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HPLC-DAD Fingerprinting Analysis, Antioxidant Activity of Phenolic Extracts from *Blighia sapida* Bark and Its Inhibition of Cholinergic Enzymes Linked to Alzheimer's Disease

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Abstract

In West Africa, the stem of *Blighia sapida* K.D. Koenig are commonly used as remedy against a variety of diseases, including neurodegenerative diseases without scientific basis. The present study characterizes the phenolic constituents, assessed the cholinergic enzymes (acetylcholinesterase and butyrylcholinesterase) and evaluated the antioxidant properties of phenolic extracts from *B. sapida* K.D. Koenig**.** Total phenol and flavonoids content was evaluated as well as antioxidants as illustrated by Fe^{2+} chelation, 2,2-diphenyl-1-picrylhydrazyl (DPPH·) radical scavenging ability and 2,2-azino-bis-(3-ethylbenthiazoline-6- sulphonic acid (ABTS·) radical scavenging ability spectophotometrically. The ability of the extract to inhibit the activities of acetylcholinesterase and butyrylcholinesterase was also evaluated. The extract was found to be rich in phenolic acid (gallic acid, ellagic acid) and flavonoids (Quercetrin and Luteolin). The results show that the phenolic extracts had DPPH radical scavenging abilities (IC_{50} = 90.71 µg/mL), ABTS· radical scavenging ability (IC_{50} = 85.47 µg/mL), iron chelation (IC_{50} = 136.61 µg/mL) and reducing power (Fe^{3+}- Fe^{2+}) (400.08 AAE mg/100g). Extracts of *B. sapida* inhibited acetylcholinesterase (AChE) (IC_{50} = 125.56 µg/mL) and butyrylcholinesterase (BChE) (IC_{50} = 230.63 µg/mL) activities in a concentration dependent manner (20-100 µg/mL). Hence, one probable means through which the stem bark execute their anti-Alzheimer's disease activity might be by inhibiting cholinesterase activities in addition to thwarting oxidative-stress-induced neurodegeneration.

Key words: *Blighia sapida*, HPLC-DAD, Acetylcholinesterase, Butyrylcholinesterase, Alzheimer Disease (AD), Antioxidant.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease associated with progressive loss of memory and cognition, and there are no definitive treatments or prophylactic agents. Cholinergic abnormalities, alongside β-amyloid plaques, neurofibrillary tangles, and extensive neuronal loss, are the major characteristics in AD (Shimohama and Kihara, 2004). AD is clinically characterized by the development of a progressive dementia, with memory loss, disturbances in language, vision spatial relations, and behavior. Among the rudimentary and common characteristics of AD is the severe deterioration of cholinergic neurons projecting from basal forebrain to cortical and hippocampal areas, associated with decrease in acetylcholine content in cholinergic target areas in AD brains (Contestabile, 2011). The biosynthetic enzyme choline acetyltransferase (ChAT) for acetylcholine (ACh), is reduced 60–90% in AD brains, and the degree of its loss is found with the severity of the observed cognitive impairments (Winkler *et al*., 1998). The action of ChAT in the synthesis of cholinergic neurotransmitter acetylcholine is inhibited by acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) (Nordberg *et al*., 2013). The deterioration of cholinergic nervous system in AD is accompanied by degeneration of many different types of neurons, with a profound loss of forebrain cholinergic neurons, which is accompanied by a progressive decline in acetylcholine. Both the acetylcholine-synthesizing enzyme ChAT, as well in the acetylcholine-hydrolyzing enzyme, AChE are

