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Neuroprotective Potentials and Antioxidant Properties of Bush Buck (*Gongronema latifolium)* and Wonder Kola (*buchholzia coriacea*)

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ABSTRACT

The study sought to evaluate the effects of phenolic extract from bush buck (*Gongronema latifolium)* and wonder kola (*buchholzia coriacea*) on key enzymes linked to neurodegeneration (acetylcholinesterase, butyrylcholinesterase and monoamine oxidase (MAO)). Ten grams each of thewonder kola and bush buck was soaked in 200 ml of mixture of methanol and 1N HCl (1:1 v/v) for 24 hours and filtered. The filtrate was evaporated at 45^{o}C to dryness and reconstituted in distilled water for subsequent assays such as cholinetsrase, monoamine oxidase and antioxidant assays. The results revealed that the phenolic extracts inhibited acetylcholinesterase, butyrycholinesterase and monoamine oxidase in a concentration-dependent manner (0 – 800 µg/ml). However, wonder kola [acetylcholinesterase (EC_{50}= 453.31 µg/ml), butyrylcholinesterase (EC_{50}= 453.72 µg/ml), monoamine oxidae (EC_{50}=954.20 µg/ml)] had significant (P< 0.05) higher inhibitory effects than bush buck kola [acetylcholinesterase (EC_{50}=1182.94 µg/ml), butyrylcholinesterase (EC_{50}=591.72 µg/ml), monoamide oxidae (EC_{50}=1041.67µg/ml]. Furthermore, incubation of brain tissue homogenate with 250µM $FeSO_4$ caused significant ($p<0.05$) increase in the malondialdehyde (MDA) content of the brain tissue homogenate compared to the basal. However, bush buck (IC_{50}= 805.24 µg/ml) had significant ($p<0.05$) higher MDA inhibitory effect than wonder kola (IC_{50} =1182.94 µg/ml). Also, wonder kola [FRAP=117.53mgAAE, $DPPH^{\bullet}$ (EC_{50}= 337.61), Fe^{2+} chelation (EC= 118.5µg/ml)] had significantly ($p<0.05$) higher antioxidant properties than bush buck [FRAP=100 mgAAE, $DPPH^{\bullet}$ (EC_{50}= 401.61 µg/ml), Fe^{2+} chelation (EC= 171.9µg/ml]. We therefore conclude that inhibition of acetylcholinesterase, butyrlcholinesterase and monoamine oxidase enzymatic activities coupled with strong antioxidant properties of bush buck (*Gongronema latifolium)* and wonder kola (*buchholzia coriacea*) could underlie their importance in neuroprotection, though, wonder kola (*buchholzia coriacea*) showed promising potential than bush buck (*Gongronema latifolium)*

Keywords: wonderful kola, bush buck, acetylcholinesterase, butyrlcholinesterase, monoamine oxidase, antioxidant

INTRODUCTION

Neurological disorders are diseases that affect the brain, central and autonomic nervous systems. Neurodegenerative disease is the deterioration of intellectual and cognitive faculties which are often irreversible. The neurodegenerative condition can either affect the entire neurological pathway or a single neuron (Jeynes and Provias 2006) and are often associated with aging (Waldemar, 2007). Aging plays vital role in the onset of cognitive dysfunction such as Alzheimer's disease, cerebrovascular impairment, seizure disorders, head injury and Parkinsonism (Ceskova 2005).

The human nervous system deteriorates with age through natural aging process and sometimes due to excessive drinking or smoking. Oxidative process is one of the major causes of the observed deterioration, which eventually damages brain cell and lead to the formation of free radicals capable of causing havoc in one's brain.

The cholinergic system plays a vital role in cognitive function, including memory and emotional processing (Perry *et al*., 1999). However, loss of activities in the cholinergic systems may occur as a result of progressive degradation of neurotransmitter, acetylcholine to acetate and choline by acetylcholinesterase and butrylcholinesterase in the synaptic nerves. Many reports have suggested that the inhibition of cholinergic enzymes (acetylcholinesterase and butrylcholinesterase) as a therapeutic approach or measure in managing neurodegenerative disorders, since this approach prevents or slows down hydrolysis of acetylcholine to choline and acetate cholinergic enzymes (Oboh *et al*., 2013). On the other hand, monoamine oxidase (MAO) is involved in the oxidation of a variety of neurotransmitters such as serotonin, norepinephrine and dopamine (Adefegha *et al*., 2016a). However, inhibition of monoamine oxidase activity has also been suggested being a treatment strategy in the management of several neurodegenerative diseases. It has been postulated that deficiency of monoamine in the brain can cause depression (Adefegha *et al*., 2016a). The monoamine oxidase inhibitors act as anti-depressants by preventing monoamine oxidase from degrading monoamine (Adefegha e*t al*., 2016a).

