East African Scholars Journal of Medical Sciences
Abbreviated Key Title: East African Scholars J Med Sci
ISSN: 2617-4421 (Print) & ISSN: 2617-7188 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-7 | Issue-2 | Feb-2024 | DOI: 10.36349/easms.2024.v07i02.001

Original Research Article

Anti-Oxytotic/Ferroptotic Neuroprotection by Medicinal Plants from Côte d'Ivoire

Maher Pamela[1], Kipré Gueyraud Rolland[2*], Bla Kouakou Brice[2], Offoumou M'Bai Rostand[2], Currais Antonio[1], Djaman Allico Joseph[2, 3]

[1]Cellular Neurobiology Laboratory, The Salk Institute for Biological Studies, 10010 N. Torrey Pines Rd. La Jolla, CA 92037, USA
[2]Laboratoire de Biologie et Santé, UFR of Biosciences, University of Felix Houphouët-Boigny, de Cocody, 22 BP 582 Abidjan 22, Côte d'Ivoire
[3]Laboratoire de Biochimie, Institut Pasteur de Côte d'Ivoire, 01 BP 490 Abidjan 01, Côte d'Ivoire

Article History
Received: 13.12.2023
Accepted: 17.01.2024
Published: 01.02.2024

Journal homepage:
https://www.easpublisher.com

Quick Response Code

Abstract: Dementia in sub-Saharan Africa has been largely underestimated, but the cases are expected to increase substantially as African countries are experiencing highrates of growthin older people. As conventional medicine is expensive and often inaccessible, the majority of the communities in Africa rely on traditional medicine for basic health care. Plants may be a valuable source of neurotherapeutics, particularly of inhibitors of oxytosis/ferroptosis, a neurodegenerative pathway associated with dementia. This study evaluated the anti-oxytotic/ferroptotic activity of different plants used in the traditional medicine of Côte d'Ivoire. Ten plant species (*Adenia cissampeloides*, *Adenia lobata*, *Entada mannii*, *Enantia polycarpa*, *Harungana madagascariensis*, *Kigelia africana*, *Terminalia ivorensis*, *Terminalia mantaly*, *Terminalia superba* and *Vernonia amygdalina*) were selected based on their traditional use and the respective parts were collected in the Agboville region of South-eastern Côte d'Ivoire. Extracts were prepared by maceration in water and tested for protection in a nerve cell culture model of oxytosis/ferroptosis. The neuroprotective effects of the extracts werefurther evaluated in additional cell-based assays, including intracellular Aβ toxicity, energy loss, inflammation and neurite differentiation. *Terminalia ivorensis*, *Terminalia mantaly* and *Terminalia superba* offered the best protection overall in the assays. They provided strong inhibition of oxytosis/ferroptosis as well as excellent protection against intracellular Aβ toxicity and energy loss. Additional studies are required to confirm the efficacy of these plants as neurotherapeutics, but the findings highlight the potential of plants used in the traditional medicine of Côte d'Ivoire to provide new treatments for dementia.

Keywords: Dementia, ethnopharmacology, inflammation, redox stress, traditional medicine, *Terminalia superba*.

INTRODUCTION

The aging of the population is a phenomenon that is gaining pace globally and that exposes everyone to an increased risk of diseases that are associated with the progressive degeneration of the body with age. A particularly challenging set of these diseases is Alzheimer's disease (AD) and related dementias, which result in severe impairments in memory, thinking and social abilities as a consequence of pathological biochemical changes in the central nervous system.

Historically, dementia in sub-Saharan Africa has been largely overlooked. This is partly explained by limitations on the diagnostic methodology, types of study settings and geographical coverage (Akinyemi *et al.*, 2022). While a lot of attention has been given to infectious diseases, non-communicable diseases such as dementia have not been a priority for governments (Guerchet *et al.*, 2017). This is despite the fact that Africa has the highest rates of human immunodeficiency virus (HIV) in the world, and that HIV patients are at an increased risk of developing cognitive dysfunction and dementia (Akinyemi *et al.*, 2022). In addition, there still

***Corresponding Author:** Kipré Gueyraud Rolland
Laboratoire de Biologie et Santé, UFR of Biosciences, University of Felix Houphouët-Boigny, de Cocody, 22 BP 582 Abidjan 22, Côte d'Ivoire

is a strong stigma among the communities towards older people with mental illnesses, which sometimes is regarded as witchery. Along with a paucity of information, all these factors translate into a lot of the dementia cases simply going undiagnosed.

It is estimated that the current prevalence of dementia in sub-Saharan people aged 60 years and over is higher than 6% (Guerchet *et al.*, 2017). But as sub-Saharan African countries are experiencing some of the fastest growth rates in older people worldwide, these countries are projected to have a rapid increase in the number of people living with dementia in the coming decades (Guerchet *et al.*, 2017). Therefore, more basic and translational research on dementia is needed in sub-Saharan Africa so that health policy decisions can be implemented adequately in the future.

As conventional medicine is expensive and often inaccessible, the majority of the rural African communities rely on traditional medicine for basic health care. This is particularly noticeable in Western Africa, where the knowledge of medicinal plants and their therapeutic effects has been preserved by the population at large and specifically by the traditional medical practitioners, who maybe consulted for treatment of a variety of ailments. Herbal medicines are central in traditional medicine, and they may be prepared from whole plants or parts of plants, including leaves, bark, berries, flowers and roots (Ozioma and Nwamaka Chinwe 2019).

Côte d'Ivoire is one of the most biodiverse countries in Western Africa as it shares the Guinean forests of West Africa, one of the biodiversity hotspots in the world. Several ethnobotanical surveys have been conducted in the country (Kerhar and Bouquet 1950; Adjanohoun and Aké Assi 1979; Vangah-Manda 1986; N'guéssan 1995; Konéet *al.*, 2002).The present study took place with plants collected in the South-eastern part of the country, in the District of Lagunes, more precisely in the Department of Agboville, located just North of Abidjan (Figure 1). The Department of Agboville is composed of 103 villages and has a population of 220,050 inhabitants (SODEFOR 1999). The largest indigenous populations are Abbey and Krobou, two ethnic entities of the Akan group, in the larger Kwa group (Sournia and Arnaud, 1978). The Abbey and Krobou are mostly landowners and farmers, but some devote themselves to hunting, fishing, sculpture, and a few work as traditional healers. The original vegetation in the region was composed of dense, evergreen humid forests (Avenard *et al.*, 1971). Today, highly degraded by agricultural and forestry exploitation, it is characterized by a dense, semi-deciduous humid forests (N'guéssan 2008) and diverse crops of coffee, cocoa, rubber and bananas. However, there still are classified forests where protected flora and a fauna can be found. Despite the rich traditional medicinal knowledge in Côte d'Ivoire, rigorous scientific experimentation must be conducted so that the ethnopharmacological value of its herbal medicines can be evaluated.