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affected. In the development of AD, therapies designed to reverse the cholinergic deficit are in large measure based on the importance of cholinergic function in cognition (García-Ayllón, 2011). The decrease of cholinergic activity can be enhanced by agents that restore or enhance cholinergic transmission in the synaptic cleft. AD and other related disorders are aimed at improving the associated cholinergic deficit by inhibiting AChE, resulting in an enhancement in the cognitive performance and in endogenous level of ACh in the brain (Elufioye *et al*., 2010). Hence, most therapeutic strategies in AD have been directed to the beta-amyloid peptides and cholinergic transmission. The first approach is to act on the Amyloid Precursor Protein (APP) processing while the later to reduce neuronal degradation or increasing cholinergic transmission which will help in the management of cognitive deficits (Mikiciuk-Olasik *et al*., 2007). Butyrylcholineterase (BuChE) has been observed to increases in the brain of AD patients and activity are commonly observed in the cerebral cortex and hippocampus (Elufioye *et al*., 2010). AChE and BuChE play a role in cholinergic signaling; BuChE can hydrolyze ACh and compensate for AChE when levels are depleted (Reid *et al*., 2013). In addition, BuChE genotype may investigate AD risk and rate of disease progression, hence, methods that increase acetylcholine levels, such as cholinesterase inhibitors, demonstrate symptomatic efficacy in AD (Nordberg *et al*., 2013). A study that investigated the effect of a selected brain-target BuChE inhibitor, cymserine analogs, revealed a long-term inhibition of butyrylcholinesterase and elevated extracellular brain acetylcholine in rats. Hence, describing the improvement from cholinergic deficit by inhibiting BuChE (Greig *et al*., 2005). In addition to factors leading to this neurodegenerative disease, oxidative stress also contributes to the development to Alzheimer's disease regarding the fact that tissues in the brain are vulnerable to Oxidative Stress (OS). Increased production of Reactive Oxygen Species (ROS), due to the level of oxygen consumption by the brain, lessen antioxidant systems, and repairing mechanisms to Alzheimer's Disease (AD). Also, mitochondrial dysfunction and ROS have been shown in the cause of AD. Impairments of mitochondrial function and oxidative stress may precede Aβ overproduction and deposition, and neurons in AD exhibit a significantly higher percentage of damaged mitochondria (Barbagallo *et al*., 2015; Pimentel *et al*., 2012). Furthermore, lipid peroxidation, causing damage to membrane phospholipids in AD, produces 4-hydroxynonenal which is found high in AD and causes neuronal death by interfering the ATPases involved in transfer of ions and calcium balance. A study showed that excessive ROS in patients with AZ was abated after the intake of an antioxidant-rich plant extract for 6 months (Christen, 2000).

Blighia sapida K.D. Koenig, commonly known as 'Akee apple', belongs to a plant family called *Sapindaceae*. *B. sapida* is a medicinal plant commonly used by traditional healers in Nigeria, and highly valued in Africa for the treatment of various diseases (John-Dewole and Popoola, 2013). The phytochemical screening of aqueous extract of *B. sapida stem bark* has been shown to contain alkaloids, saponins, cardiac glycosides, reducing sugars and carbohydrates, and it was stated to have several ethnomedicinal purposes; the pulp and leafy juice are used as eye drops in ophthalmic lesions and conjunctivitis (Saidu *et al*., 2013; Hamzah *et al*., 2013). The extracts from stem bark of *B. sapida* have been reported to be used by indigenes of Sagamu, Nigeria, as an antiaging by mixing it with black soap and bathing daily, and were shown to exhibit anti-microbial activity against *S. aureus* and *B. subtilis* (Elufioye, 2012; Udobi *et al*., 2013) and also to ameliorative pancreatic β-cell dysfunction in diabetic rats (Ojo *et al*., 2017). Hence, since limited information is known about the activity of *B. sapida* in management of AD, there is a curiosity to examine the inhibitory activity of phenolic extract from *B. sapida* bark against cholinergic enzymes associated with AD, AChE and BuChE. In addition, AD is associated with increased oxidative stress. Investigating the antioxidant activity of the extracts from *B. sapida* bark against free radicals is essential to reveal its ability to counteract excessive ROS in AD patients.