Medicinal plants are plants with health promoting properties. Medicinal plants have been used in preventing, management and treatment of various illnesses and diseases such as malaria, diabetes, erectile dysfunction, Alzheimer disease, mental disorder etc across various cultures and tribes (Stafford *et al*., 2007). A good number of plants have been used to treat cognitive disorders, including neurodegenerative diseases such as Alzheimer's disease (AD) and other memory related disorders (Adefegha *et al*., 2016a; Rickert *et al*., 1999). .

Gongronema latifolium Benth (bush buck) belongs to the family *asclepiadaceae*. It is an edible nutritional/medicinal plant mostly found in the rain forest zones in Nigeria and other tropical African countries (Adefegha and Oboh, 2012). The leaves of *G. latifolium* are used as vegetables in preparation of soups to which they add a bitter-sweet flavor (Chattopadhyah, 1999; Morebise and Fafunso, 1998). The leaves are also sometimes used to spice locally brewed beer (Edim *et al*., 2012). Essien *et al* (2007) reported that *G. latifolium* is used to treat cough in Nigeria (Essien *et al*., 2007). The antioxidant property (Ugochukwu and Babady, 2002), antimicrobial activity (Morebise and Fafunso, 1998)., anti-inflammatory activity (Morebise *et al*., 2006), anti diabetes (Sylvester et al., 2015) and anti-cancer activity (Iweala et al 2015) of *G. latifolium* have been reported. In the folklore, *G. latifolium* has been used in the management of various cases of mental illness.

Buchholzia coriacea (wonder Kola) belongs to the family capparidaceae. *Buchholzia coriacea* has multiple medicinal values. These seeds are commonly referred to as wonder kola because of its usage in traditional medicine. The seed of the plant is commonly eaten either cooked or raw (Lemmens, 2013). In Africa, *Buchholzia coriacea* is useful in the treatment of hypertension and also in the management and prevention of premature aging. *Buchholzia coriacea* has been termed as brain food because it promotes memory. The leaf infusions of *buchholzia coriacea* are applied to the eyes against filarial nematodes, and powdered or pulped leaves are applied to treat fever, ulcers, boils and haemorrhoids. Fruit kernels are chewed to treat angina and nose bleeding, and fruit extracts are taken as anthelmintic. Fruit scrapings are administered to treat asthma and cough. Seed

preparations are taken to treat; fever, diabetes (Adisa *et al*., 2011), hypertension, cough, psychiatric disorders and impotence. Although, bush buck and wonder kola have been shown to possess anti-Alzheimer's properties, but, there is limited information on how they exert this effects. This study was designed to investigate and compare the effect of phenolic extracts from wonder kola and bushbuck on cholinesterases and monoamine oxidase (MAO) activities as well as pro-oxidant induced lipid peroxidation in the isolated rat's brain *in vitro*, in order to provide possible mechanisms of action for the use of these plants in folklore medicine.

MATERIALS AND METHODS

Materials

The sample bush buck (*Gongronema latifolium)* and wonder kola (*buchholzia coriacea*) were obtained from local market, in Akure, Ondo state. Authentication was carried out at the Department of Crop, Soil and Pest management (CSP), Federal University of Technology, Akure, Nigeria. The samples were dried and blended into powdery form using warring commercial heavy duty bender (model 37BL18; 240CB6).

Chemicals and reagents

All chemicals used in this study were of analytical grade and glass-distilled water was used.

Sample preparation

Phenolics were extracted according to method previously describe by Oboh and Ogunruku (2010). Ten gram each of bush buck and wonder kola was soaked with 200 ml of the mixture of methanol: 1N HCl (1:1, v/v) for 24 hours and filtered. The filtrate was evaporated at 45^{o}C and reconstituted in distilled water. The extract was kept in the refrigerator at 4°Cfor subsequent analysis.

Determination of total phenol content

The total phenol content of the phenolic extract from bush buck and wonder kola were determined according to the method of Singleton *et al*, (1999). Briefly, appropriate dilutions of the extracts were oxidized with 2.5 ml of 10 % Folin-Ciocalteau's reagent (v/v) and neutralized by 0.2 ml of 7.5 % sodium carbonate. The reaction mixture was incubated for 40 minutes at 45°C and absorbance was measured at 765 nm in the spectrophotometer. The total phenol was subsequently calculated as gallic acid equivalent (GAE).

