Received: 18 August 2017 | Revised: 6 November 2017 | Accepted: 15 December 2017

DOI: 10.1111/jfbc.12510

FULL ARTICLE

WILEY Journal of Food Biochemistry

Effects of drying on cholinesterases and angiotensin-I converting enzyme inhibitory potential and phenolic constituents of African mistletoe (*Loranthus bengwensis L*) leaves from kolanut host tree

Olasunkanmi S. Omojokun[1,2] | Ganiyu Oboh[2] | Adedayo O. Ademiluyi[2]

[1]Biochemistry Unit, Department of Chemical Sciences, Elizade University, Ilara-mokin P.M.B., 002, Ondo State, Nigeria

[2]Functional Foods and Nutraceuticals Unit, Department of Biochemistry, Federal University of Technology, Akure, Nigeria P.M.B., 704, Akure 340001, Nigeria

Correspondence
Olasunkanmi S. Omojokun, Biochemistry Unit, Department of Chemical Sciences, Elizade University, Ilara-mokin, P.M.B., 002, Ondo State, Nigeria.
Email: sunjoks@live.com; olasunkanmi.omojokun@elizadeuniversity.edu.ng

Abstract

This study investigated the most appropriate drying method (sun drying, oven drying, and shade drying) for mistletoe leaves obtained from kolanut tree. The phenolic constituents were characterized using high performance liquid chromatography–diode array detector, while the inhibitory effect of the aqueous extracts of the leaves on cholinesterases and angiotensin-I converting enzyme (ACE) and antioxidant activities were determined in vitro. The extracts inhibited acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and ACE in dose-dependent manner. However, extract from sun-dried sample exhibited the highest AChE, BChE, and ACE inhibitory effect while extract from shade-dried sample had the least. Likewise, sun-dried sample exhibited the highest antioxidant properties as exemplified by Fe^{2+}-chelating, 1,1-diphenyl-2 picrylhdrazyl, OH, and nitric oxide radical scavenging abilities. This study also revealed the presence of 20 phenolic compounds with caffeic acid being the most predominant. Conclusively, kolanut host tree mistletoe leaves can be used as therapeutic agent in the management of Alzheimer's disease and hypertension.

Practical application

The best drying method for mistletoe harvested from kolanut host tree was proposed in this study. Having identified the best drying method that retained phenolic constituents, antioxidant activities, anticholinesterase, and antihypertensive potential of kolanut host tree mistletoe leaves, this work maybe applicable in pharmaceutical industry for development of anticholinesterase and antihypertensive drugs with little or no side effect as well as in the food industry as complementary functional food/medicine in the management of Alzheimer's disease and hypertension.

KEYWORDS

angiotensin-I converting enzyme, cholinesterase, drying, kolanut, mistletoe, phenolics

1 | INTRODUCTION

Plant foods and herbs have been used in folklore and traditional medicine to treat and manage hypertension (Saliu, Ademiluyi, Akinyemi, & Oboh, 2012). However, previous findings have revealed that these plant foods are rich sources of phytochemicals such as phenolics; with strong antioxidant properties and many researchers have attributed the health promoting effects of plant foods to its phenolic constituents (Oboh, Ademosun, et al., 2014).

African mistletoe (*Loranthus bengwensis* L.) is an evergreen, semi-parasitic plant which depends on the host tree for nutrients and water and it has been employed in sub-Saharan African folklore for treatment of many degenerative diseases such as hypertension (Obatomi, Aina, & Temple, 1996). The leaves of this parasitic plant are usually made into concoctions, infusions, or teas which are taken to manage these diseases and this has been demonstrated in hypertensive rats (Obatomi et al., 1996). The extract of the plant have been confirmed to have both cytolytic and immune modulatory properties (Kienle & Kiene,

2007; Melzer, Iten, Hostanska, & Saller, 2009). The numerous biological activities of mistletoe leaves have necessitated its need for all year round availability especially during dry seasons hence, the need to employ drying for longer shelf-life.