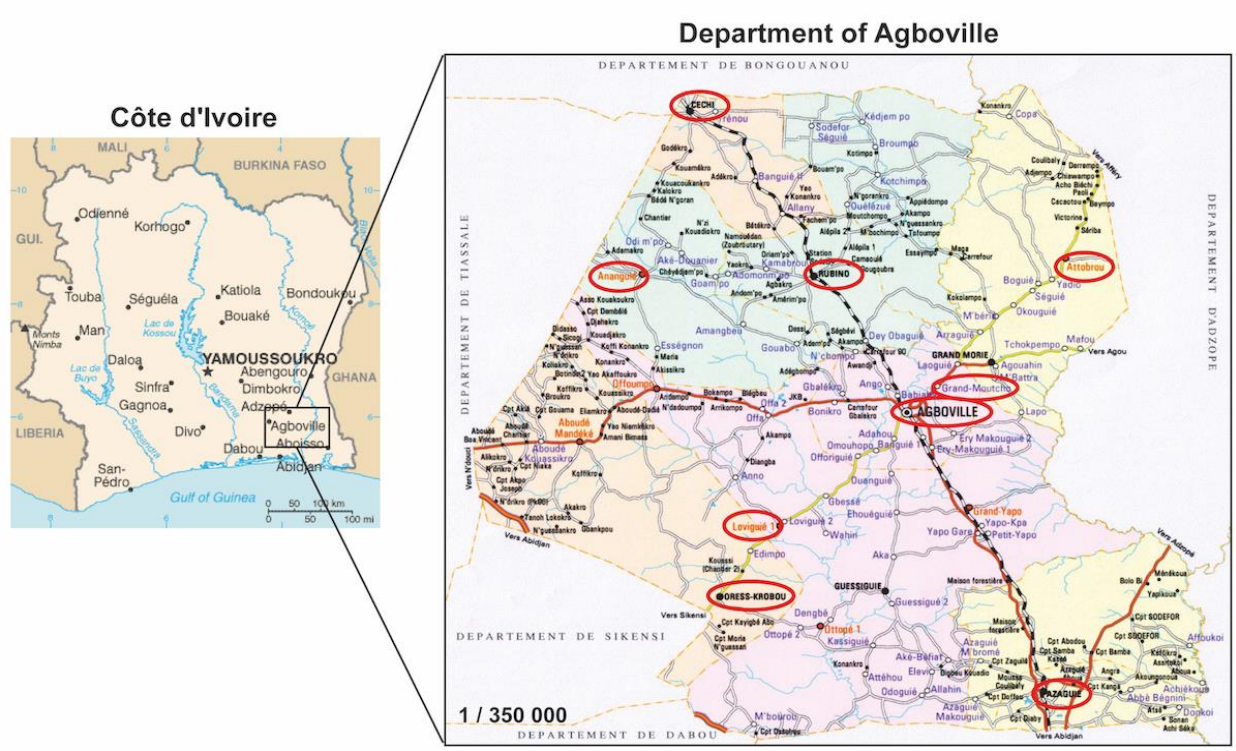

Figure 1: Geographical and administrative context of the Department of Agboville in Côte d'Ivoire. The specific locations where the plants were collected are indicated with red circles (Agboville, Ananguié, Attobrou, Azaguié, Cechi, Grand-Moutcho, Loviguié, Oress-Krobou, Rubino)

Because old age is the greatest risk factor for AD and related dementias, we have been combining cell culture and animal models of brain aging to understand AD and to develop medicines that are derived from natural products. These efforts have identified a unique mechanism of neurodegeneration called oxytosis/ferroptosis as a possible link between aging and AD (Lewerenz *et al.*, 2018; Maher *et al.*, 2020a). Oxytosis/ferroptosis is a form of non-apoptotic regulated cell death characterized by glutathione (GSH) depletion and dysregulated production of reactive oxygen species (ROS) from mitochondria that results in lethal lipid peroxidation. All of these changes are detected in the brain with aging and exacerbated in AD (Currais and Maher 2013; Maher *et al.*, 2020a), where oxytosis/ferroptosis may manifest itself over an extended time period thereby offering a significant window for therapeutic intervention. We have shown that inhibitors of oxytosis/ferroptosis are not only protective in transgenic mouse models of AD (Ates *et al.*, 2020; Currais *et al*., 2014b) but also prevent dementia in SAMP8 mice (Currais *et al.*, 2015; Currais *et al.*, 2019), a model of accelerated aging.

Plants display a great diversity of biochemicals to deal with physiological stresses, some of which are relevant to human medicine. Importantly, the oxytosis/ferroptosis pathway and associated toxic lipid peroxidation also occur in plants and many natural products are likely made to overcome these stresses (Conrad *et al.*, 2018; Soriano-Castell *et al.*, 2021b). We have now demonstrated that inhibitors of oxytosis/ferroptosis can be identified from libraries of plant compounds (Soriano-Castell *et al.*, 2021a), plant extracts (Fischer *et al.*, 2019), herbarium collections (Maher *et al.*, 2020b)and plants used in traditional medicine (Currais *et al.*, 2014a). Once identified, these compounds are tested in animal models and used to uncover regulatory molecular pathways. Therefore, the potential for discovering new anti-oxytotic/ferroptotic compounds from plants is immense.

In the present study, ten plant species that are used in the traditional medicine of Western Africa and Côte d'Ivoire to treat symptoms relevant to aging and AD were evaluated for protection against oxytosis/ferroptosis in nervecell culture. Additional assays were used to test the effects of extracts from these plants against other toxicities, such as toxic intracellular accumulation of amyloid beta (Aβ), energy loss and inflammation. These findings result in one of the first seminal studies to investigate the value of medicinal plants from Côte d'Ivoire to treat dementia.