Determination of total flavonoids content

The total flavonoids content of the phenolic extract of the bush buck and wonder kola were 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, 0.5 µl of 1 M potassium acetate and 1.4 ml of distilled water, and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm. The total flavonoids content was subsequently calculated. The non-flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid contents.

Enzyme Inhibition Assays

Acetylcholinesterase assay

Acetylcholinesterase (AChE) activity was assessed by a modified spectrophotometry method of Ellman *et al* (1961). The AChE activity was determined in a reaction mixture containing 200 µL of the S1 preparation from the brain homogenate (AChE source) in 0.1 M phosphate buffer (pH8.0), 100 µL of 3.3 mM solution of 5,5-dithio-bis(2-nitrobenzoic)acid (DTNB), in the same buffer solution containing 6 mM $NaHCO_3$ and 500 µL of phosphate buffer (pH 8.0). After incubation for 20 min at 25°C, 100 µL of 0.05 mM acetylthiocholine iodide solution was added and AChE activity will be determined as a change in absorbance at 412 nm for 3 min at 25°C.

Butyrylcholinesterase assay

Butyrylcholinesterase (BChE) activity was assessed by a modified spectrophotometry method of Ellman *et al* (1961). The BChE activity was determined in a reaction mixture containing 200

µL of the S1 preparation from the brain homogenate (BChE source) in 0.1 M phosphate buffer (pH8.0), 100 µL of 3.3 mM solution of 5,5-dithio-bis(2-nitrobenzoic) acid (DTNB), in the same buffer solution containing 6 mM $NaHCO_3$ and 500 µL of phosphate buffer (pH 8.0). After incubation for 20 min at 25 ^oC, 100 µL of 0.05 mM acetylthiocholine iodide solution was added and BChE activity was determined as a change in absorbance at 412 nm for 3 min at 25^oC.

Monoamine Oxidase Assay

MAO (EC 1.4.3.4) activity was determined according to the methods of Green and Haughton (1961) with slight modifications. Briefly, the reaction mixture contained 0.025 M phosphate buffer pH 7, 0.0125 M of semicarbazide, 10 mM benzylamine (pH 7.0), and 0.67 mg of enzyme and (0-500µg/ml) of the extracts in a total reaction volume of 2 mL. After, 30 min of incubation, 1 mL of acetic acid was added and boiled for 3 min, followed by centrifugation. The supernatant (1 mL) was mixed with equal volume of 0.05 % of 2, 4-di-nitrophenyl hydride (DNPH) and 2.5 mL of benzene (absolute) was added after 10 min. After separation, the benzene layer was mixed with equal volume of 0.1 N NaOH. The alkaline layer was decanted and heated at 80 ^oC for 10 min. The absorbance of the orange-yellow colour developed was measured at 450 nm and the percentage MAO inhibition was subsequently calculated.

In Vitro Antioxidant Studies

Inhibition of lipid peroxidation and Thiobarbituric acid reactions

Male adult albino rat was decapitated under mild diethyl ether anaesthesia and the brain was rapidly isolated and placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1/10 w/v) with about 10-up and down strokes at approximately 1200 rev/min in a Teflon glass homogenizer. The homogenate was centrifuged for 10 min at 3000 × g to yield a pellet that was discarded, and the low-speed supernatant (S1) was kept for lipid peroxidation assay (Belle *et al*., 2004). The lipid peroxidation assay was carried out using the modified method of Ohkawa *et al*., 1979. Briefly 100 µl S1 fraction was mixed with a reaction mixture containing 30 µl of 0.1 M pH 7.4 Tris – HCl buffer, extract (0 – 100 µl) and 30 µl of 250 µM freshly prepared $FeSO_4$ (the procedure was also carried out using 5 µM sodium nitroprusside). The volume was made up to 300 µl water before incubation at 37^oC for 1hr. The colour reaction was developed by adding 300 µl 8.1 % SDS (Sodium dodecyl sulphate) to the reaction mixture containing S1, this was subsequently followed by the addition of 600 µl of acetic acid/HCl (pH 3.4) mixture and 600 µl 0.8 % TBA (Thiobarbituric acid). This mixture was incubated at 100^oC for 1hr. Thiobarbituric acid reactive species (TBARS) produced was measured at 532 nm and expressed using MDA (Malondialdehyde) equivalent.

Determination of ferric reducing antioxidant power (FRAP) assay

The reducing properties of the extracts were determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu (1986). A 2.5 ml aliquot was mixed with 2.5 ml of 200 mmol/l 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 centrifuged at 650 rpm for 10 min. 5 ml of the supernatant 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 antioxidant property was subsequently calculated using ascorbic acid as standard.

