.61***	346.36±4.49	0.566±0.012	> 100
Senna alata	Leaves	95.35±1.43***	290.60±2.78	0.439±0.047	63.15±0.21***
Spilanthes Africana	Flowers	> 100	210.90±4.71	0.284±0.007	> 100
Vernonia guineensis	Bark	> 100	295.15±0.42	0.311±0.003	> 100
Annona muricata	Seeds	> 100	193.93±3.85	0.162±0.015	> 100
Allanblackia floribunda	Leaves	> 100	149.39±0.64	0.220±0.031	> 100
Annona senegalensis	Bark	**7.40±0.00ns**	318.19±8.44	**0.520±0.010**	29.20±2.61***
Annona senegalensis	Leaves	48.04±0.10***	135.41±18.26	0.512±0.004	> 100
Draceana deisteliana	Leaves	> 100	98.05±11.78	0.258±0.033	> 100
Ocimum gratissimum	Leaves	56.40±3.28***	87.22±8.24	0.383±0.006	11.20±0.35***
Erythrina senegalensis	Bark	**12.20±1.74ns**	83.33±5.89	0.333±0.010	Nd
Anthocleista sweinfurthii	Bark	**11.03±0.51ns**	192.22±7.85	0.532±0.022	> 100
Ascorbic acid		8.41±0.205	Nd	0.655±0.028	Nd
Trolox		Nd	Nd	Nd	4.35±0.00

Results are represented as means ± standard deviation, n = 3. Nd: Not determined. ns: not significative difference, **: significative difference ($p<0.001$); ***: significative difference ($p<0.0001$) compare to standard (ascorbic acid and trolox).

Table 5. Phytochemical content (Total phenolics and total flavonoids) of plants extracts

Medicinal plants	Plants part used	Total phenolics (mg GAE/g extract)	Total flavonoids (mg QE/g extract)
Acanthus montanus	Leaves	25.30±1.49	1.45±0.12
Ageratum conyzoides	Aerial part	33.48 ± 1.92	1.57±0.08
Alchornea laxiflora	Leaves	**92.12±1.71**	3.92±0.55
Voacanga africana	Bark	42.72±1.71	0.15±0.02
Annona muricata	Leaves	35.60±1.78	1.33±0.14
Bidens pilosa	Whole plant	75.45±1.28	**6.43±0.47**
Dacryodes edulis	Bark	85.45±7.71	0.27±0.00
Cola acuminata	Bark	**93.18±2.78**	0.50±0.01
Crinum purpurascens	Bulbs	27.87±4.71	0.76±0.04
Croton macrosthachyus	Root bark	22.12±0.85	0.11±0.01
Emilia coccinea	Leaves	15.75±0.00	3.57±0.33
Euphorbia hirta	Aerial part	42.27±0.21	3.88±0.53
Ficus exasperata.	Leaves	13.93±0.42	1.98±0.35
Nicotiana tabacum	Leaves	24.54±3.42	1.48±0.26
Piliostigma thonningii	Leaves	36.51±1.92	5.02±0.56
Piliostigma thonningii	Bark	46.51±4.07	0.21±0.00
Piper umbellatum	Leaves	33.78±6.64	3.73±0.56
Polyscias fulva	Bark	62.12±1.71	0.47±0.00
Senna alata	Leaves	26.81±1.07	3.26±0.45
Spilanthes africana	Flowers	36.06±3.42	2.78±0.38
Vernonia guineensis	Rhizomes	22.72±2.99	0.21±0.00
Annona muricata	Seeds	7.42±0.21	0.19±0.04
Allanblackia floribunda	Leaves	20.90±0.85	2.26±0.38
Annona senegalensis	Bark	**100±1.28**	2.95±0.01
Annona senegalensis	Leaves	90.45±0.21	3.46±0.00
Draceana deisteliana	Leaves	52.27±5.78	2.88±0.01
Ocimum gratissimum	Leaves	81.96±1.49	4.36±0.07
Erythrina senegalensis	Bark	85.90±0.21	3.69±0.01
Anthocleista sweinfurthii	Bark	**91.81±1.28**	2.67±0.00

Results are represented as means ± standard deviation, n = 3.
GAE = gallic acid equivalents; QE = quercetin equivalents.

Conclusions

The primary findings of this study suggest that all plants used in this study exhibited to some extent AChE inhibitory, anti-inflammatory and antioxidant properties. This result supports the use of these medicinal plants by traditional healers to improve memory loss or to treat neurological disorders. The extracts of *C. purpurascens, D. edulis* and *A. laxiflora* have promising potential to be valorised for use against neurodegenerative diseases. Therefore, the isolation of active compound(s) from these extracts will help to identify the potent natural antioxidant, anti-inflammatory and AChEI compounds, which can be used in the near future to prevent and/or to treat neurodegenerative diseases.

