Aqueous extract of Securidaca longipendunculata Oliv. and Olax subscropioidea inhibits key enzymes (acetylcholinesterase and butyrylcholinesterase) linked with Alzheimer’s disease in vitro

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ABSTRACT

Context: Plants have historically been used to treat neurodegenerative diseases which include Alzheimer’s disease.

Objective: This study investigated the antioxidant properties and inhibitory effect of aqueous extracts of Securidaca longipendunculata root and Olax subscropioida leaf on the cholinergic system in rat brain in vitro.

Materials and methods: Aqueous extracts (1:20 w/v) of S. longipendunculata root and O. subscropioida leaf were prepared and the ability of the extract to inhibit the activities of acetylcholinesterase and butyrylcholinesterase was evaluated as well as antioxidants as typified by 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid (ABTS*) radical scavenging ability and Fe chelation spectrophotometrically.

Results: ABTS* radical scavenging ability showed that S. longipendunculata (0.075 Mmol TEAC/100 g) had a higher scavenging ability than O. subscropioida (0.07 Mmol TEAC/100 g). Also, the Fe** chelating ability of both extracts revealed that S. longipendunculata (IC\textsubscript{50} = 105.57 g/mL) had a significantly (p < 0.05) higher Fe** chelating ability than O. subscropioida (IC\textsubscript{50} = 255.84 g/mL). Extracts of S. longipendunculata and O. subscropioida inhibited acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities. However, S. longipendunculata (IC\textsubscript{50} = 108.02 g/mL) has the higher AChE inhibitory activity than O. subscropioida (IC\textsubscript{50} = 110.35 g/mL). Also, both extracts inhibit BChE activity in vitro but S. longipendunculata (IC\textsubscript{50} = 82.55 g/mL) had a higher BChE inhibitory activity than O. subscropioida (IC\textsubscript{50} = 108.44 g/mL).

Discussion and conclusions: The mechanism by which S. longipendunculata root and O. subscropioida leaf perform their anti-Alzheimer’s disease activity may be by their inhibition on the key enzymes linked to this disease.

Introduction

Neurodegeneration is a process involved in both neuropathological conditions and brain ageing. It is known that brain pathology in the form of cerebrovascular and neurodegenerative disease is a leading cause of death all over the world (Kolominsky-Rabas et al. 2001). Mental disorder, also called a mental illness or psychiatric disorder, is a mental or behavioural pattern that causes impaired ability to function in ordinary life (disability). This may be associated with particular regions or functions of the brain or the rest of the nervous system. Cell damage caused by free radicals appears to be a major contributor to aging (Finkel & Holbrook 2000, Halliwell 2006), diabetes, cardiovascular and several diseases which are debilitating. The term that encompasses all highly reactive, oxygen-containing molecules (including free radicals), peroxides, the superoxide anion radicals, hydroxyl radicals, nitric oxide radicals, singlet oxygen and hypochlorite radicals, all of which can react with the membrane lipids, proteins, enzymes, nucleic acids and other smaller molecules leading to oxidative stress.

Oxidative stress originating from the body is a feature of life in the modern world. Sustained oxidative stress from a heavy cumulative burden of oxidants may deplete the body antioxidants reserves to a point beyond which the antioxidant defences can quench (Kidd 1991). Oxidative damage to DNA, proteins and other macromolecules may lead to a wide range of human diseases most notably, heart disease and cancer (Ananya et al. 2004). Antioxidants stabilize or deactivate free radicals before they attack the cell and there are several foods (especially of plant origin) that contain these antioxidants. Vitamin E is the most important lipid soluble antioxidant, while vitamin C helps to neutralize ROS in water and aqueous phase before it attacks lipids. β-Carotene and other carotenoids also have antioxidant properties.