Determination of Fe^{2+} chelating ability

The Fe^{2+} chelating ability of the extracts was determined using a modified method of Minotti and Aust (1987) with a slight modification by

Puntel *et al*., (2005). Freshly prepared 500 µM $FeSO_4$ (150 µl) was added to a reaction mixture containing 168 µl 0.1M Tris–HCl (pH 7.4), 218 µl saline and the extracts (0 – 25 µl). The reaction mixture was incubated for 5min, before the addition of 13 µl 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm. The Fe^{2+} chelating ability was subsequently calculated.

Determination of DPPH free radical scavenging ability

The free radical scavenging ability of the phenolic extract against DPPH* (1, 1-diphenyl–2 picrylhydrazyl) free radical was evaluated as described by Gyamfi *et al*., (1999). Briefly, appropriate dilution of the extracts (1 ml) was mixed with 1ml, 0.4 mM DPPH in methanolic solution, the mixture was left in the dark for 30 min and the absorbance was read at 516 nm. The DPPH free radical scavenging ability was subsequently calculated.

Data Analysis

The values of replicate experiments (n = 3) were pooled and expressed as mean ± standard deviation (SD) (Zar, 1984). The means were analyzed using one-way analysis of variance (ANOVA) and Duncan test was used for the post hoc treatment. Significance was accepted at $p \leq 0.05$.

RESULTS

Effect of phenolic extracts from bush buck and wonder kola on acetylcholinesterase (AChE), butrylcholinesterase (BChE) and monoamine oxidase activities *in vitro* were investigated and are presented in figure 1, 2, & 3 respectively. The results revealed that phenolic extracts from wonder kola and bush buck inhibited acetylcholinesterase (AChE), butrylcholinesterase (BChE) and monoamine oxidase (MOA) in dose-dependent manner in the range (0 – 800 µg/ml). However, as revealed by their EC_{50} (concentration causing 50% enzyme inhibition) wonder kola [acetylcholinesterase (EC_{50}= 453.31 µg/ml), butyrylcholinesterase (EC_{50}= 453.72 µg/ml), monoamine oxidae (EC_{50}=954.20 µg/ml)] had significant ($P< 0.05$) higher inhibitory effect than bush buck [acetylcholinesterase (EC_{50}=1182.94 µg/ml), butyrylcholinesterase (EC_{50}=591.72 µg/ml), monoamine oxidase (EC_{50}=1041.67 µg/ml].

Furthermore, effect of bush buck and wonder kola phenolic extracts on Fe^{2+} induced lipid peroxidation in rat brain homogenate *in vitro* are presented in figure 4. Incubation of rat brain homogenates in the presence of 250 µM Fe^{2+} caused a significant ($P<0.05$) increase in the brain malodiaaldehyde (MDA) content. However, phenolic extracts from bush buck and wonder kola inhibited MDA production in the rat brain tissue homogenate in a dose-dependent manner (0-800 µg/ml). Nevertheless, bush buck (EC_{50}= 805.24 µg/ml) had significant ($P<0.05$) higher inhibitory effect on Fe^{2+} induced lipid peroxidation in rat brain than wonderful kola (EC_{50}=1182.94 µg/ml).

Furthermore**,** the Ferric reducing antioxidant property (FRAP) reported as ascorbic acid equivalents (AAE) for the phenolic extract of bush buck (*Gongronema latifolium*) and Wonder kola (*Buchholzia coriacea*) was investigated and is presented in Table 2. The results revealed that wonder kola extract (117.53 mgAAE/100g) had a significantly ($P<0.05$) higher ferric reducing power than bush buck extract (100 mgAAE/100g).

Also, the Fe^{2+} chelating ability of the phenolic extract of bush buck and wonder kola was investigated *in vitro* and presented in Figure 5. The results showed that both phenolic extracts chelated Fe^{2+} in a concentration-dependent manner (0–160 µg/ml). However, bush buck (EC_{50}= 118.57 µg/ml) had significantly ($P<0.05$) higher chelating ability than wonder kola (EC_{50}=171.94 µg/ml).

DPPH$^{\bullet}$ radical scavenging ability of phenolic extracts from bush buck and wonder kola was investigated *in vitro* and presented in figure 6. The result revealed that bush buck and wonder kola phenolic extracts scavenged DPPH$^{\bullet}$ radicals in a dose-dependent manner (0-800 µg/mL). However, IC_{50} value revealed that wonder kola extract (IC_{50} =337.61 µg/mL) had a significantly ($P< 0.05$) higher DPPH$^{\bullet}$ radical scavenging ability than bush buck extract (IC_{50} = 401.61 µg/mL).