Di Cesare, Forni, Viscardi, and Nani (2003) stated that drying is one of the most widely used conventional treatments for preserving seasonal medicinal herbs and that plant materials of herbs inhibits microbial growth and forestalls certain biochemical changes. Furthermore, drying is observed to alter the quality of herbs such as changes in appearance and aroma (Di Cesare et al., 2003; Hossain, Barry-Ryan, Martin-Diana, & Brunton, 2010). Certain compounds like eugenol in bay leaf, thymol in thyme, and some sesquiterpenes in different herbs have been observed to increase after drying (Venskutonis, 1997; Yousif, Scaman, Durance, & Girard, 1999), while the bioactive compounds and antioxidant capacities of the herbs might as well be lost during the process of drying as reported by Hung and Tran (2012).

Alzheimer's disease (AD) is a neurodegenerative disease that affects the brain (Cummings & Cole, 2002). Pharmacotherapeutic approach in the management of AD involved the use of cholinesterase inhibitors that block the breakdown of acetylcholine (Giacobini, 2002). Cholinesterases which are classified as either acetylcholinesterase (AChE) or butyrylcholinesterase (BChE) based on their substrate and inhibitor specificity belong to a family of proteins that is widely distributed throughout the body in both neuronal and nonneuronal tissues (Anglister, Etlin, Finkel, Durrant, & Lev-Tov, 2008). AChE inhibitors such as donepezil and prostigmine are commonly used synthetic drugs for the treatment of AD; however, these drugs are limited in use due to their adverse side effects, hence the need for alternative from natural sources.

Hypertension, referred to as high blood pressure, is a medical condition in which the blood pressure is chronically elevated (Go et al., 2013). Persistent hypertension is one of the risk factors for strokes, heart attacks, heart failure and is a leading cause of chronic renal failure (Jung et al., 2006). Angiotensin-I converting enzyme (ACE), an enzyme involved in maintaining vascular tension, converts angiotensin I to II, the later which is a very potent vasoconstrictor and stimulator of aldosterone secretion by the adrenal gland (Skeggs, Kahn, & Shumway, 1956). ACE inhibition has been identified as a vital therapeutic approach in the management of high blood pressure and phenolics from the diet has shown promising potential in this regard (Oboh, Omojokun, & Ademiluyi, 2016). Interestingly, a strong correlation between ACE and AD has been reported by Kehoe and Passmore (2012) stating that ACE inhibitors are associated with reduced risk of AD.

African mistletoe is used in traditional medicine for management of neurological disorders with no scientific basis. Our previous finding (Oboh, Akinyemi, et al., 2014) reported the potential of kolanut in the management of AD. A report by Watson (2001) stated that the biological activity/health benefit of mistletoe may be dependent on the host plant it is found, this informed of our decision to harvest mistletoe from kolanut tree and assess its possible antihypertensive and anticholinesterase potential taking into consideration the influence of some conventional drying methods (sun drying, oven drying, and shade drying).

2 | MATERIALS AND EXPERIMENTAL METHODS

2.1 | Sample collection and preparation

Mistletoe leaves from kolanut (*Kola nitida*) were harvested from a farm location at Ilara-mokin in Nigeria. The authentication of the plants was done at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria. A voucher specimen was deposited at the Department of Crop, Soil and Pest Management Herbarium FUTA/HB/101013LB2. Subsequently, the leaves were rinsed under running tap and then divided into three portions. The first part was dried to constant weight using direct sunlight at an average temperature of 35°C, the second part was dried to constant weight using a laboratory heat drying oven at a temperature of 50°C, and the third portion shade dried to constant weight at average room temperature between 25 and 28°C kept away from the intensity of the sun. The dried samples were then milled into fine powder of about 0.25 mm particle size using Panasonic blender with model no AC300 (Panasonic Manufacturing, Taytay, Rizal, Philippines). The aqueous extraction was carried out as described by Oboh, Puntel, and Rocha (2007).

2.2 | Chemicals and reagents

All chemicals used were sourced from Sigma Co. (St Louis, MO) and of analytical grade, while the water used was glass distilled.