Phytochemicals present in vegetables and fruits are believed to reduce the risk of several major diseases including cardiovascular diseases, cancers as well as neurodegenerative disorders (Selvam 2008). In traditional practices of medicine, numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases such as Alzheimer’s disease (AD) and other memory-related disorders. Herbal products contain complicated mixtures of organic chemicals, which may include fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, tannins, terpenes and so forth. The phenolic compounds (catechins and epicatechins) of green tea are capable to protect neurons against a range of oxidative and metabolic insults (Rogério et al. 2008).
Cholinesterases belong to a family of protein that is widely distributed throughout the body in both neuronal and non-neuronal tissues and is classified as either acetylcholinesterase (AChE) or butyrylcholinesterase (BChE). Acetylcholinesterase is a key enzyme in the cholinergic nervous system. During the progression of AD, many different types of neurons deteriorate, although there is profound loss of forebrain cholinergic neurons, which is accompanied by a progressive decline in acetylcholine which accounts for the cholinergic dysfunction associated with the disease. The most prescribed drug class in pharmacotherapy of AD is the cholinesterase inhibitors (ChEIs) that block the breakdown of ACh (Giacobinini 2002). BChE is involved in the breakdown of certain drugs, including muscle relaxant drugs called choline esters used during general anaesthesia. It also breaks down toxic substances before they reach the nerves. These substances include certain pesticides, poisons that attack the nerves, and specific natural toxins including a compound called solanine found in green potatoes skin. BChE is found in significantly higher quantities in AD plaques than in plaques of age-related non-demented brains (Schneider 2001).

Securidaca longipendunculata Oliv. (Polygalaceae) root and Olax subscropioidea (Olacaceae) leaf have been adopted in folklore in the prevention/management of Alzheimer’s disease with no or little scientific basis. Therefore, this study sought to evaluate the inhibitory property of S. longipendunculata root and O. subscropioidea leaf on the key enzymes (acetylcholinesterase and butyrylcholinesterase) linked with Alzheimer’s disease in vitro.

Materials and methods

Chemicals and reagents

Chemicals and reagents used such as thiobarbituric acid (TBA), 1,10-phenanthroline, gallic acid, Folin–Ciocalteau’s reagent were procured from Sigma-Aldrich, Inc. (St. Louis, MO), trichloroacetic acid (TCA) was sourced from Sigma-Aldrich, Chemie GmbH (Steinheim, Germany), hydrogen peroxide, methanol, acetic acid, hydrochloric acid, sodium carbonate, potassium ferricyanide, hydrogen peroxide, ferric chloride, aluminium chloride, potassium acetate, sodium dodecyl sulphate, iron (II) sulphate, potassium ferricyanide and ferric chloride were sourced from BDH Chemicals Ltd, (Poole, England). Except when stated otherwise, all other chemicals and reagents were of analytical grades and the water was glass distilled.

Sample collection and identification

Securidaca longipendunculata and O. subscropioidea were obtained in September 2015 from the botanical garden of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko. Nigeria. The identification and authentication was carried out by Dr Obembe of Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba Akoko. Nigeria. Voucher specimens of S. longipendunculata and O. subscropioidea have been deposited in the Herbarium.

Preparation of aqueous extract of the samples

The selected herbs were collected, air-dried and milled into powder to prepare the aqueous extracts in distilled water (1:20 w/v). The samples were soaked for 4 h after which they were filtered using Whatman (No. 1) filter paper and centrifuged (Model KX3400C) to obtain a clear supernatant. The supernatants were freeze-dried (Lab-Kit FD-10-MR model; Lab-Kits, Utherm International, Xiangtan City, Hunan Province, China) and then stored in a refrigerator for subsequent analysis (Oboh et al. 2007).

Determination of total phenol content

The total phenol content was determined according to the method of Singleton et al. (1999). Briefly, appropriate dilutions of S. longipendunculata and O. subscroioidea extracts were oxidized with 2.5 mL 10% Folin–Ciocalteau’s reagent (v/v) and neutralized by 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45 °C and the absorbance was measured at 765 nm in the UV-Visible spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent (GAE).