The total phenol and total flavonoid content of the phenolic extracts from wonder kola and bush buck are presented in Table 1. The results revealed that wonder kola (140.33 mgGAE/g) had significant ($P< 0.05$) higher total phenol content than Bush buck seed (55.32 mgGAE/g). In a similar manner, wonder kola (61.12 mgQUE/g) had significant ($P< 0.05$) higher flavonoid content than Bush buck (38.89 mgQUE/g).

Table 1: The total phenol (mg GAE/g) and total flavonoids (mg QUE/g) of extract of Bush buck seed (*Gongronema latifolium*) and Wonder kola (*Buchholzia coriacea*)

Samples	Total phenol	Total flavonoids
Bush buck seed	55.32 ± 1.67^{b}	38.89 ± 1.50^{b}
Wonderful kola	140.33 ± 2.28^{a}	61.12 ± 1.86^{a}

Values represent mean ± standard deviation (n=3). Values with the same superscript alphabet down the same column are not significantly different ($P < 0.05$)

Table 2: The Ferric reducing antioxidant property (mgAAE/g) extract of Bush buck seed (*Gongronema latifolium*) and Wonder kola (*Buchholzia coriacea*)

Samples	FRAP
Bush buck seed	100.65 ± 1.33^{a}
Wonderful kola	117.53 ± 0.92^{b}

Values represent mean ± standard deviation (*n=3*). Values with the same superscript alphabet along the same column are not significantly different ($P < 0.05$)

Table 3: EC_{50} values for Iron chelation and for inhibition of Fe^{2+}-induced lipid peroxidation of phenolic extract (*Gongronema latifolium*) and Wonder kola (*Buchholzia coriacea*)

Samples	DPPH	Iron chelation (µg/mL)	Lipid peroxidation (µg/mL)
Bush buck seed	401.61±1.62	$118.57.65\pm 1.33^{a}$	805 ± 0.83^{a}
Wonderful kola	337.61±1.90	$171.94.53\pm0.92^{b}$	1182.945 ± 0.76^{b}

Values represent mean ± standard deviation (*n=3*). Values with the same superscript alphabet down the same column are not significantly ($P < 0.05$) different

Table 4: EC_{50} values for acetylcholinestaerase, butrylcholinestaerse, monoamine oxide (µg/ml) inhibitory ability of phenolic extract of bush buck (*Gongronema latifolium*) and Wonder kola (*Buchholzia coriacea*)

Samples	AChE (µg/mL)	BChE (µg/mL)	MAO (µg/mL)
Bush buck seed	657.89	591.72	1041.62
Wonderful kola	453.31	453.72	954.20

Values represent mean ± standard deviation (*n=3*). Values with the same superscript alphabet along the same column are not significantly different ($P < 0.05$)

DISCUSSION

Inhibition of key enzymes linked with neurodegenerative disease by phenolic extracts from wonder kola and bush buck justify their usage in the management of mental illness as reported in the folklore. This experimental study revealed that phenolic extracts from wonder kola and bush buck inhibited acetylcholinesterase (AChE), butrylcholinesterase (BChE) and monoamine oxidase (MAO) activities. However, wonder kola exhibited higher inhibitory effects on acetylcholinesterase, butyrylcholinesterase and monoamine oxidase than bush buck as shown in Fig 1, 2 & 3 respectively. AChE and BChE have been implicated in the pathogenesis and progression of neurodegenerative dysfunction such as Alzheimer's disease (Oboh *et al*., 2013; Adefegha *et al*., 2016a). The inhibition of AChE and BChE activities is of health promoting benefit since AChE and BChE are involved in breaking down acetylcholine in the brain and inhibiting these enzymes enhance the concentration of the neurotransmitter acetylcholine reside at the synaptic cleft. Also, this brings about proper communication mechanisms between the nerve cells that use acetylcholine as a chemical messenger. Recent studies have shown that ample concentration of neurotransmitter brings about relief and improve symptoms of Alzheimer's disease (Howes *et al*., 2003). The observed difference in the inhibitory effect of wonder kola and bush buck on the AChE, BChE and MAO activities (Fig 1, 2, 3) could be accounted for by their phytochemical constituents which act synergistically or additively in eliciting inhibitory effects. Recent reports have showed that extracts from two Aframomum species (Adefeha and Oboh, 2012), some leafy vegetables (Nwanna *et al*., 2016) and avocado pear (Oboh *et al*., 2016) exhibited anti-cholinesterase inhibitory properties.