2.3 | Assays

2.3.1 | Anticholinesterase assays

Inhibition of AChE was assessed by a modified spectrophotometric method of Perry et al. (2001). The AChE activity was determined in a reaction mixture containing 200 μL of a solution of AChE (0.415 U/mL in 0.1 M phosphate buffer, pH 8.0), 100 μL of a solution of 5,5′-dithiobis(2-nitrobenzoic) acid (3.3 mM in 0.1 M phosphate buffered solution, pH 7.0) containing NaHCO3 (6 mM), extract dilutions (0–100 μL), and 500 μL of phosphate buffer, pH 8.0. After incubation for 20 min at 25°C, acetylthiocholine iodide (100 μL of 0.05 mM solution) was added as the substrate, and AChE activity was determined with an ultraviolet visible spectrophotometer from the absorbance changes at 412 nm for 3.0 min at 25°C. Butyrylthiocholine iodide (100 μL) was used as a substrate to assay BChE enzyme, while all the other reagents and conditions were the same. The AChE and BChE inhibitory activities were expressed as percentage inhibition.

2.3.2 | ACE assay

Appropriate dilution of the mistletoe leaves extracts (0–500 μL) and ACE solution (50 μL, 4 mU) was incubated at 37°C for 15 min. The enzymatic reaction was initiated by adding 150 μL of 8.33 mM of the substrate Bz-Gly-His-Leu in 125 mM Tris-HCl buffer (pH 8.3) to the mixture. After incubation for 30 min at 37°C, the reaction was stopped by adding 250 μL of 1 M HCl. The Bz-Gly produced by the enzymatic reaction was extracted with 1.5 mL ethyl acetate, centrifuged to separate the ethyl acetate layer; then 1 mL of the ethyl acetate layer was transferred to a

clean test tube and evaporated. The residue was redissolved in distilled water and absorbance measured at 228 nm (Cushman & Cheung, 1971) using Jenway 6315 UV/Visible spectrophotometer. The ACE inhibitory activity was expressed as percentage (%) inhibition.

2.3.3 | Lipid peroxidation and thiobarbituric acid reactions

Rats were anaesthesized with mild diethyl ether and the heart and kidney were rapidly isolated and placed in ice and weighed. These tissues were subsequently homogenized differently in cold saline (1/10 wt/vol) with about 10-up-and-down strokes at approximately 1,200 rev/min in a Teflon glass homogenizer. The homogenate was centrifuged for 10 min at 3,000 × g to yield a pellet that was discarded, and a low-speed supernatant (Sl) was kept for lipid peroxidation assay (Belle, Dalmolin, Fonini, Rubim, & Rocha, 2004). The lipid peroxidation assay was carried out using the modified method of Ohkawa, Ohishi, and Yagi (1979). Briefly 100 μL Sl fraction was mixed with a reaction mixture containing 30 μL of 0.1 M Tris-HCl buffer (pH 7.4), extracts (0–100 μL) and 30 μL of 250 μM freshly prepared $FeSO_4$ (the procedure was also carried out using 7 μM sodium nitroprusside [SNP]). The volume was made up to 300 μL by water before incubation at 37°C for 1 hr. The reaction was developed by adding 300 μL 8.1% sodium dodecyl sulfate to the reaction mixture followed by the addition of 600 μL of acetic acid/HCl (pH 3.4) and 600 μL 0.8% thiobarbituric acid (TBA). This mixture was incubated at 100°C for 1 hr and the TBA reactive species produced were measured at 532 nm using Jenway 6315 UV/Visible spectrophotometer. Subsequently, the lipid peroxidation was calculated as malondialdehyde (MDA) produced (percentage of control) using MDA as the standard.

2.3.4 | Inhibition of Fenton reaction (degradation of deoxyribose)

The method of Halliwell and Gutteridge (1981) was used to determine the ability of the mistletoe leaves extracts to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose. The extract (0–100 μL) was added to a reaction mixture containing 120 μL of 20 mM deoxyribose, 400 μL of 0.1 M phosphate buffer, 40 μL of 500 μM $FeSO_4$, and the volume were made up to 800 μL with distilled water. The mixture was incubated at 37°C for 30 min and the reaction was stopped by the addition of 0.5 mL of 2.8% trichloroacetic acid (TCA). This was followed by addition of 0.4 mL of 0.6% TBA solution. The tubes were subsequently incubated in boiling water for 20 min and the absorbance was measured at 532 nm using Jenway 6315 UV/Visible spectrophotometer.