Determination of total flavonoid content

The total flavonoid content was determined using a slightly modified method reported by Meda et al. (2005). Briefly, 0.5 mL of appropriately diluted sample was mixed with 0.5 mL methanol, 50 μL 10% AlCl3, 50 μL of 1 M potassium acetate and 1.4 mL distilled water, and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm in the UV-Visible spectrophotometer; the total flavonoid content was subsequently calculated using quercetin (QA) as standard.

Reducing property

The reducing property of aqueous extracts of S. longipendunculata and O. subscroioidea were determined by assessing the ability of the extracts to reduce FeCl3 solution as described by Oyaizu (1986). A 2.5 mL aliquot was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min and then 2.5 mL of 10% trichloroacetic acid was added. This mixture was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm and ferric reducing power was subsequently calculated using ascorbic acid equivalent (AAE).

2,2-Azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS+) radical scavenging ability

The ABTS+ scavenging ability of the extracts was determined according to the method described by Re et al. (1999). The ABTS+ was generated by reacting 7 mM ABTS aqueous solution with K2S2O8 (2.45 mM, final concentration) in the dark for 16 h and adjusting the Abs 73 nm to 0.700 with ethanol. Thereafter, 200 μL of appropriate dilution of the extracts were added to 2.0 mL ABTS+ solution and the absorbance were measured at 734 nm after 15 min. The Trolox equivalent antioxidant capacity (TEAC) was subsequently calculated using Trolox as the standard.

Iron (Fe2+) chelation assay

The Fe2+ chelating ability of the extracts were determined using the method by Minotti and Anst (1987) with a slight modification by Puntel et al. (2005). Freshly prepared 500 μmolL⁻¹ FeSO4 (150 μL) was added to a reaction mixture containing 168 μL of 0.1M Tris-HCl (pH 7.4), 218 μL saline and the extracts (0–100 μL). The reaction mixture was incubated for 5 min, before the addition of 13 μL of 0.25% 1,10-phenanthroline (w/v). The
absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe$^{3+}$ chelating ability was subsequently calculated.

**Lipid peroxidation and thiobarbituric acid reactions**

Male Wister strain albino (200 g) rats were decapitated under mild diethyl ether anaesthesia and the cerebral tissue (whole brain) was rapidly dissected, placed on ice and weighed. This tissue was subsequently homogenized in cold saline (9%) with about 10-up- and down-strokes at approximately 1200 rev/min in a Teflon glass homogenizer. The homogenate was centrifuged about 10-up- and down-strokes at approximately 1200 rev/min using a modified method as described by Ohkawa et al. (1979).

Briefly, 100 l of the tissue supernatant was mixed with a reaction mixture containing 30 l of 0.1 M Tris–HCl buffer (pH 7.4), appropriate dilutions of aqueous extracts of *S. longipendunculata* and *O. subscorpioides* and 30 l of 250 lM freshly prepared FeSO$_4$. The volume was made up to 300 l with distilled water before incubation at 37°C for 1 h. Subsequently, 300 l of 8.1% sodium dodecyl sulphate (SDS), 500 l of acetic acid/HCl buffer (pH 3.4) and 500 l of 0.8% thiobarbituric acid (TBA) were added to the reacting mixture. This mixture was incubated at 100°C for 1 h and thiobarbituric acid reactive species (TBARS) produced were measured at 532 nm using a spectrophotometer.