MATERIAL AND METHODS

All reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise stated.

Plant material

The fresh stem/bark from *Adenia cissampeloides* (AC), *Adenialobata* (AL), *Entada mannii* (EM), *Enantia polycarpa* (EP), *Kigelia africana* (KA), *Terminalia ivorensis* (TI), *Terminalia mantaly* (TM) and *Terminalia superba* (TS), and fresh leaves from *Harungana madagascariensis* (HM) and *Vernonia amygdalina* (VA) were collected in the Agboville region of South-eastern Côte d'Ivoire between November and December 2020 (Table 1). The identification and authentication were done by a botanist from the National Floristic Center (CNF) at the University of Felix Houphouët-Boigny. The nomenclature follows the African plant database (APD) and PROTA4U (PROTA4U).

Table 1: Descriptions of the medicinal plants used in the study

ID	Botanical name	Family	Common name	Part used	Indications
AC	*Adenia cissampeloides* (Planch. ex Hook.) Harms	Passifloraceae	Ekêlé	Stem + Bark	Anaemia, cholera, depression, fever, gastro-intestinal disorders, inflammatory illnesses, insanity, malaria, pain, respiratory problems, rheumatism.
AL	*Adenia lobata* (Jacq.) Engl.	Passifloraceae	Ayêlêgnaman	Stem + Bark	Fever, insanity,malaria,pain, respiratory problems.
EM	*Entada mannii* (Oliv.) Tissèrent	Mimosaceae	Poûta	Bark	Diabetes, high blood pressure, malaria.
EP	Enantia polycarpa (DC.) Engl. et Diels	Annonaceae	Pkawouê	Bark	Fever, jaundice, leprosy, malaria, ophthalmia, skin infections, sores, ulcers.
HM	*Harungana madagascariensis* Lam. ex Poir.	Hypericaceae	Ennvi-vi/ Wombê	Leaves	Anemia, angina, asthma, diarrhea, dysentery, fever, gonorrhea, malaria, parasitic skin diseases, syphilis, tuberculosis, wounds.
KA	*Kigelia africana* (Lam.) Benth.	Bignoniaceae	Gborô	Bark	Anaemia, epilepsy, bacterial and fungal infections, fainting, gastric disorders, gynaecological disorders, hepatic and cardiac disorders, infectious diseases, malignant neoplasms, pain, respiratory problems, rheumatism, skin problems, weakness, wounds.

ID	Botanical name	Family	Common name	Part used	Indications
TI	*Terminalia ivorensis* A. Chev.	Combretaceae	Gbô-ti	Bark	Haemorrhoids, malaria, pain, rheumatism, sores, ulcers, wounds, yellow fever.
TM	*Terminalia mantaly*	Combretaceae	Etagedyirini (Bambara)	Bark	Cutaneous and genital problems, diabetes, dysentery, gastroenteritis, hypertension, infections, oral problems.
TS	*Terminalia superba*	Combretaceae	Pai	Bark	Aphthae, analgesic, bronchitis, diarrhea, dysentery, gingivitis, haemorrhoids, malaria, ovarian problems, sores, swellings, vomiting, wounds.
VA	*Vernonia amygdalina* Delile	Asteraceae	Abowé/ Wahorvi	Leaves	Cough, diarrhea, dysentery, fever, hepatitis, malaria, pain.

Preparation of extracts

Dried material was powdered and 100 g of vegetable powder were macerated in 1 L of distilled water for 24 hours at room temperature. The homogenate obtained was filtered once with cloth, three times with absorbent cotton and once with Whatman 3 mm paper. The filtrate obtained was then dried in a "Venticel" oven at 55°C for 48 hours. The dried product was solubilized in water at 50 mg/mL. Samples were frozen at -80 ºC for long-term storage.

Screening assays

Cell culture:

HT22 mouse hippocampa l nerve cells and MC65 human neuroblastoma cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA, United States) supplemented with 10% fetal calf serum (FCS) (Hyclone, Logan, UT, United States), and incubated at 37ºC in an atmosphere with 10% CO_2. BV2 mouse microglial cells were grown in low glucose DMEM supplemented with 10% FCS, and incubated in similar conditions. PC12 rat pheochromocytoma cells were grown in high-glucose DMEM supplemented with 10% FCS and 5% horse serum.

Oxytosis/ferroptosis:

5 x 10^3 HT22 cells were plated per well in 96 well plates. After 24 h of culture, the medium was exchanged with fresh medium, and 5 mM glutamate or 500 nM RSL3 were added alone or in combination with the plant extracts at the indicated concentrations, as previously described (Maher *et al.*, 2020b; Soriano-Castell *et al.*, 2021a). 24 h later, the cellular viability was measured by the 3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay. In the absence of a protective extract ≥ 90% of the cells die under these conditions. In all cases, cells in the dishes were examined microscopically before the addition of the MTT reagent to ensure that any positive results in the MTT assay are not an artifact due to interaction of the extracts with the assay chemistry.

Intracellular Aβ toxicity:

MC65 cells were regularly grown with 2 µg/mL tetracycline (Soriano-Castell *et al.*, 2021a). For the assay, cells were dissociated, plated at 4 × 10^5 cells per 35 mm tissue culture dish and grown for 24 h. The next day, the cells were washed with PBS and placed in Opti-minimal essential media (Opti-MEM, Invitrogen) in the presence (no induction) or absence (APP-C99 induced) of 2 µg/mL tetracycline in combination with the plant extracts. At day 3, the control cells in the absence of tetracycline were dead, and cell viability was determined by the MTT assay and confirmed by visual inspection.

Protection against energy loss:

HT22 cells were seeded onto 96 well plates as described in the oxytosis/ferroptosis assay. The medium was exchanged 24 h later with fresh medium and the cells were treated with 15 µM iodoacetic acid (IAA) alone (which results in 90–95% cell death) or in combination with the plant extracts at the indicated concentrations (Soriano-Castell *et al*., 2021a). After 2 h, the medium was replaced with fresh medium without IAA but containing the compounds. 24 h later, the cellular viability was measured by the MTT assay.