2.3.5 | Nitric oxide radical scavenging assay

The scavenging effect of the mistletoe leaves extracts on nitric oxide (NO) radical was measured according to the method of Marcocci, Maguire, Droy-Lefaix, and Packer (1994). Samples of 100–400 μL of the extracts were added in the test tubes to 1 mL of SNP solution (25 mM) and tubes were incubated at 37°C for 2 hr. An aliquot (0.5 mL) was removed and diluted with 0.3 mL Griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% naphthlyethylenediamine dihydrochloride). The absorbance of the chromophore formed was immediately read at 570 nm against distilled water as blank using Jenway 6315 UV/Visible spectrophotometer. Results were expressed as percentage radical scavenging activity.

2.3.6 | 1,1-Diphenyl-2 picrylhydrazyl free radical scavenging ability

The free radical scavenging ability of the mistletoe leaves extracts against 1,1-diphenyl-2 picrylhdrazyl (DPPH) free radical was evaluated as described by Gyamfi, Yonamine, and Aniya (1999). Briefly, appropriate dilution of the extracts (0–500 μL) was mixed with 1 mL, 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was taken at 516 nm using Jenway 6315 UV/Visible spectrophotometer. The DPPH free radical scavenging ability was subsequently calculated.

$$\%\ \text{scavenging ability} = (\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}})/\text{Abs}_{\text{ref}} * 100$$

where, Abs_{ref} = absorbance of reference and Abs_{sam} = absorbance of sample.

2.3.7 | Fe^{2+} chelating assay

The Fe^{2+} chelating ability of the mistletoe leaves extracts was determined using a modified method of Minotti and Aust (1987). Freshly prepared 500 μM $FeSO_4$ (150 μL) was added to a reaction mixture containing 168 μL 0.1 M Tris-HCl (pH 7.4), 218 μL saline, and the extracts (0–25 μL). The reaction mixture was incubated for 5 min, before the addition of 13 μL 0.25% 1,10-phenanthroline (wt/vol). The absorbance was subsequently measured at 510 nm using Jenway 6315 UV/Visible spectrophotometer. The Fe^{2+} chelating ability was subsequently calculated as percentage (%).

$$\%\ Fe^{2+}\text{chelating ability} = (\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}})/\text{Abs}_{\text{ref}} * 100$$

where, Abs_{ref} = absorbance of reference and Abs_{sam} = absorbance of sample.

2.3.8 | Determination of ascorbic acid content

Vitamin C (ascorbic acid) content of the mistletoe leaves extracts was determined using the method of Benderitter et al. (1998). Briefly, 75 μL DNPH (2 g dinitrophenylhydrazine, 230 mg thiourea, and 270 mg $CuSO_4 \cdot 5H_2O$ in 100 mL of 5 M H_2SO_4) was added to 500 μL of the reaction mixture (300 μL of the test sample with 100 μL of 13.3% TCA and water). The reaction mixture was subsequently incubated for 3 hr at 37°C, then 0.5 mL of 65% H_2SO_4 (v/v) was added to the medium and the absorbance was measured at 520 nm using Jenway 6315 UV/Visible spectrophotometer. The vitamin C content of the samples was expressed as ascorbic acid equivalent.

2.3.9 | Determination of total phenol content

The total phenol content was determined according to the method of Singleton, Orthofor, and Lamuela-Raventos (1999). Briefly, appropriate dilutions of the extracts (200 μL) were oxidized with 2.5 mL 10% Folin–Ciocalteau's reagent (v/v) and neutralized by the addition of 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 using Systonic S-910 digital colorimeter. The total phenol content was subsequently calculated as gallic acid equivalent.

2.3.10 | Determination of total flavonoid content

The total flavonoid content was determined using a slightly modified method reported by Meda, Lamien, Romito, Millogo, and Nacoulma (2005). Briefly 0.5 mL of appropriately diluted sample was mixed with 0.5 mL methanol, 50 μL 10% A1C1$_3$, 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 using Jenway 6315 UV/Visible spectrophotometer; the total flavonoid content calculated. The non-flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid content.