2. Material and Methods

2.1. Chemicals and Reagents

All chemicals and reagents used in the present study were of analytical grade. Methanol, formic acid, acetic acid, gallic acid and ellagic acid were purchased from Merck (Darmstadt, Germany). Folin-Ciocalteu reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), linoleic acid, and α-tocopherol, quercetin and luteolin were acquired from Sigma Chemical Co. (St. Louis, MO, USA). Acetylcholinesterase (EC.3.1.1.7) and butrylcholinesterase (EC 3.1.1.8) were products of Fluka Co., Germany. Acetylthiocholine iodide (ATChI), butyrylthiocholine chloride (BTChCl), 5:5-dithiobis-2-nitrobenzoic acid (DTNB); eserine and sodium bicarbonate were from Sigma Co. UK. High performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software.

2.2. Plant Material and Preparation

The plant was purchased from Bisi Market, Ado-Ekiti, Ekiti State, Nigeria. The herberia specimen of the plants was prepared and voucher specimens deposited at the Herbarium located at the Biology Department, Afe Babalola University, Ado-Ekiti. The stem barks were washed, air-dried and milled into a fine powder and thereafter weighed.

2.3. Extraction of Phenol Extract

Phenol-rich extract from *B. sapida* K.D. Koenig stem bark was acquired by dissolving the powder in an 80% acetonic solution for 72 h. It was then filtered employing a cheese cloth, concentrated to a small volume to remove the entire acetone using rotary evaporator. Then, it was transferred into a 500 mL beaker and placed in a water bath (40 ˚C) to evaporate to dryness. The extract was kept in a closed container and kept inside the fridge at 4 ˚C for further studies (Marimoutou *et al*., 2015).

2.4. Cholinesterase Activity Assay

Acetycholinesterase and Butyrylcholinesterase activity were, respectively, carried out using the colorimetric method of (Tor *et al*., 1994). The reaction assay mixture consisted 2000 mL 100 mM phosphate butter pH 8.0, 100 mL of test phenol extracted, 100 mL, of enzyme AChE or BuCHE solution at a final concentration of 0.03 U/mL and 0.01 µ/mL, respectively, 100 µL of DTNB (0.3 mM) prepared in 100 M phosphate buffer pH 7.0 containing 120 mM sodium bicarbonate. The mixture was vortexed and then pre-incubated in a water bath at 37^{o}C for 30 min. The reaction was then investigated by the adding 100 µL of ATCI or BTCI at a final concentration of 0.5 mM as a negative control. The absorbance change at wavelength 412 nm was then measured for a period of 5 min. All assays were done in triplicate. Eserine (-) physiotigmine) was used as positive control. The % inhibition was calculated as follows:

% = [(a-b)/a] x 100

where: a = ΔA/min of control; b = ΔA/min of test sample; ΔA = Change in absorbance.

2.5. *Determination of Total Phenolic Content*

All the phenolic contents present in the phenol extract were determined using Folin–Ciocalteu method, slightly modified as described by Boligon *et al*. (2009). Gallic acid was the standard and samples were carried out in triplicate at wavelength 730 nm in a spectrophotometer. The total phenolic content of sample was expressed in mg/100g gallic acid (GAE).

2.6. Determination of Total Flavonoid Contents

Total flavonoid contents were carried according to a slightly modified colorimetric method described by Woisky and Salatino (1998). Briefly, each sample (0.5 mL) was added to $AlCl_3$ (2%, w/v, 0.5 mL). The absorbance was measured at wavelength 420 nm against the blank. Quercetin was used as standard and samples were performed in triplicate. The flavonoid content was expressed in mg/100g quercetin (QE).

2.7. *Radical-Scavenging Activity—DPPH Assay*

The phenol extract was evaluated for its antioxidant activity by monitoring its potency in scavenging the stable free radical DPPH, according to Choi *et al*. (2002). The DPPH quenching ability was expressed as IC50 (the extract concentration required to inhibit 50% of the DPPH in the assay medium). The absorption was measured at 518 nm and ethanol was used to calibrate the spectrophotometer. The test was performed in triplicate and the antioxidant activity was
calculated by the equation:

$$\%\text{inhibition} = 100 - [((Abs_{sample} - Abs_{blank}) * 100)/ Abs_{control}]$$

where Abs_{sample} is absorbance of sample; Abs_{blank} is absorbance of fractions without adding the DPPH; $Abs_{control}$ is absorbance the solution of negative control.