Inflammation:

BV2 cells were plated at 5 × 10^5 cells in 35 mm tissue culture dishes (Maher *et al*., 2020b). After growth overnight, the cells were treated with 25 µg/mL bacterial lipopolysaccharide (LPS) alone or in the presence of the extracts. After 24h, the medium was removed, spun briefly to remove floating cells and 100 µL assayed for nitrite using 100 µL of the Griess Reagent in a 96 well plate. After incubation for 10 min at room temperature the absorbance at 550 nm was read on a microplate reader. The absorbance was normalized to the cell viability as determined using the MTT assay.

PC12 differentiation:

PC12 cells were plated in 35 mm tissue culture dishesand the assay carried out as described previously (Currais *et al*., 2014a). Briefly, after 3 days of growth, the medium was replaced with serum-free N2 medium (Invitrogen, Carlsbad, CA, USA) and the cells were treated with the extracts. After 24 h, the cells were scored for the presence of neurites. PC12 cells produce neurites much more rapidly when treated in N2 medium than when treated in regular growth medium. For each treatment, 100 cells in each of three separate fields were counted. Cells were scored positive if one or more

neurites longer than one cell body diameter in length were observed.

Statistical analysis

The EC_{50}s were determined from sigmoidal dose response curves using GraphPad Prism 9. Experiments were done at least three independent times.

RESULTS AND DISCUSSION

RESULTS

Plant selection and extract preparation

In this study, ten species of plants used in the traditional medicine of Côte d'Ivoire were selected to be evaluated for their protection against toxic insults that are characteristic of aging and AD. The selection criteria were mostly based on the reported therapeutic effects of these plants on ailments that may have translational value to dementia, such as memory problems, headaches, vision decay, neuropathies, different types of pain, paralysis and movement disorders (Soriano-Castell *et al.*, 2021b). Diseases associated with old age were also considered, such as arthritis, body pain, frailty, heart and kidney problems, inflammation and rheumatism. This approach rendered the following plants that were harvested in the Department of Agboville of Côte d'Ivoire (Figure 1) but that can be found in other Western African countries (Figure 2).

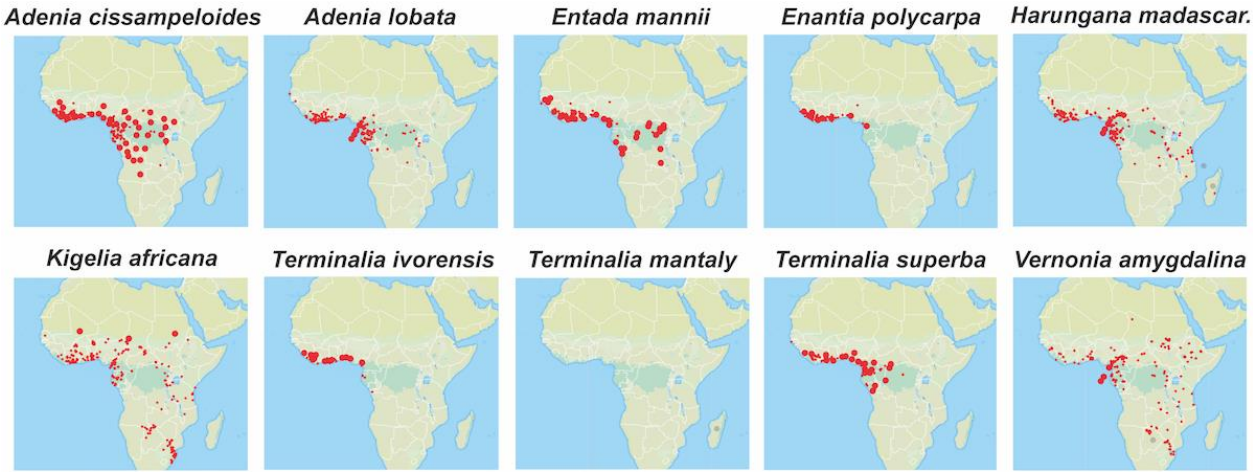

Figure 2: Distribution across Africa of the different plant species used in this study (APD). *Terminalia mantaly* is endemic to Madagascar but it has been introduced into Western Africa

Adenia cissampeloides (AC) is a robust liana up to many meters long with numerousapplications in traditional medicine. Its roots, stems and leaves are commonly used in infusions or decoctions to treat gastro-intestinal disorders (abdominal pain, constipation, diarrhoea and dysentery), inflammatory illnesses, respiratory problems, fever, malaria, cholera, anaemia, and various forms of pain such as rheumatism, headaches and back pain (PROTA4U). It can also be used as a stimulant to treat depression and insanity.

Adenia lobata (AL) is also a large liana, whose leaf decoction is used to treat fever,insanity, cough and bronchitis (PROTA4U). In Côte d'Ivoire the leaves are eaten with palm oil and salt to treat palpitations. The leaf sap is used against rheumatic, rib and abdominal pains. The stem sap is taken to treat gastrointestinal problems, headaches, neck pain and ear inflammation. Other treated ailments include bacterial infections and malaria.

Entada mannii (EM) is a shrub that can be scandent or arborescent (JSTOR). Its leaves and bark are used to treat diabetes, high blood pressure and malaria in Côte d'Ivoire (Kassi Bosson *et al.*, 2020).

Enantia polycarpa (EP) is a tree whose bark is used for preparing various traditional medicines. Its bark decoction is used to treat sores, ulcers, leprosy, ophthalmia, skin infections,jaundice, fever and malaria (PROTA4U).

Harungana madagascariensis (HM) is a small tree. Its leaves and bark are used to treat anemia, asthma, tuberculosis, fever, angina, diarrhea, dysentery, syphilis, gonorrhea, malaria, parasitic skin diseases, and wounds, with analgesic and anti-inflammatory activities (Iwalewa *et al.*, 2009).

Kigelia Africana (KA) is a tree with a wide distribution in Africa (Figure 2) with considerable pharmacological properties that have been studied due to its medicinal applications. Its roots, bark, leaves, stems and fruits can all be used, mostly through decoctions or topical ointments, to treat gastric disorders, skin problems, wounds, fainting, anaemia, sickle-cell anaemia, gynaecological disorders, epilepsy, respiratory ailments, infectious diseases, hepatic and cardiac disorders, bacterial and fungal infections, malignant neoplasms and weakness (PROTA4U). The leaves are sometimes used to prepare a tonic for general health. In Côte d'Ivoire, renal and bladder problems are treated with medicine containing the bark and leaves of KA

combined with other medicinal plants. KA is also used for their analgesic and anti-inflammatory properties to relieve rheumatism, sprains, haematoma, bruising, toothaches and headaches.