**Acetylcholinesterase (AChE) and butyrylcholinesterase inhibition assay**

Inhibition of AChE was assessed by a modified colorimetric method as described by Ellman et al. (1961). The AChE activity was determined in a reaction mixture containing 200 l of a solution of AChE (0.415 U/mL in 0.1 M phosphate buffer, pH 8.0), 100 l of a solution of 5,5’-dithiobis (2-nitrobenzoic) acid (DTNB) (3.3 mM in 0.1 M phosphate-buffered solution, pH 7.0) containing NaHCO$_3$ (6 mM), aqueous extracts of *S. longipendunculata* and *O. subscorpioides*, and 500 l of 4 phosphate buffer, pH 8.0. After incubation for 20 min at 25°C, 100 l of 0.05 mM acetylthiocholine iodide solution was added as the substrate, and AChE activity was determined as changes in absorbance reading at 412 nm for 3 min at 25°C using a spectrophotometer. Also, inhibition of BChE was assessed by a modified colorimetric method as described (Ellman et al. 1961). The BChE activity was determined in a reaction mixture containing 200 l of a solution of BChE (0.415 U/mL in 0.1 M phosphate buffer, pH 8.0), 100 l of solution of 5, 5’-dithiobis (2-nitrobenzoic) acid (DTNB) (3.3 mM in 0.1 M phosphate-buffered solution, pH 7.0) containing NaHCO$_3$ (6 mM), aqueous extracts of *S. longipendunculata* and *O. subscorpioides*, and 500 l of 4 phosphate buffer, pH 8.0. After incubation for 20 min at 25°C, 100 l of 0.05 mM butyrylthiocholine iodide solution was added as the substrate, and BChE activity was determined as changes in absorbance reading at 412 nm for 3 min at 25°C using a spectrophotometer. The AChE and BChE inhibitory activities were expressed as percentage inhibition (%).

**Data analysis**

The result of replicate experiments were pooled and expressed as mean ± standard deviation (SD). The means were analyzed using one-way analysis of variance (ANOVA) and the Duncan test was performed using the free software R version 3.1.1.

**Results**

The results of the total phenol, total flavonoid of aqueous extracts of *S. longipendunculata* root and *O. subscorpioides* leaf is as shown in Figure 1. The result revealed that *S. longipendunculata* (40 mg GAE/g) had significantly ($p < 0.05$) higher total phenol content than *O. subscorpioides* (30 mg GAE/g). The result also revealed that *S. longipendunculata* (10 mg QE/g) had significantly ($p < 0.05$) higher total flavonoid content than *O. subscorpioides* (4.26 mg QE/g) as presented in Figure 2. As revealed by the results, *S. longipendunculata* (25.53 mg AEE/g) had significantly ($p < 0.05$) higher reducing property (Figure 3) than *O. subscorpioides* (19.74 mg AEE/g). The results of the 2,2-azino-bis (3-ethylbenthiazoline-6-sulphonic acid (ABTS$^+$)) radical scavenging ability of the aqueous extracts of *S. longipendunculata* and *O. subscorpioides* are presented in Figure 4. The result showed that the extracts are able to scavenge ABTS$^+$ radicals, however, *S. longipendunculata*
(0.075 Mmol TEAC/100 g) had a higher ABTS* scavenging ability than \textit{O. subscropioidea} (0.68 Mmol TEAC/100 g).

Furthermore, the \( \text{Fe}^{2+} \) chelating ability of both extracts is presented in Figure 5 and their IC\textsubscript{50} values in Table 1. The result revealed that the aqueous extracts of \textit{S. longipendunculata} (IC\textsubscript{50} = 105.57 g/mL) had a significantly \((p < 0.05)\) higher \( \text{Fe}^{2+} \) chelating ability than \textit{O. subscropioidea} (IC\textsubscript{50} = 255.84 g/mL). The inhibition of \( \text{FeSO}_{4} \)-induced lipid peroxidation of aqueous extracts of \textit{S. longipendunculata} and \textit{O. subscropioidea} are presented in Figure 6 with their IC\textsubscript{50} values in Table 1. The result revealed that both extracts were able to inhibit \( \text{FeSO}_{4} \)-induced lipid peroxidation in a dose-dependent manner; however, \textit{S. longipendunculata} (IC\textsubscript{50} = 67.15 g/mL) had a higher inhibition of \( \text{Fe}^{2+} \)-induced lipid peroxidation than \textit{O. subscropioidea} (IC\textsubscript{50} = 111.03 g/mL).