2.8. Determination of Ferric Ion Reducing Antioxidant Power: FRAP Assay

The reducing property of the *B. sapida* stem bark extract was studied by assessing the ability of the extract to scale back $FeCl_3$ solution as delineated by Mukhopadhyay *et al*. (2015). 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 solution was incubated for 20 min at 50 °C in a water bath and then 2.5 mL of 10% trichloroacetic acid was added. The sample was then centrifuged at 650 g for 10 min. After that, 5 mL of the supernatant was mixed with an equal water volume and one mL, 0.1% $FeCl_3$. The above-stated process was conjointly applied to a standard ascorbic acid solution, and finally the absorbance was read at 700 nm. The reducing ability was calculated as percentage inhibition.

2.9. Ferrous Ion Chelating Activity Assay

The ferrous ion chelating activity of the phenol extract of *B. sapida* was determined according to Mukhopadhyay *et al*. (2015). In this process, ferrozine, the reaction initiator of the assay, combines with divalent iron to form a stable magenta complex species, the absorbance of which is measured at 562 nm.

The chelating activity of the sample extract was calculated as:

Ferrous ion chelating ability in % = [1-(test sample absorbance/blank sample absorbance)] X 100%

2.10. ABTS Radical Scavenging Assay

ABTS radical scavenging assay was determined by the method of Re *et al*. (1999). Firstly, stock solutions that included 7 mM ABTS solution and 2.4 mM potassium persulfate solution were prepared, mixed together in equal quantities and allowed to react for 12 h at room temperature in the dark. Sample (1 mL) was added to 1 mL of the ABTS solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer:

%inhibition= [(Abs control –Abs sample)]/ (Abs control)] x 100

Abs control is the absorbance of ABTS radical + methanol; absorbance sample is the absorbance of ABTS radical + sample extract /standard.

2.11. HPLC-DAD Fingerprinting

Bliglia sapida bark extract at 12 mg/mL was taken onto reversed phase Phenomenex C_{18} column (4.6 mm x 250 mm) packed with 5 µm diameter particles. The mobile phases were 0.5% (v/v) aqueous formic acid (solvent A) and 1% (v/v) acetic acid in methanol (solvent B). The binary elution system was as follows: 2% B at initial 5 min used to wash the column, a linear gradient 8% B (25 min), 12% B (45 min), 24% B (60 min). After 80 min, organic phase concentration was taken back to 2% (B) and lasted 10 min for column equilibration. Flow rate of 0.6 mL/min and injection volume 40 µl (Ademosun *et al*., 2016). Thereafter, quantifications were carried out by integrating the peaks using the external standard method, at 257 nm for gallic and ellagic acids; and 366 for quercetin and luteolin. The extract and mobile phase were filtered using 0.45 µm membrane filter (Millipore) and degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.030 – 0.500 mg/mL. Chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 700 nm). All chromatography operations were carried out at ambient temperature and in triplicate.

2.12. Data Analysis

The results obtained for DPPH, total flavonoids, total phenols, FRAP, iron chelation, ABTS assays and HPLC were assessed by an analysis of variance model and Tukey's test (HSD multiple range post hoc test). The level of significance for the analyses was set to $p < 0.05$. These analyses were performed by using the free software R version 3.1.1. (R Core Team, 2014) and expressed as mean ± standard error of mean.

3. Results

3.1. Cholinesterase Assay

Acetylcholinesterase inhibitory properties of *B. sapida* phenolic stem bark extract was examined and the result is displayed in Figure 1; the result shows that the phenol extract inhibited AChE activity in a concentration-dependent manner (20–100 μg/mL), having an IC_{50} (extract concentration causing 50% inhibition) value of 125.16 μg/mL as displayed in Table 1. Likewise, the ability of the phenolic extract to inhibit butyrylcholinesterase activity *in vitro* was also assessed, and the result is displayed in Figure 2. The result reveals that the phenol extract inhibited BChE in a concentration-dependent manner (20– 100 μg/mL) having an IC_{50} (extract concentration causing 50% inhibition) value of 230.63 μg/mL as revealed in Table 2.