Terminalia ivorensis (TI) is a medium-sized to large tree whose bark decoctions or powdered bark are used in traditional medicine to treat wounds, sores, ulcers, haemorrhoids, malaria, yellow fever, rheumatism and muscular pain (PROTA4U).

Terminalia mantaly (TM) is a tree endemic to Madagascar but it has been introduced into Western Africa and Côte d'Ivoire. It is used against diverse infections, dysentery, gastroenteritis, hypertension, diabetes, and oral, dental, cutaneous and genital problems (Tchuente Tchuenmogne *et al.*, 2017). Studies on the extracts of this plant have shown antibacterial and antifungal activities.

Terminalia superba (TS) is a tree widespread in Western and Central Africa (Figure 2). Bark decoctions and macerations are used to treat wounds, sores, haemorrhoids, diarrhoea, dysentery, malaria, vomiting, gingivitis, bronchitis, aphthae, swellings and ovarian troubles, and as an analgesic (PROTA4U).

Vernonia amygdalina (VA) is a shrub or small tree whose leaves are consumed in many dishes. In traditional medicine, leaf decoctions are used to treat fever, malaria, diarrhoea, dysentery, hepatitis, cough, headaches and abdominal pain (PROTA4U).

In the present study, the extracts of all these plants were prepared by maceration in water, reflecting the methodsof extraction used in traditional practice.

Determination of anti-oxytotic/ferroptotic activity

Oxytosis/ferroptosis can be triggered by inhibiting cystine uptake via system xc- with glutamate, which subsequently depletes intracellular GSH (Maher *et al.*, 2020a). This leads to inhibition of the GSH-dependent enzyme GSH peroxidase 4 (Gpx4) and activation of lipoxygenases (LOXs). Gpx4 can also be directly inhibited with the chemical RSL3. In both cases, ROS and lipid hydroperoxides are generated, leading to cell death. The plant extracts were screened for their ability to protect HT22 cells from oxytosis/ferroptosis induced by glutamate and RSL3. In order to assess the anti-oxytotic/ferroptotic potency, the half maximal effective concentrations (EC_{50}s) of the extracts were determined (Table 2). No protection was observed with KA and VA at the concentrations tested. On the other hand, TI, TS and TM, which belong to the same genus *Terminalia*, offered by far the best protection of all of the extracts against both glutamate and RSL3, with EC_{50}s within the range of 19-59 µg/mL. The other extracts (AC, AL, EM, EP and HM) also protected, but to a much lesser extent.

Table 2: Biological activity in the oxytosis/ferroptosis assay

ID	Glutamate EC_{50} (µg/mL)[a]	RSL3 EC_{50} (µg/mL)[a]
AC	498±68	> 500
AL	219 ±30	340 ±28
EM	178 ±39	249 ±14
EP	279 ±50	511 ±43
HM	558 ±24	490 ±40
KA	no[b]	no[b]
TI	29 ±1	19 ±1
TM	41 ±1	45 ±6
TS	59 ±8	42 ±2
VA	no[b]	no[b]

[a] EC_{50} (µg/mL) – half maximal effective concentration.
[b] no – no effect.

Assessment of additional bioactivities relevant to neuroprotection

We have hypothesized previously that drug candidates that possess multiple biological activities against different toxicities associated with aging and AD offer a greater therapeutic advantage to fight the physiological complexity of the disease (Prior *et al.*, 2014). Therefore, we tested the plant extracts in additional cell culture assays that mimic other toxicities that can be found in the brains of AD patients. These are intracellular Aβ toxicity, energy loss and inflammation (Table 3). In addition, we also assessed the potential of the extracts to induce neurite outgrowth in PC12 cells.

A significant body of evidence indicates that Aβ accumulates within neurons of AD patients before the appearance of plaques, and that this accumulation may play a central role in driving the disease (Currais *et al.*, 2017). To determine the effects of the plant extracts on intracellular Aβ toxicity we used the MC65 nerve cell model. MC65 cells expresses the C99 fragment of the amyloid precursor protein (APP) under the control of a tetracycline-sensitive promoter (Sopher *et al.*, 1994). When tetracycline is withdrawn, cells express C99 which is then converted to Aβ by γ-secretase and the cells die within several days due to Aβ aggregation within the cells. As shown in Table 3, all plant extracts protected the cells to some degree. However, similar to the oxytosis/ferroptosis assay, TI, TM and TS showed the best protection against Aβ toxicity. EM was also very protective, at similar levels to the *Terminalia*.

Table 3: Biological activity in the intracellular Aβ toxicity, energy loss, inflammation and neurite differentiation assays

ID	Aβ toxicity EC_{50} (µg/mL)[a]	Energy loss EC_{50} (µg/mL)[a]	Inflammation EC_{50} (µg/mL)[a]	Differentiation EC_{50} (µg/mL)[a]
AC	53.81± 6.59	452 ± 49	no[b]	no[b]
AL	8.62 ± 3.73	217 ± 10	no[b]	no[b]
EM	0.97 ± 0.29	367 ± 17	161 ± 66	no[b]
EP	18.34± 6.34	no[b]	no[b]	no[b]
HM	4.80± 0.57	464 ± 41	no[b]	no[b]
KA	12.59± 1.23	437 ± 26	369 ± 21	no[b]
TI	0.75± 0.02	197 ± 10	no[b]	no[b]
TM	0.40± 0.08	176 ± 6	99 ± 11	no[b]
TS	0.53± 0.09	210 ± 46	no[b]	no[b]
VA	11.95± 3.25	no[b]	83 ± 2	125 ± 25

[a] EC_{50} (µg/mL) – half maximal effective concentration.
[b] no – no effect.

Energy metabolism in the brain decreases with age and is associated with nerve cell damage and death in AD (Currais 2015). The loss of energy can be mimicked using an *in vitro* ischemia model, in which HT22 cells are treated with IAA, a well-known irreversible inhibitor of the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Soriano-Castell *et al.*, 2021a). The effects of the extracts in this model were evaluated. Again, extracts from TI, TM and TS protected HT22 cells the best against energy depletion (Table 3). Although not as good, with the exceptions of EP and VA that had no effect, the rest of the extracts also offered some protection.