Abbreviations

AD: Alzheimer's disease; PD: Parkinson's disease; AChE: acetylcholinesterase; LPS: Lipopolysaccharide; MTT: Methyl thiazol tetrazolium; NO: Nitric oxide; LOX: Lipoxygenase; ROS:

Reactive Oxygen Species; DPPH: 2,2-diphenyl-1-picrylhydrazyl; MDA: Malondialdehyde.

Authors' Contribution

VLNN designed, carried out the experiment and wrote the manuscript; EMN carried out the cell culture and biochemical experiments. AFE contributed to plant collection and extraction. SNF, LJM and PFM supervised the work and provided the facilities for the study. All authors read the manuscript and approved the final version.

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Conflict of interest

The authors declare that they have no competing interests.

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References

1. Elujoba AA, Odeleye O. M., Ogunyemi C. M. Traditional medicine development for medical and dental primary health care delivery system in Africa. Afr J Tradit Complement Altern Med. 2005; 2(1):46-61.
2. Kantati YT, Kodjo KM, Dogbeavou KS, Vaudry D, Leprince J, Gbeassor M. Ethnopharmacological survey of plant species used in folk medicine against central nervous system disorders in Togo. J Ethnopharmacol. 2016; 181:214-220.
3. Bum EN, Taiwe GS, Moto FCO, Ngoupaye GT, Vougat RRN, Sakoue VD, Gwa C, Ayissi ER, Dong C, Rakotonirina A, et al. Antiepileptic Medicinal Plants used in Traditional Medicine to Treat Epilepsy. In: Clinical and Genetic Aspects of Epilepsy. Edited by Afawi Z. Rijeka: InTech; 2011: Ch. 08.
4. Niranjan R. The role of inflammatory and oxidative stress mechanisms in the pathogenesis of Parkinson's disease: focus on astrocytes. Mol Neurobiol. 2014; 49(1):28-38.
5. Singh S. Antioxidants as a preventive therapeutic option for age related neurodegenerative diseases. Ther Targets Neurolog Dis. 2015: 2:e592.
6. Pagano G, Rengo G, Pasqualetti G, Femminella GD, Monzani F, Ferrara N, Tagliati M: Cholinesterase inhibitors for Parkinson's disease: a systematic review and meta-analysis. J Neurol Neurosur Psych. 2015; 86(7):767-773.
7. Anand R, Gill KD, Mahdi AA. Therapeutics of Alzheimer's disease: Past, present and future. Neuropharmacol. 2014, 76, Part A: 27-50.
8. Bishara D, Sauer J, Taylor D. The pharmacological management of Alzheimer's disease. Prog Neurolo Psych. 2015; 19(4):9-16.
9. Henderson EJ, Lord SR, Brodie MA, Gaunt DM, Lawrence AD, Close JCT, Whone AL, Ben-Shlomo Y. Rivastigmine for gait stability in patients with Parkinson's disease (ReSPonD): a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Neurolo. 2016; 15(3):249-258.
10. Mukherjee PK, Kumar V, Mal M, Houghton P.J. Acetylcholinesterase inhibitors from plants. Phytomed. 2007; 14:289-300.
11. Čolović MB, Krstić DZ, Lazarević-Pašti TD, Bondžić AM, Vasić VM: Acetylcholinesterase Inhibitors: Pharmacology and Toxicology. Curr Neuropharmacol. 2013; 11(3):315-335.
12. Prakash A, Kalra J, Mani V, Ramasamy K, Majeed ABA. Pharmacological approaches for Alzheimer's disease: neurotransmitter as drug targets. Expert Rev of Neurotherap. 2015; 15(1):53-71.