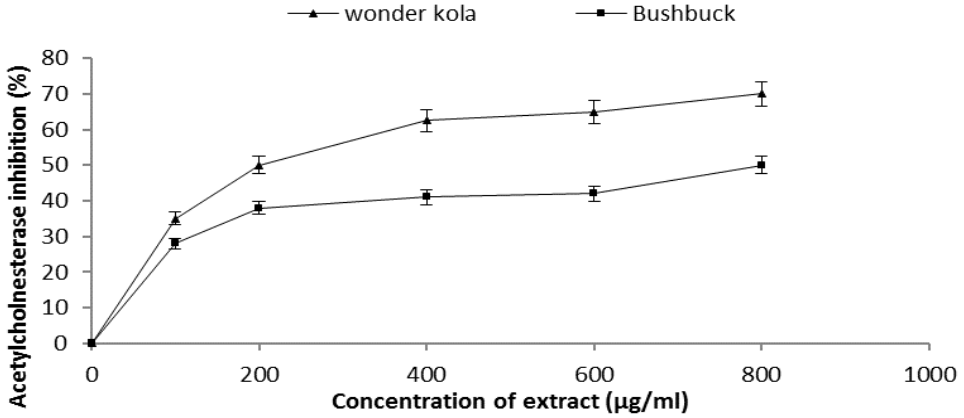

Figure 1: Acetylcholinesterase inhibitory effects of Bush buck seed and Wonder kola extracts.

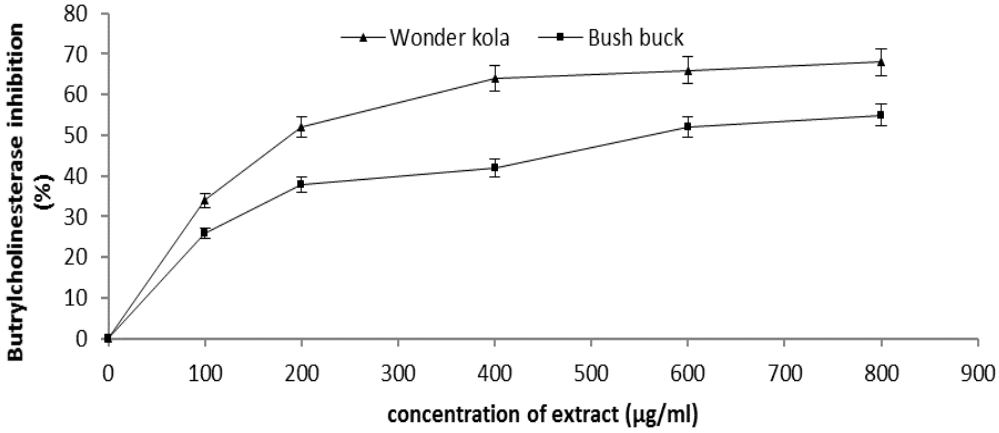

Figure 2: Butrylcholinesterase inhibitory effects of Bush buck seed and Wonder kola extracts.

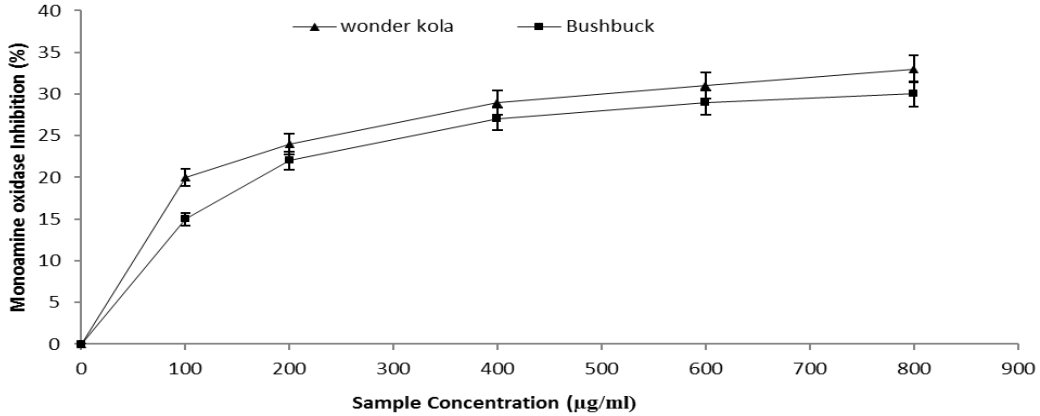

Figure 3: Monoamine oxidase inhibitory effects of Bush buck seed and Wonder kola extracts.