Inflammation is a major feature in AD (Wyss-Coray and Rogers, 2012). Activated brain microglia have been implicated in the pathogenesis of AD, as they produce a wide array of pro-inflammatory and cytotoxic factors, including cytokines, that may work in concert to promote neurodegeneration. Thus, inhibiting the activation of microglia is another important therapeutic target. Most of the plant extracts were not active in this assay (Table 3). Only EM, KA, TM and VA were able to prevent the production of nitric oxide by microglia stimulated with LPS.

Connections between nerve cells are impaired in AD. Thus, promoting the regeneration of these connections might be of particular benefit. As a model for this property, we use neurite outgrowth in PC12 cells, a well-studied model system of neuronal differentiation. In response to neurotrophic factors such as nerve growth factor (NGF), PC12 cells undergo a series of physiological changes culminating in a phenotype resembling that of sympathetic neurons (Keegan and Halegoua, 1993). With the exception of VA, no other extract induced the differentiation of PC12 cells (Table 3).

Overall, our data show that, out of the ten different plants studied, the three species belonging to the genus *Terminalia* – TI, TM and TS – showed the best activities in our assays although no extract was effective in all of the assays.

DISCUSSION

In this study, we evaluated the neuroprotective properties of ten plants used in the traditional medicines of Western Africa and Côte d'Ivoire. The selection of these plants was based on their historical use to treat symptoms relevant to aging and AD, and the preparation of their extracts for testing took into account the traditional methods. It is shown that three plants belonging to the genus *Terminalia*– TI, TM and TS – offered the best protection overall in our assays.

Some of the most common methods of preparing medicines involve the extraction of the active components using water through decoctions or macerations. Therefore, it is important to generate extracts for pharmacological testing using similar solvents to ensure that the plant secondary metabolites (PSMs) in the extract resemble more precisely those that are present in the traditional medicine. This allows the investigation of the value of these medicines in a specific disease context. In this study, all plant extracts were prepared by maceration in water. We have also purposely selected the plant parts for extraction that correspond to the ones used in thepractice.

Our primary screening assay for identifying neuroprotective therapeutics relies on the oxytosis/ferroptosis pathway, a key cell death mechanism that we think may bridge the contribution of aging to AD (Lewerenz *et al.*, 2018; Maher *et al.*, 2020a). Since this pathway is characterized by endogenous ROS production and lipid peroxidation, the fact that TI, TM and TS strongly inhibited oxytosis/ferroptosis in HT22 cells, indicates that they may be preventing the redox dysregulation in this model. In order to understand how TI, TM and TS may be working, future experiments should address this, as well as their effects on the levels of intracellular GSH.

In previous reports, we have tested extracts from plants and showed that neuroprotective compounds can be identified fromthe extractsthat are potent and have EC_{50}s ranging 0.3-50 μg/mL in the oxytosis/ferroptosis assay (Currais *et al.,* 2014a; Fischer *et al.,* 2019; Maher *et al.,* 2020b). The anti-oxytotic/ferroptotic activities of TI, TM and TS that we measured were within this range. It should be noted that the plant extracts in our previous studies were prepared using either ethanol or dichloromethane, which are solvents stronger than water in addition to extracting a set of PSMs that differ in polarity.

Given the growing role that oxytosis/ferroptosis is being reported to play in a diversity of human diseases, the value of having new therapeutics that target this pathway cannot be overstated. Features of oxytosis/ferroptosis have also been observed in heart, liver, vascular and kidney diseases (Han *et al.,* 2020; Li *et al*., 2020). Oxytosis/ferroptosis has been described in rodent models of ischaemia/reperfusion injury in the heart (Gao *et al.*, 2015; Fang *et al.*, 2019), liver (Friedmann Angeli *et al.,* 2014; Wang *et al*., 2019), acute kidney injury (Friedmann *et al.,* 2014; Martin-Sanchez *et al.,* 2017), and haemolytic disorders (NaveenKumar *et al.,* 2018). Therefore, the value of plant-based oxytosis/ferroptosis inhibitors may thus extend beyond diseases of the nervous system.

TI, TM and TS were also the most effective extracts in the intracellular Aβ toxicity assay. We have recently demonstrated a mechanistic overlap between this toxicity and oxytosis/ferroptosis (Huang *et al.,* 2020). Therefore, our results with TI, TM and TS were not completely surprising. It is also interesting that the three *Terminalia* offered the best protection against the loss of energy induced by the IAA. Therefore, it is possible that some of the active PSMs in these extracts are common to the three species. Future studies could address this question through a combination of fractionation, re-testing and compound identification with mass spectrometry and nuclear magnetic resonance, as we have described before (Soriano-Castell *et al.,* 2021b). It would also be important to test other *Terminalia* species. For instance, *Terminalia chebula* Retz is widely used in traditional Indian and Iranian medicine to treat dementia (Das *et al.,* 2020).

One of the clearest therapeutic effects of traditional medicines that can be detected is anti-inflammatory action. This is because the inflammatory process is often acute and can be visually monitored. Although our assay for inflammation uses brain microglia, since these cells behave similarly to other tissue-specific macrophages, the assay is also a good surrogate for inflammation in general that is caused by a bacterial pathogen (mimicked by the LPS). Of all the plants tested, TM and VA performed the best. Because TI and TS showed no inhibition of inflammation in culture, it is possible that the effects of TM are explained by PSMs specific to this species. This illustrates the type of pharmacological information that could be additionally used to select candidate plants in the future for testing in patients. The lack of anti-inflammatory activity observed with the other plant extracts, even though they may have traditional medical usages associated with inflammatory illnesses, could be explained by the fact that many of these plants are also excellent antibacterial agents. So, their effect could be not on the inflammatory processes directly but rather upon the primary causes related to the infection.

In summary, the present studies confirm the value of plants used in the traditional medicine of Côte d'Ivoire as a potential source for new neuroprotective therapeutics to treat dementia and possibly other diseases of old age. More studies are necessary and should focus on screening additional species. The identification of the active compounds would also be an important step, but is not strictly necessary, as the plants are immediately available to the communities and are already likely safe due to their history of administration. Therefore, future research can use the approach described here to identify additional candidate plants that could be immediately tested in human patients. Overall, our main goal is to accelerate the discovery of new treatments for dementia from the available medicinal plants of Western Africa while at the same time raising awareness to this devastating disease.