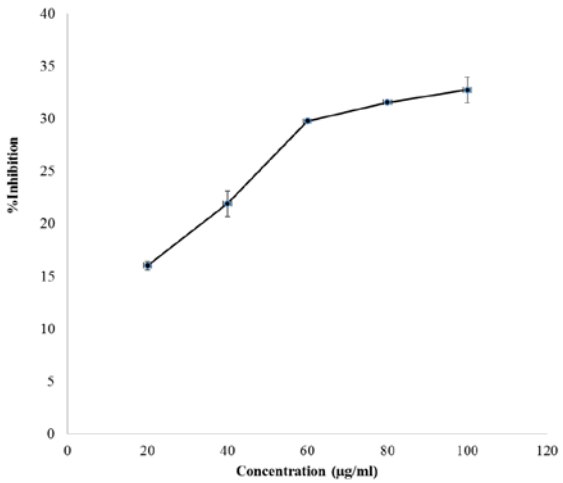

Figure 1. Acetylcholinesterase inhibition by phenolic extract from *Blighia sapida* stem bark
Values are expressed as mean ± SEM for three determinations

Table 1. IC_{50} values for Fe^{2+} chelating ability, DPPH·, ABTS· as well as acetylcholinesterase and butyrylcholinesterase inhibitory activities

Sample	IC_{50} (µg/ml)
Fe Chelation	136.61 ± 7.30
DPPH·	90.71 ± 0.35
ABTS·	85.47 ± 0.30
Acetylcholinesterase	125.16 ± 7.07
Butyrylcholinesterase	230.63 ± 9.07

Values represent means ± standard mean of error

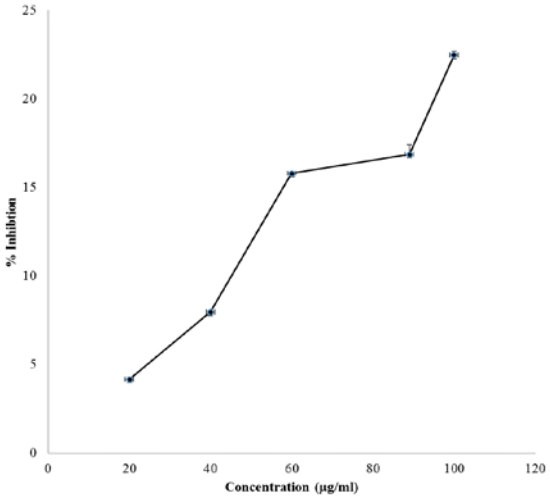

Figure 2. Butyrylcholinesterase inhibition by phenolic extract from *Blighia sapida* stem bark
Values are expressed as mean ± SEM for three determinations

Table 2. Total phenol content, total flavonoid content and reducing properties (FRAP) values of aqueous phenolic extract of *Blighia sapida*

Parameters	Value
Total phenol content (GAE mg/100g)	133.02 ± 32.83
Total flavonoid content (QUE mg/100g)	10.01 ± 1.78
Ferric reducing antioxidant property (AAE mg/100g)	400.08 ± 24.41

* Values represent means ± standard mean of error of triplicate readings. AAE=Ascorbic Acid Equivalent; QUE=Quercetin Equivalent and GAE=Gallic Acid Equivalent

3.2. Phenolic and Flavonoid Content

The results for the total phenol and total flavonoid content of the phenolic extract of stem bark of *B. sapida* are presented in Table 2. The results reveal that *B. sapida* had a total phenol content of 133.02 mg GAE/100g and the total flavonoid content of 10.01 mg QUE/100g.

3.3. DPPH Scavenging Activity

DPPH radical scavenging ability is displayed in Figure 3, with its IC_{50} values (90.71 µg/mL) in Table 2. The result reveals that the phenolic extracts from *B. sapida* scavenged free radicals in a concentration-dependent manner (20-100 µg/mL).