The ability of the aqueous extracts of \textit{S. longipendunculata} and \textit{O. subscropioidea} to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities \textit{in vitro} was investigated and the result are shown in Figures 7 and 8, respectively, with their IC\textsubscript{50} in Table 1. The result revealed that both extracts inhibited AChE activity in a dose-dependent manner. However, \textit{S. longipendunculata} (IC\textsubscript{50} = 108.02 g/mL) has the higher AChE inhibitory activity than \textit{O. subscropioidea} (IC\textsubscript{50} = 110.35 g/mL). Also, the result revealed that both extracts inhibit BChE activity \textit{in vitro}. However, \textit{S. longipendunculata} (IC\textsubscript{50} = 82.55 g/mL) had a higher BChE inhibitory activity than \textit{O. subscropioidea} (IC\textsubscript{50} = 108.44 g/mL).

\textbf{Discussion}

Mental disorder, also called a mental illness or psychiatric disorder, is a mental or behavioural pattern of brain function in life that causes either suffering or an impaired ability to retain information for a while in the brain. Inhibition of the enzyme linked to Alzheimer’s disease with the use of synthetic drugs has been associated with some side effects which include headache, diarrhea, drowsiness and vomiting among others unlike the use of natural products. The plant parts used in this research have been a bailout for those suffering from Alzheimer’s disease in folklore medicine but the mechanism of action remains unknown.

The antioxidant properties showed a promising result to fight free radicals in the body system. It revealed that aqueous extract of \textit{S. longipendunculata} had a higher total phenolic constituent than \textit{O. subscropioidea} extract. Phenolic compounds have been reported to protect the human body from free radicals, the formation of which is associated with the normal natural metabolism of aerobic cells. They are strong antioxidants capable of removing free radicals; they may chelate metallic catalysts,
activate antioxidant enzymes, reduce \( \alpha \)-tocopherol radicals and inhibit oxidases (Amic et al. 2003). Phenolic constituents of plants are considered as antioxidants and they carry out their protective properties on cells either by preventing the production of free radicals or by neutralizing scavenging free radicals produced in the body (Oboh 2006). The extracts also contain an ample amount of flavonoids. Flavonoids are regarded as antioxidant molecules and could therefore lower cellular oxidative stress (Oboh et al. 2007). The antiradical activity of flavonoids and phenolics is principally based on the redox properties of their hydroxyl groups and the structural relationships between different parts of their chemical structure (Rice-Evans et al. 1996). It is also well-known that phenolic compounds contribute to the quality of food in terms of modifying colour, taste, aroma and flavour (Mennune et al. 2009).

Reducing power is another antioxidation defence mechanism; the two mechanisms available to affect this property are by electron transfer and by hydrogen atom transfer (Dastmalchi et al. 2007). This is because the ferric-to-ferrous iron reduction occurs rapidly with all reductants with half reaction reduction potentials above that of Fe\(^{3+}/Fe^{2+}\), the values in the ferric reducing antioxidant property (FRAP) assay will express the corresponding concentration of electron-donating antioxidants (Halvorsen et al. 2002). The reducing powers of the aqueous extracts of \( S. \) longipendunculata and \( O. \) subscropioidea were assessed based on their ability to reduce Fe\(^{3+}\) to Fe\(^{2+}\). As revealed by the results, \( S. \) longipendunculata (25.53 mg AAE/g) had significantly (\( p < 0.05 \)) higher reducing property than \( O. \) subscropioidea (19.74 mg AAE/g).

Metal ions such as Fe\(^{3+}\) which results in the induction of oxidative stress have been reported to be associated with Alzheimer’s disease (Tabert et al. 2005); the extracts were also screened for their antioxidant activity. Therefore, the free radical scavenging ability of the \( S. \) longipendunculata and \( O. \) subscropioidea extracts was studied using a moderately stable nitrogen-centred radical species. The results of the 2,2’-azino-bis (3-ethylbenthiazoline-6-sulphonic acid (ABTS\(^*\)) radical scavenging ability of the aqueous extracts of \( S. \) longipendunculata and \( O. \) subscropioidea showed that the extracts are able to scavenge ABTS\(^*\) radicals, however, \( S. \) longipendunculata had a higher ABTS\(^*\) radical scavenging ability than \( O. \) subscropioidea. The antioxidant activities of plant phytochemicals occur by preventing the production of free radicals or by neutralizing/scavenging free radicals produced in the body or reducing/chelating the transition metal composition of foods (Amic et al. 2003; Oboh et al. 2007). This is an important antioxidant mechanism demonstrated by these plant parts and could play some part in the prevention of oxidative stress-induced neurodegeneration. \( Securidaca \) longipendunculata extract displayed a stronger Fe\(^{3+}\) chelating ability than \( O. \) subscropioidea extract.