Funding: This work was supported by the National Institutes of Health (AG067331 for CA; AG069206 for MP) and Africa Green Solution (AGS-2021 for KGR)

ACKNOWLEDGEMENTS

Team researchers work in full partnership with local institutions, traditional healers and communities in order to respectfully conduct research in the area of Indigenous Knowledge, assuring the intellectual property rights and the sharing of benefits that may arise as a result of this study.

Conflict of Interest Statement: The authors declare that there are no conflicts of interest.

List of Abbreviations: Aβ: amyloid beta; AC: *Adenia cissampeloides*; AD: Alzheimer's disease; AL: *Adenialobata*; APD: African plant database; APP: amyloid precursor protein; DMEM: Dulbecco's modified Eagle's medium; EC50: half maximal effective concentration; EM: *Entada mannii*; EP: *Enantia polycarpa*; FCS: fetal calf serum; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; Gpx4: glutathione peroxidase 4; GSH: glutathione; HIV: human immunodeficiency virus; HM: *Harungana madagascariensis*; IAA: iodoacetic acid; KA: *Kigelia Africana*; LOX: lipoxygenase; LPS: lipopolysaccharide; MTT: 3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide; NGF: nerve growth factor; Opti-MEM: opti-minimal essential media; PSM: plant

secondary metabolite; ROS: reactive oxygen species; TI: *Terminalia ivorensis*; TM: *Terminalia mantaly*; TS: *Terminalia superba*; VA: *Vernonia amygdalina*.

REFERENCES

- Adjanohoun, E., & Aké, A. L. (1979). Contribution au recensement des plantes médicinales de la Côte-d'Ivoire. Centre National de Floristique, Côte-d'Ivoire.
- Akinyemi, R. O., Yaria, J., Ojagbemi, A. … & Ogunniyi, A. (2022). Dementia in Africa: Current evidence, knowledge gaps, and future directions. *Alzheimers Dement, 18*(4), 790-809.
- APD, African Plant Database (version 3.4.0) (2022). Conservatoire et Jardin botaniques de la Ville de Genève and South African National Biodiversity Institute, Pretoria, retrieved April 2022, from http://africanplantdatabase.ch.
- Ates, G., Goldberg, J., Currais, A., & Maher, P. (2020). CMS121, a fatty acid synthase inhibitor, protects against excess lipid peroxidation and inflammation and alleviates cognitive loss in a transgenic mouse model of Alzheimer's disease. *Redox Biol,* 36, 101648.
- Avenard, J. M., Eldin, M., Girard, G., … & Perraud, A. (1971). Le milieu naturel de la Côte d'Ivoire. IRD Editions. Document ORSTOM N°50.
- Conrad, M., Kagan, V. E., Bayir, H., … & Stockwell, B. R. (2018). Regulation of lipid peroxidation and ferroptosis in diverse species. *Genes Dev,* 32(9-10), 602-619.
- Currais, A. (2015). Ageing and inflammation - A central role for mitochondria in brain health and disease. *Ageing Res Rev,* 21, 30-42.
- Currais, A., Chiruta, C., Goujon-Svrzic, M. … & Maher, P. (2014a). Screening and identification of neuroprotective compounds relevant to Alzheimers disease from medicinal plants of S. Tome et Principe. *J Ethnopharmacol, 155*(1)**,** 830-840.
- Currais, A., Fischer, W., Maher, P., & Schuber, D. (2017). Intraneuronal protein aggregation as a trigger for inflammation and neurodegeneration in the aging brain. *FASEB J, 31*(1), 5-10.
- Currais, A., Goldberg, J., Farrokhi, C., … & Schuber, D. (2015). A comprehensive multiomics approach toward understanding the relationship between aging and dementia. *Aging (Albany NY), 7*(11), 937-955.
- Currais, A., Huang, L., Goldberg, J., … & Maher, P. (2019). Elevating acetyl-CoA levels reduces aspects of brain aging. *Elife,* 8, e47866.
- Currais, A., & Maher, P. (2013). Functional consequences of age-dependent changes in glutathione status in the brain. *Antioxid Redox Signal, 19*(8), 813-822.
- Currais, A., Prior, M., Dargusch, R., … & Maher, P. (2014b). Modulation of p25 and inflammatory pathways by fisetin maintains cognitive function in Alzheimer's disease transgenic mice. *Aging Cell, 13*(2), 379-390.
- Das, G., Kim, D. Y., Fan, C., … & Patra, J. K. (2020). Plants of the Genus *Terminalia* : An Insight on Its Biological Potentials, Pre-Clinical and Clinical Studies. *Front Pharmacol,* 11, 561248.
- Fang, X., Wang, H., Han, D., … & Wang F. (2019). Ferroptosis as a target for protection against cardiomyopathy. *Proc Natl Acad Sci U S A, 116*(7), 2672-2680.
- Fischer, W., Currais, A., Liang, Z., … & Maher, P. (2019). Old age-associated phenotypic screening for Alzheimer's disease drug candidates identifies sterubin as a potent neuroprotective compound from Yerba santa. *Redox Biol,* 21, 101089.
- Friedmann, A. J. P., Schneider, M., Proneth, B., … & Wang, J. G. (2014). Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nat Cell Biol,* 16(12), 1180-1191.
- Gao, M., Monian, P., Quadri, N., … & Jiang X. (2015). Glutaminolysis and Transferrin Regulate Ferroptosis. *Mol Cell, 59*(2), 298-308.
- Guerchet, M., Mayston, R., Lloyd-Sherlock, P., … & Ezeah, P. (2017). Dementia in sub-Saharan Africa Challenges and opportunities. *Alzheimer's Disease International* (ADI), 1-69.
- Han, C., Liu, Y., Dai, R. I., … & Li, B. (2020). Ferroptosis and Its Potential Role in Human Diseases. *Front Pharmacol,* 11, 239.
- Huang, L., McClatchy, D. B., Maher, P., … & Currais, A. (2020). Intracellular amyloid toxicity induces oxytosis/ferroptosis regulated cell death. *Cell Death Dis, 11*(10), 828.
- Iwalewa, E. O., Suleiman, M.M., Mdee, L. K., & Eloff, J. N. (2009). Antifungal and antibacterial activities of different extracts of *Harungana madagascariensis* stem bark. *Pharmaceutical Biology, 47*(9), 878–885.
- JSTOR, Global plants, https://plants.jstor.org.
- Kassi-Bosson, J. A. J., Doumbia, I.,, Bédou, K. D., … & N'Guessan, J. D. (2020). Evaluation of cardiac biotolerance from total aqueous extract of *Entada mannii* (Fabaceae) at the wistar rat. *Journal of Pharmacognosy and Phytochemistry, 9*(5), 16-20.
- Keegan, K., & Halegoua, S. (1993). Signal transduction pathways in neuronal differentiation. *Curr Opin Neurobiol, 3*(1), 14-19.
- Kerhar, J., & Bouquet, A. (1950). Plantes médicinales et toxiques de la Côte d'Ivoire-Haute Volta. Vigot Frères (éd.), Paris 250.
- Koné, M. W., Kamanzi A. K., & Traoré, D. (2002). Plantes et médecine traditionnelle dans la région de Ferkessédougou (Côte d'Ivoire). *Annales de Botanique de l'Afrique de l'Ouest,* 2, 13-23.
- Lewerenz, J., Ates, G., Methner, A. … & Maher, P. (2018). Oxytosis/Ferroptosis-(Re-) Emerging Roles for Oxidative Stress-Dependent Non-apoptotic Cell Death in Diseases of the Central Nervous System. *Front Neurosci,* 12, 214.