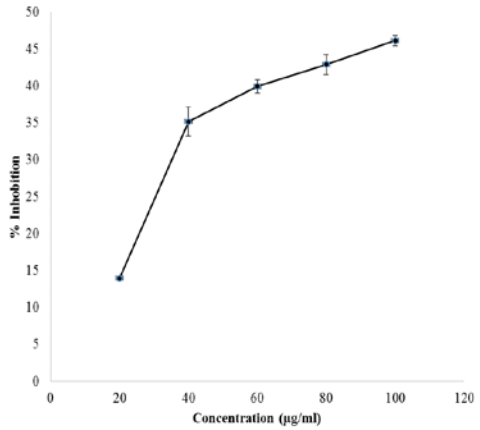

Figure 3. DPPH· scavenging ability of phenolic extract from *Blighia sapida* stem bark.
Values are expressed as mean ± SEM for three determinations

3.4. Reducing Property

The free radical scavenging ability of the *B. sapida* was assessed and the results are presented in Table 2. The results reveal that the *B. sapida* extract (400.08 AAEmg/100g) reduced Fe^{3+} to Fe^{2+} as shown in Table 2.

3.5. Iron (Fe2+) Chelating Ability

Fe^{2+} chelating ability of phenolic extracts from *B. sapida* is presented in Figure 4 and its IC_{50} values of 136.61 µg/mL. The result reveals that the phenolic extract of *B. sapida* exhibited metal chelating activity in a concentration-dependent manner, as shown in Figure 4.

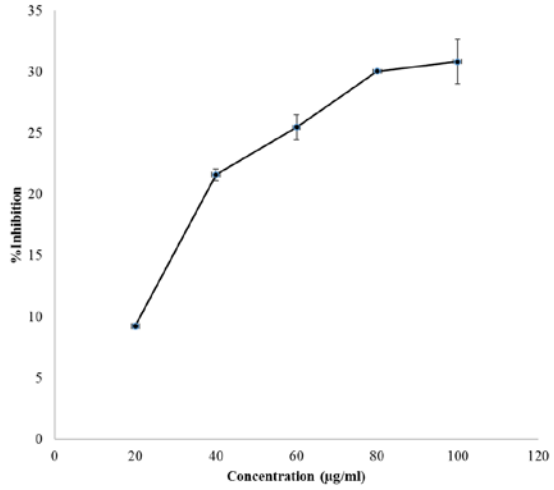

Figure 4. Fe^{2+} chelating ability of phenolic extract from *Blighia sapida* stem bark.
Values are expressed as mean ± SEM for three determinations

3.6. 2,2-Azino-bis3-ethylbenthiazoline-6sulphonic acid (ABTS·) Radical Scavenging Ability

The free radical scavenging ability of the *B. sapida* phenolic stem bark extract was consequently evaluated using the moderately stable ABTS radical (ABTS·) and displayed in Figure 5 with IC_{50} values of 85.47 µg/mL. The results show that the *B. sapida* phenolic extract quenched ABTS radical in a concentration-dependent manner (20-100 µg/mL).

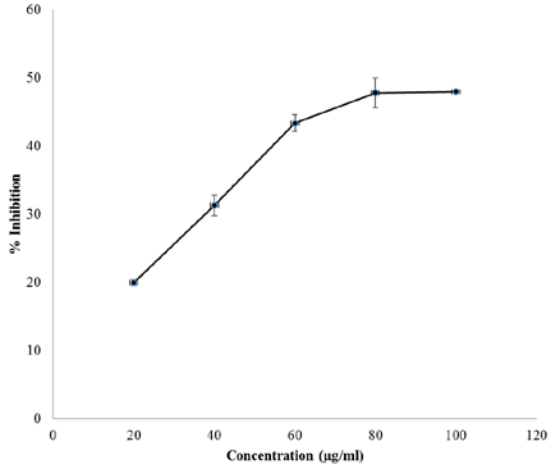

Figure 5. ABTS· scavenging ability of phenolic extract from *Blighia sapida* stem bark
Values are expressed as mean ± SEM for three determinations

3.7. HPLC-DAD Analysis of Phenolic Composition

The phenolics (flavonoids and phenolic acids) composition of *B. sapida* bark as quantified using HPLC-DAD are presented in Table 3. The major phenolic acid was gallic acid, ellagic acid, whereas quercetin and luteolin were the major flavonoids. The result show that the stem bark had high levels of some major flavonoids and phenolic acids of pharmacological importance, including gallic acid, ellagic acid, quercetin and luteolin (Figure 6).