In this study, incubation of rat brain tissues in the presence of 250 \( \mu \)M FeSO\(_4\) caused a significant increase in the MDA content of the brain as presented in Figure 6. This finding agreed with earlier report by Oboh et al. (2012) where significant increase in MDA production in rat brain was observed in the presence of Fe\(^{2+}\). Elevated Fe\(^{2+}\) content in the brain had been linked to a host of neurodegenerative diseases and high Fe contents have been localized to degenerate regions of brains from Alzheimer’s disease patients, a finding which demonstrated in animal models of the disease. However, \( S. \) longipendunculata and \( O. \) subscropioidea extracts inhibited MDA production in rat brain in a dose-dependent manner. \( Securidaca \) longipendunculata had a higher inhibition of Fe\(^{3+}\)-induced lipid peroxidation than \( O. \) subscropioidea considering their IC\(_{50}\) values. This finding is consistent with earlier report where plant extracts inhibited Fe\(^{2+}\)-induced lipid peroxidation in rat brain in \textit{vitro} (Zago et al. 2000).

Furthermore, aqueous extracts of \( S. \) longipendunculata and \( O. \) subscropioidea inhibited both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The result revealed that both extracts inhibited AChE and BChE activities in a dose-dependent manner (0–160 g/mL). Nevertheless, \( S. \) longipendunculata had the higher AChE and BChE inhibitory activities than \( O. \) subscropioidea. Inhibition of AChE is considered as a promising
approach for the treatment of Alzheimer’s disease (AD) and for possible therapeutic applications in the treatment of Parkinson’s disease, ageing and myasthenia gravis (Quinn 1987). Meanwhile, BChE has been considered to be directly associated with the side effects of the AChE inhibitors and the existing drugs of Alzheimer’s disease (Quinn, 1987). It has been shown that BChE is found in significantly higher quantities in AD plaques than in the plaques of age-related non-demented brains. AChE is an important regulatory enzyme that controls the transmission of nerve impulses across cholinergic synapses by hydrolyzing the excitatory transmitter acetylcholine (Schetinger et al. 2000). Neurodegeneration due to oxidative stress has been implicated in the pathogenesis and progression of Alzheimer’s disease, with selective loss of cholinergic neurons in the brain being the most prominent. Studies have reported the Alzheimer’s brain disease to be under intensive oxidative stress (Massaad 2011) and decrease in the cholinergic neurons has been shown to promote amyloid protein deposition in the Alzheimer’s brain disease which in turn favour amyloid protein-associated oxidative stress and neurotoxicity (Buttfield & Lauderback 2002). The inhibition of AChE and BChE by aqueous extracts of S. longipendunculata and O. subscropioidea is a pointer to the neuroprotective abilities of the extracts.

Conclusions

Aqueous extracts of S. longipendunculata root and O. subscropioidea leaf are rich in phenolic compounds and exhibited both anticholinesterase and antioxidant activity with S. longipendunculata displaying a stronger neuroprotective effect than O. subscropioidea extract. These herbs showed potential in the management of Alzheimer’s disease as it exhibited inhibitory activity on key enzymes (acetylcholinesterase and butyrylcholinesterase) linked to this disease. Therefore, the possible mechanism through which the extracts exerted their neuroprotective properties may be by inhibiting cholinesterases activities as well as preventing oxidative stress-induced neurodegeneration. However, this is a preliminary study with possible physiological implications.

Disclosure statement

The authors report no declarations of interest.

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