13. Hirsch EC, Vyas S, Hunot S. Neuroinflammation in Parkinson's disease. Parkinsonism Relat Disord. 2012; 18.
14. Chen Z, Zhong C. Oxidative stress in Alzheimer's disease. Neurosci Bull. 2014; 30(2):271-281.
15. Diana Gallego, Rojas M, Orozco C. Free Radicals, Neuronal Death and Neuroprotection, Neurodegenerative Diseases - Processes, Prevention, Protection and Monitoring. Dr Raymond Chuen-Chung Chang (Ed), ISBN: 978-953-307-485-6, InTech, 2011.
16. Hwang O. Role of oxidative stress in Parkinson's disease. Exp Neurobiol. 2013; 22(1):11-17.
17. Gandhi S, Abramov A.Y. Mechanism of Oxidative Stress in Neurodegeneration. Oxi Med Cell Longevity. 2012; Article ID 428010:11 pages.
18. Zhao Y, Zhao B. Oxidative Stress and the Pathogenesis of Alzheimer's Disease. Oxi Med Cell Longevity. 2013; Article ID 316523,:10 pages.
19. Elena CI, Annalisa L., Cecilia C., Daniele O., Giulia R., Michelangelo M., S. G. Oxidative Stress Treatment for Clinical Trials in Neurodegenerative Diseases. J Alzheimer's Dis. 2011; 24:111-126.
20. Madeo J, Elsayad C. The Role of Oxidative Stress in Alzheimer's Disease. J Alzheimers Dis Parkinsonism. 2013; 3(2):116.
21. Kuete V, Efferth T. Cameroonian medicinal plants: pharmacology and derived natural products. Front Pharmacol. 2010; 1(Article 123).
22. Ellman GL, Courtney KD, Andres V, Feather-stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7.
23. Adewusi EA, Fouche G, Steenkamp V. Antioxidant, acetylcholinesterase inhibitory activity and cytotoxicity assessment of the crude extracts of Boophane disticha. Afr J Pharmacol Ther. 2012; 1(3):78-83.
24. Mu MM, Chakravortty D, Sugiyama T, Koide N, Takahashi K, Mori I, Yoshida T, Yokochi T. The inhibitory action of quercetin on lipopolysaccharide-induced nitric oxide production in RAW 264.7 macrophage cells. J Endotoxin Res. 2001; 7(6):431-438.
25. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunolog Meth. 1983; 65(1):55-63.
26. Dzoyem JP, Eloff JN. Anti-inflammatory, anticholinesterase and antioxidant activity of leaf extracts of twelve plants used traditionally to alleviate pain and inflammation in South Africa. J Ethnopharmacol. 2015; 160:194-201.
27. Magama S, Lieta M.I., Asita AO: Antioxidant and free radical scavenging properties of four plant species used in traditional medicine in Lesotho. Int J Med Plant Res 2013; 2(3):170-178.
28. Moyo M., Ndhlala A.R., Finnie J.F., Van Staden J. Phenolic composition, antioxidant and acetylcholinesterase inhibitory activities of Sclerocarya birrea and Harpephyllum caffrum (Anacardiaceae) extracts. Food Chem. 2010; 123:69-76.
29. Asaduzzaman M., Uddin M., Kader M., Alam A., Rahman A.A., Rashid M., Kato K., Tanaka T., Takeda M., Sadik G. In vitro acetylcholinesterase inhibitory activity and the antioxidant properties of Aegle marmelos leaf extract: implications for the treatment of Alzheimer's disease. Psychogeriatrics. 2014; 14(1):1-10.
30. Singleton V.L., Rossi J.A.: Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am J Enol Vitic. 1965; 16.
31. Eckert G.P.. Traditional used plants against cognitive decline and Alzheimer disease. Front Pharmacol. 2010; 1(138):1-10.