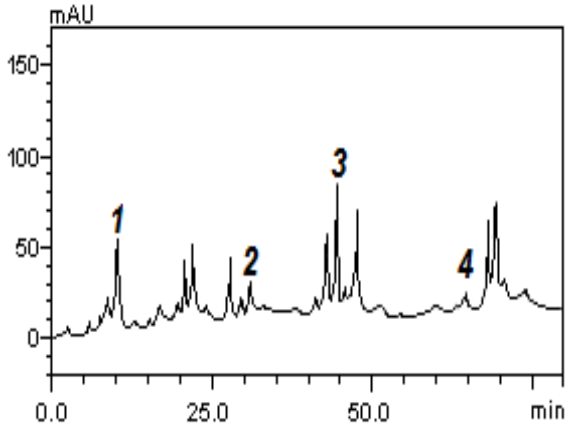

Figure 6. Representative high performance liquid chromatography profile of *Blighia sapida*. Gallic acid (peak 1), ellagic acid (peak 2), quercetin (peak 3) and luteolin (peak 4)

4. Discussion

Mental disorder, also called mental ailment, is a mental or a behavioral pattern of brain functions in life that lead to 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 modern drugs has been linked with some side effects that include headache, diarrhoea, drowsiness and vomiting, among others, unlike the use of natural products. The plant parts employed in the present study have been a bailout for those suffering from Alzheimer's disease in traditional medicine although

the mechanism of action remains unknown. Medicinal plants are rich sources of phytochemical, and intakes of these plant chemicals have a protective potential against neurodegenerative diseases (Chu *et al*., 2002; Ojo *et al*., 2013). However, phenolic extracts of *B. sapida* bark inhibited both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity. Inhibition of acetylcholinesterase is well thought out as a probable method for the management of Alzheimer's disease and for doable therapeutic applications in the treatment of Parkinson's disease and ageing (Nochi *et al*., 1995). However, BChE has been considered to be directly connected with the side effects of the AChE inhibitors and the existing drugs of Alzheimer's disease (Tong *et al*., 1996). Medicinal plants that possess a high phenolic content have been reported to inhibit AChE activity *in vitro* (Benamar *et al*., 2010; Adefegha and Oboh, 2012). Cholinesterases inhibition by the *B. sapida* may be of great importance because it could be an appropriate therapeutic approach in the management or treatment of neurodegenerative disorders. Similarly, in certain forms of Alzheimer's disease, BChE variant has been revealed to upsurge brain susceptibility, thus making BChE inhibition of the extract another means in managing neurodegenerative ailments. As soon as AChE is inhibited, acetylcholine degradation in the brain becomes impossible. The subsequent increase in the brain neurotransmitter acetylcholine concentrations enhances the communication between nerve cells that use acetylcholine as a chemical messenger, and this may recover or soothe the symptoms of Alzheimer's disease momentarily (Howes *et al*., 2003). Phenolic extract of *B. sapida* was able to inhibit AChE and BChE activities in a concentration-dependent manner *in vitro*. This AChE and BChE inhibition is partially in agreement with some earlier reports where plant phytochemicals revealed a considerable improvement in the cognitive performance and memory (Maruyama *et al*., 2006). The ability of the extract to inhibit AChE and BChE may be owing to the antioxidant ability of the plant. Phenolic compounds can protect the human body from free radicals, whose formation is associated with the convectional metabolism of aerobic cells. They are strong antioxidants capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals and inhibit oxidases (Marin *et al*., 2004). The antioxidant properties showed a promising result to fight free radicals in the body system. It revealed that phenolic extract of *B. sapida* had a total phenolic (133.02 mg GAE/100g) and total flavonoid (10.01 mg QUE/100g) constituent (Table 2). Flavonoids are regarded as antioxidant molecules and could, hence, reduce cellular oxidative stress (Oboh *et al*., 2007).