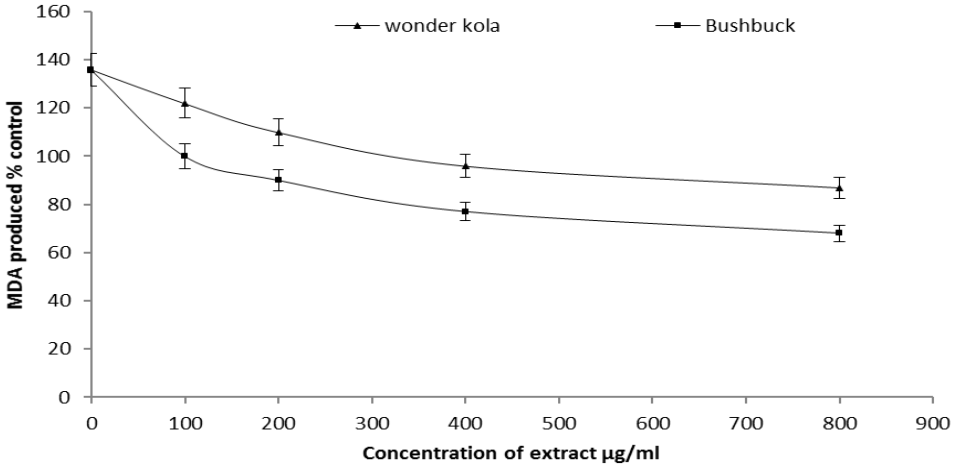

Figure 4: Inhibition of Fe^{2+} induced lipid peroxidation in rat's brain by extracts of Bush buck seed and Wonder kola.

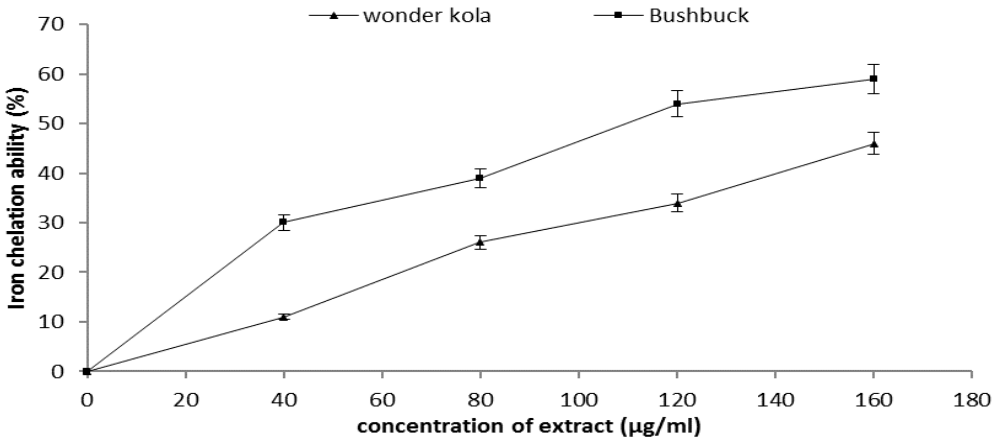

Figure 5: Fe^{2+} chelating ability of extracts of Bush buck seed and Wonderful kola

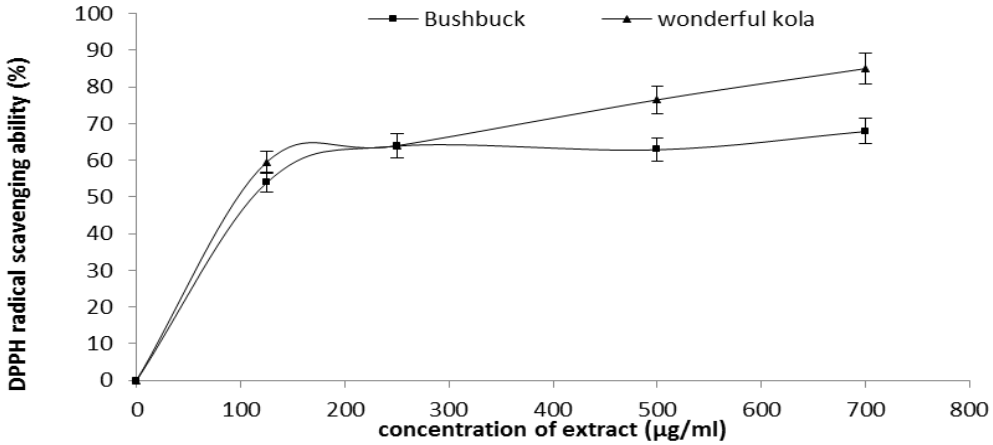

Figure 6: DPPH radical scavenging ability of extracts of Bush buck seed and wonder kola