32. Dastmalchi K., Dorman H.J.D., Vuorela H., Hiltunen R. Plants as potential sources for drug development against Alzheimer's disease. Int J Biomed Pharm Sci. 2007; 1(2): 83-104.
33. Calderon A.I., Cubilla M., Espinosa A., Gupta M.P. Screening of plants of Amaryllidaceae and related families from Panama as sources of acetylcholinesterase inhibitors. Pharm biol. 2010; 48(9): 988-993.
34. Ding Y., Qu D., Zhang K., Cang X., Kou Z., Xiao W., Zhu J. Phytochemical and biological investigations of Amaryllidaceae alkaloids: a review. J Asian Nat Prod Res. 2017. 19(1):53-100.
35. Marston A., Van der Westhuizen J., Zietsman P. Investigation of South African Amaryllidaceae for inhibitors of acetylcholinesterase. Planta Med. 2009. 75(09): PC20.
36. Abou-Donia A.H., Shawky E.A., El-Din M.M.M., Takayama H., El Din A.A.S. Bio-guided isolation of acetylcholinesterase inhibitory alkaloids from the bulbs of Crinum bulbispermum. Nat Prod: An Indian J. 2012; 8(3):107-114.
37. Elgorashi E., Stafford G., Van Staden J. Acetylcholinesterase enzyme inhibitory effects of amaryllidaceae alkaloids. Planta Med. 2004; 70(3):260-262.
38. Houghton P.J., Agbedahunsi J.M., Adegbulugbe A. Choline esterase inhibitory properties of alkaloids from two Nigerian Crinum species. Phytochemistry. 2004; 65(21):2893-2896.
39. Martha Perez Gutierrez R., Maria Neira Gonzalez A., Hoyo-Vadillo C. Alkaloids from piper: a review of its phytochemistry and pharmacology. Mini Rev Med Chem. 2013; 13(2):163-193.
40. Ndam L., Mih A., Fongod A., Tening A., Tonjock R., Enang J., Fujii Y. Phytochemical screening of the bioactive compounds in twenty (20) Cameroonian medicinal plants. Int J Curr Microbiol App Sci. 2014; 3(12): 768-778.
41. Togola A., Austarheim I., Theïs A., Diallo D., Paulsen B.S. Ethnopharmacological uses of Erythrina senegalensis: a comparison of three areas in Mali, and a link between traditional knowledge and modern biological science. J. Ethnobiol Ethnomed. 2008; 4(1):6.
42. Simplice F.H., Abdou B.A., Abaissou N., Hervé H., Lucy M.F., Annabel M.N., Nyenti S., Neh P., Acha A.E. Neuroprotective and memory improvement effects of a standardized extract of Emilia coccinea (SIMS) G. on animal models of anxiety and depression. J Pharmacog Phytochem. 2014; 3(3):146-154.
43. Shakir T., Coulibaly A.Y., Kehoe P.G. An exploration of the potential mechanisms and translational potential of five medicinal plants for applications in Alzheimer's disease. Am J Neurodegener Dis. 2013; 2(2):70-88.
44. Biradar S., Joshi H.K. Psychopharmacological investigations on the benefits of Ageratum conyzoides in the modulation of neurodegenerative disorder of Alzheimer's type. Int J Green Pharm (IJGP). 2011; 5(3).
45. Yuste J.E., Tarragon E., Campuzano C.M., Ros-Bernal F. Implications of glial nitric oxide in neurodegenerative diseases. Front Cell Neurosci. 2015; 9(322).
46. Yang E-J., Yim E-Y., Song G., Kim G-O., Hyun C-G. Inhibition of nitric oxide production in lipopolysaccharide-activated RAW 264.7 macrophages by Jeju plant extracts. Interdisciplinary Toxicol. 2009; 2(4): 245-249.
47. Joo T., Sowndhararajan K., Hong S., Lee J., Park S-Y., Kim S., Jhoo J-W. Inhibition of nitric oxide production in LPS-stimulated RAW 264.7 cells by stem bark of Ulmus pumila L. Saudi J Biol Sci. 2014; 21(5): 427-435.
48. Owona B.A., Njayou N.F., Laufer S., Moundipa P.F., Schluesener H.J. A fraction of stem bark extract of Entada africana suppresses lipopolysaccharide-induced inflammation in RAW 264.7 cells. J Ethnopharmacol. 2013; 149(1):162-168.
49. Eleftheriadis N., Poelman H., Leus N.G.J., Honrath B., Neochoritis C.G., Dolga A., Dömling A., Dekker F.J. Design of a novel Thiophene Inhibitor of 15-Lipoxygenase-1 with both Anti-inflammatory and Neuroprotective Properties. Eur J Med Chem. 2016; 122:786-801.
50. Van Leyen K., Arai K., Jin G., Kenyon V., Gerstner B., Rosenberg P.A., Holman T.R., Lo E.H. Novel Lipoxygenase Inhibitors as Neuroprotective Reagents. J Neurosci Res. 2008; 86(4): 904-909.
51. Praticò D., Sung S. Lipid Peroxidation and Oxidative imbalance: Early functional events in Alzheimer's disease. J. Alzheimer's Dis. IOS Press 2004; 6: 171-175
52. Butterfield D.A., Lauderback C.M. Lipid peroxidation and protein oxidation in Alzheimer's Disease brain. Potential causes and consequences involving Amyloid -β peptide associated free radical oxidative stress. Free Rad Biol Med. 2002; 32(11): 1050 -1060.
53. Dimitrios B. Sources of natural phenolic antioxidants. Trends Food Sci Technol. 2006; 17:505–512.
54. Ogunlade I., Awosanmi I.A., Osukoya O.A. Antioxidant activity and total phenolic content of some nuts commonly consumed in South-Western Nigeria. J Phytopharmacol. 2014; 3(4):248-253.
55. Oboh G., Akinyemi A.J., Omojokun O.S., Oyeleye I.S. Anticholinesterase and antioxidative properties of aqueous extract of Cola acuminata seed in vitro. Int J Alzheimer's Dis. Vol. 2014, Article ID 498629, 8 pages, 2014.
56. Lowe H.I., Watson C.T., Badal S., Peart P., Toyang N.J., Bryant J. Promising efficacy of the Cola acuminata plant: a mini review. Adv Biol Chem. 2014; 4:240-245
57. Jitin Ahuja, J. Suresh, A. Deep, Madhuri, Pratyusha, Ravi. Phytochemical Screening of Aerial Parts of Artemisia parviflora Roxb.: A medicinal plant. Der Pharmacia Lett. 2011; 3 (6):116-124.
58. Costa S.L., Silva V.D.A., dos Santos Souza C., Santos C.C., Paris I., Muñoz P., Segura-Aguilar J. Impact of plant-derived flavonoids on neurodegenerative diseases. Neurotox Res. 2016; 30(1): 41-52.