DPPH abstracts hydrogen or electrons from stable molecules, turning them into free radicals, as it becomes a stable diamagnetic molecule (Gyamfi *et al*., 1999; Shim *et al*., 2003). Hence, phenolic scavenge DPPH by donating electrons or hydrogen to stabilize the radical. The present investigation reveals that phenolic extracts from *B. sapida* stem bark scavenged DPPH free radicals in a concentration dependent manner (20–100 µg/mL). However, the radical scavenging abilities of the phenol extracts correlate with the total phenolic contents of the stem. Thus, the observed DPPH radical scavenging ability might be attributed to the abundant phenolic constituents in the extracts. This is consistent with the previous studies (Ojo *et al*., 2013; Ojo *et al*., 2014).

The reducing powers of the phenolic extracts of *B. sapida* were evaluated based on their ability to reduce Fe^{3+} to Fe^{2+}. As displayed by the results, *B. sapida* had a reducing property of (400.08 mg AAE/100g). Reducing power is an antioxidant defense system; the two mechanisms that have an effect on this property are electron transfer and hydrogen atom transfer (Tanaka *et al*., 1998; Dastmalchi *et al*., 2007). The reducing capacity of the extracts may be a sign of its potential antioxidant activities due to the presence of reductants.

Metal ions, for example Fe^{2+}, which results in the induction of oxidative stress, have been reported to be coupled with Alzheimer's disease (Tabert *et al*., 2005). The phenolic extract of *B. sapida* significantly chelate Fe^{2+} in a concentration dependent manner (20–100 µg/mL). By chelating Fe^{2+}, the generation of hydroxyl radicals in the Fenton reaction may be attenuated and thus prevent possible damage of hydroxyl radicals to biomolecules. Accumulation of iron has been reported to lead to an increase in the free radicals and development of oxidative stress (Shim *et al*., 2003).

The free radical scavenging ability of the *B. sapida* extracts was studied employing a moderately stable nitrogen-centred radical species (Re *et al*., 1999). The results of the 2,2′-azino-bis (3-ethylbenthiazoline-6-sulphonic acid (ABTS·) radical scavenging ability of the phenolic extracts of *B. sapida* disclose that the extracts are able to scavenge ABTS· radicals in a concentration-dependent manner (20 - 100 µg/mL). The ABTS· scavenging ability of the stem bark might be due to the hydrogen donating ability of the phenolics present in the extract of *B. sapida* to the single pair of ABTS radical.

The antioxidant properties of plant foods have been associated with the presence of an array of important phenolic and nonphenolic phytochemicals, including phenolic acids and flavonoids (Cheplick *et al*., 2007). However, characterization of the extract with HPLC revealed that the major constituents of the phenolic extract from *B. sapida* bark are gallic acid, ellagic acid, quercetin and luteolin of which the level of gallic acid and quercetin were high. The high amounts of gallic acid (2.07 ± 0.01 mg/g) observed in *B. sapida* may be linked to the biological effects of the plant. These phenolic compounds are well acknowledged as potential antioxidants, free radical scavengers, metal chelation agents and inhibitors of lipid peroxidation (Rice-evans *et al*., 1997; Pereira *et al*., 2009).

5. Conclusion

Phenolic extracts of *B. sapida* stem bark are rich in both phenolic and flavonoids compounds that exhibit both anticholinesterase and antioxidant activity. These herbs show a great potential in the management of Alzheimer's disease as it exhibited an inhibitory activity on cholinergic enzymes (acetylcholinesterase and butyrylcholinesterase) in a concentration-dependent manner and exhibit radical scavenging ability due to the phytochemicals present in the extract.

Conflict of Interest

The authors declare that there is no conflict of interests whatsoever throughout the compilation of the manuscript.

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