Monoamine oxidase (MAO) catalyzes oxidative deamination of neuroactive amines to aldehyde and ammonia. The aldehyde and ammonia formed are involved in the production of ROS in the brain of AD patient (Cases *et al*., 1995; Ademosun *et al*., 2015). Monoamine oxidase preferentially degrades monoamine neurotransmitters (dopamine, serotonin, and norepinephrine) in the brain lead to the manifestation of depression observed in AD and other neurodegenerative diseases (Huang *et al*., 2012, Zheng *et al*., 2012). Moreso, MAO activity in the brain tissue has been characterized by the production of reactive oxygen species (Thomas, 2000). Therefore, inhibiting MAO activity prevents degradation of monoamine neurotransmitter and the generation of reactive oxygen species; co-inhibition of MAO and cholinesterases by phenolic extract of bush buck and wonder kola (Fig 1, 2, 3) could justify their use in the management of Alzheimer's disease in folklore.

Antioxidants are widely used as ingredients in dietary supplements to maintain health and prevent diseases such as cancer, Alzheimer's disease and coronary heart disease. The oxidative damage induced in the brain by free radicals has been linked to the pathogenesis of neurodegenerative disease such as Alzheimer's disease (Munch *et al*., 1996). The polyunsaturated

fatty acids in the brain are prone to oxidative damage due to brain's low antioxidant status (Oboh *et al*., 2012). Therefore, antioxidants prevent oxidative stress through free radical scavenging, metal chelating and ability to prevent lipid oxidation which helps effectively in the management and preventing neurodegenerative diseases. The inhibition of Fe^{2+} induced malondiaadehyde (MDA) production in the rat brain homogenate by wonder kola and bush buck phenolic content supports the fact that phenols possess strong antioxidant properties. Also, Fe^{2+} was able to induced lipid peroxidation in the brain owing to Fe^{2+} ability to initiate oxidation of membrane fatty acids through Fenton reaction. The Fenton reaction results in the generation of hydroxyl radical ($OH^{\bullet}$) from H_2O_2. However, MDA concentration in the brain homogenate was used to monitor the extent of oxidative damaged caused by the pro-oxidant (Fe^{2+}).

Moreso, recent findings have shown that polyphenols are capable of crossing the blood-brain barrier where they exhibit neuro-protective property through their free radical scavenging, metal chelation ability and inhibition of lipid peroxidation (Gomez-Pinilla and Nguyen, 2012).

In the same vein, phenolic extracts from bush buck and wonder kola chelated Fe^{2+} from solution in a dose dependent manner as presented in Figure 5. An increase in Fe^{2+} concentration in the brain of AD patient suggests that Fe^{2+} could initiate the generation of reactive oxygen species (hydroxyl radical) via iron-mediated Fenton reaction; this gave credence to Fe^{2+} chelation ability of phenolic extracts from wonder kola and bushbuck in the management and in neuroprotection. The Fe^{2+} chelating ability of the phenolic extract from wonder kola and bush buck could have been an additional mechanism by which they elicit their protective mechanism. Also, to buttress this view, phenolic extract from wonder kola and bushbuck are able to reduce Fe^{3+} to Fe^{2+} as presented in Table 2, which further prevents trivalent iron ion (Fe^{3+}) in reacting with the hydroxyl group of water to produce a powerful hydroxyl radical (Lipinski and Pretorius, 2013), which is capable of causing neuron damages. Nevertheless, the polyphenolic constituents of the extracts have the ability to remove hydroxyl radicals (Zielonka *et al*., 2003; Lipinski, 2011).

The free radical scavenging ability of the phenolic extract from wonder kola and bush buck extracts were studied using various antioxidant assays. However, the DPPH* radical scavenging ability of the phenolic extract from wonderful kola and bush buck presented in Figure 1 showed that the phenolic extracts are able to stabilize DPPH* radical by donating hydrogen molecule to the solution. The observed higher DPPH* scavenging ability of wonder kola also agree with the total phenol, flavonoid content where wonder kola had higher content than bush busk as presented in Table 1 & 2 (Tepe *et al*., 2005).

CONCLUSION

This study has been able to show and establish that phenolic extract of bush buck and wonder kola were able to inhibit acetylcholinesterase, butrylcholinesterase and monoamide oxidase activities and possess antioxidant properties *in vitro.* Wonder kola phenolic extract had higher AChE, BChE, MAO inhibitory and antioxidant activities than bush buck phenolic extracts. Therefore, *in vitro* mechanistic studies of these plants gave credence to their usage in folklore for the management of cognitive dysfunction.

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