- Li, J., Cao, F., Yin, H. L., … & Wang, W. (2020). Ferroptosis: past, present and future. *Cell Death Dis* 11(2), 88.
- Maher, P., Currais, A., & Schubert, D. (2020a). Using the Oxytosis/Ferroptosis Pathway to Understand and Treat Age-Associated Neurodegenerative Diseases. *Cell Chem Biol*, 27(12), 1456-1471.
- Maher, P., Fischer, W., Liang, Z. … & Currais, A. (2020b). The Value of Herbarium Collections to the Discovery of Novel Treatments for Alzheimer's Disease, a Case Made With the Genus Eriodictyon. *Front Pharmacol*, 11, 208.
- Martin-Sanchez, D., Ruiz-Andres, O., Poveda, J., … & Sanz A. B. (2017). Ferroptosis, but Not Necroptosis, Is Important in Nephrotoxic Folic Acid-Induced AKI. *J Am Soc Nephrol,* 28(1), 218-229.
- N'guéssan, K. (1995). Contribution à l'étude ethnobotanique chez les Krobou de la Sous-Préfecture d'Agboville (Côte-d'Ivoire). Thèse 3e Cycle, Université Nationale, Côte-d'Ivoire.
- N'guéssan, K. (2008). Plantes médicinales et pratiques médicales traditionnelles chez les peuples Abbey et Krobou du Département d'Agboville (Côte-d'Ivoire).Thèse de Doctorat ès Sciences Naturelles. Université de Cocody-Abidjan, U.F.R. Biosciences, Laboratoire de Botanique.
- Naveen-Kumar S. K., Sharath-Babu B. N., Hemshekhar, M. … & Mugesh, G. (2018). The Role of Reactive Oxygen Species and Ferroptosis in Heme-Mediated Activation of Human Platelets. *ACS Chem Biol, 13*(8), 1996-2002.
- Ozioma, E. J., & Nwamaka Chinwe O. A. (2019). Herbal Medicines in African Traditional Medicine. IntechOpen.
- Prior, M., Chiruta, C., Currais, A. … & Schuber, D. (2014). Back to the future with phenotypic screening. *ACS Chem Neurosci,* 5, 7503-7513.
- PROTA4U, Plant Resources of Tropical Africa, https://www.prota4u.org/database/.
- SODEFOR. (1999). Plan d'aménagement de la forêt classée de Bamo (Agboville). . Société de développement des forêts, Côte d'Ivoire.
- Sopher, B. L., Fukuchi, K., Smith, A. C. … & Martin, G. M. (1994). Cytotoxicity mediated by conditional expression of a carboxyl-terminal derivative of the beta-amyloid precursor protein. *Brain Res Mol Brain Res, 26*(1-2), 207-217.
- Soriano-Castell, D., Liang, Z., Maher, P., & Currais, A. (2021b). The search for anti-oxytotic/ferroptotic compounds in the plant world. *Br J Pharmacol, 178*(18), 3611-3626.
- Sournia, G., & Arnaud J.C. (1978). Les ethnies de Côte-d'Ivoire. In Atlas de la Côte-d'Ivoire. Édition Groupe JA — 51, avenue des ternes — 75017 Paris.
- Tchuenmogne, M. A. T., Kammalac, T. N., Gohlke, S. … & Boyom, F. F. (2017). Compounds from *Terminalia mantaly* L. (Combretaceae) Stem Bark Exhibit Potent Inhibition against Some Pathogenic Yeasts and Enzymes of Metabolic Significance. *Medicines (Basel), 4*(1).
- Vangah-Manda M. O. (1986). Contribution à la connaissance des plantes médicinales utilisées par les ethnies Akans de la région littorale de la Côte-d'Ivoire. Thèse 3e Cycle, Université Nationale, Côte d'Ivoire.
- Wang, L., Zhang, Z., & Li, M. (2019). P53-dependent induction of ferroptosis is required for artemether to alleviate carbon tetrachloride-induced liver fibrosis and hepatic stellate cell activation. *IUBMB Life, 71*(1), 45-56.
- Wyss-Coray, T., & Rogers J. (2012). Inflammation in Alzheimer disease-a brief review of the basic science and clinical literature. *Cold Spring Harb Perspect Med, 2*(1), a006346.

Cite This Article: Maher Pamela, Kipré Gueyraud Rolland, Bla Kouakou Brice, Offoumou M'Bai Rostand, Currais Antonio, Djaman Allico Joseph (2024). Anti-Oxytotic/Ferroptotic Neuroprotection by Medicinal Plants from Côte d'Ivoire. *East African Scholars J Med Sci, 7*(2), 18-27.