2.3.11 | High performance liquid chromatography—diode array detector characterization of the phenolic constituents

Two stage extraction procedures followed for the removal of the phenolic compounds as described by Kelley, Coffey, and Mueller (1994) and Provan, Scobbie, and Chesson (1994). After extraction, the sample was centrifuged (5,000 × g), rinsed with water, centrifuged again, and the supernatants combined and placed in a disposable glass test tube followed by heating at 90°C for 2 hr to release the conjugated phenolic compounds. The heated extract was cooled, titrated with 4 M HCl to pH <2.0, diluted to 10 mL, with deionized water and centrifuged to remove the precipitate. The supernatant was saved for subsequent purification and the residue was extracted further in Stage 2.

Stage 2: The residue from stage 1 above was extracted with 5 mL of 4 M NaOH, heated to 160°C in Teflon as described by Provan et al. (1994). After cooling, the mixture was filtered, supernatant was collected, and the residue washed with water (deionized). The supernatants were combined and adjusted to pH < 2.0 with 4 M HCl. The filtrate was combined for further preparation.

Purification: An aliquot (5–15 mL) of the various supernatants was passed through a conditioned varian (Varian Assoc., Harbor City, CA) Bond Elut PPL (3 mL size with 200 mg packing) solid-phase extraction tube at ~5 mL min^{-1} attached to a Visiprep (Supelco, Bellefonte, PA). The tubes were placed under a vacuum (−60 kPa) until the resin was thoroughly dried after which the phenolic compounds were eluted with 1 mL of ethyl vials. The PPL tubes were conditioned by first passing 2 mL of ethyl acetate followed by 2 mL of water (pH < 2.0). Purified phenolic extracts (1 μL: 10:1 split) were analyzed by comparison with phenolic standards (Aldrich Chemical Co., Milwaukee, W1) with standards on a Waters 600 High Performance Liquid Chromatograph LCD System equipped with Waters 515 HPLC pump, Waters 2487 UV/VIS detector, C18 column with dimensions 5 μm, 4.6 × 250 mm with Hamilton microliter syringe and injection volume of 20 μL. The following conditions were employed per separation; wavelength, 280 nm; flow rate, 1.0 mL/min; gradient elution total run time of 31 min, having Solvent A as acetonitrile, Solvent B as 0.1% phosphoric acid in deionized water which was started with 85% A and held at this for 13 min, followed by 75% eluent B for 10 min. The concentration of B was increased to 85% for another 8 min.

2.4 | Data analysis

The results of replicate experiments were pooled and expressed as mean ± standard deviation (*SD*). A one-way analysis of variance was used to analyze the mean, significance was accepted at $p < .05$ (Zar, 1984). The EC_{50} (extract concentration causing 50% enzyme inhibition/antioxidant activity) was performed using non-linear regression analysis.

3 | RESULTS AND DISCUSSION

3.1 | Effect of drying methods on the anticholinesterase and antihypertensive potential of kolanut host tree African mistletoe leaves

Figure 1 presents the effect of the drying methods on the inhibitory effect of mistletoe leaves on AChE activity in rat brain in vitro. The result revealed that extracts inhibited brain AChE activity in a dose-dependent manner (0–390.26 μg/mL). Furthermore, as revealed by the EC_{50} values (Table 1), shade-dried sample exhibited the least AChE inhibitory activity. However, the sun-dried (241.18 μg/mL) extract had the highest inhibitory effect on AChE activities. Same trend was observed in the brain BChE activity with shade-dried extract having the least BChE inhibitory activity while sun-dried extract had the highest (Figure 2 and Table 1).

Similarly, Figure 3 presents the effect of drying methods on the inhibitory effect of extracts on ACE in rat kidney in vitro. The result revealed that the extracts inhibited kidney ACE activity in a dose-dependent manner (0–354.1 μg/mL). Furthermore, the EC_{50} values (Table 1) revealed that shade-dried sample exhibited least ACE inhibitory activity. However, sun-dried (260.7 μg/mL) extract had the highest inhibitory effect on kidney ACE activities. Same trend was observed in the lung ACE activity with shade-dried extract having the least ACE inhibitory activity while sun-dried extract exhibited the highest (Figure 4 and Table 1). Nevertheless, the ACE inhibitory activities of the samples were more pronounced in the kidney than the lungs.

Ogunmefun, Fasola, Saba, and Oridupa (2013) reported the phytoconstituents (alkaloids, phenolic acids, flavonoids, etc.) of a certain specie (*Phragmanthera incana*) of mistletoe and proposed it may be responsible for various health promoting benefits elicited by the plant.

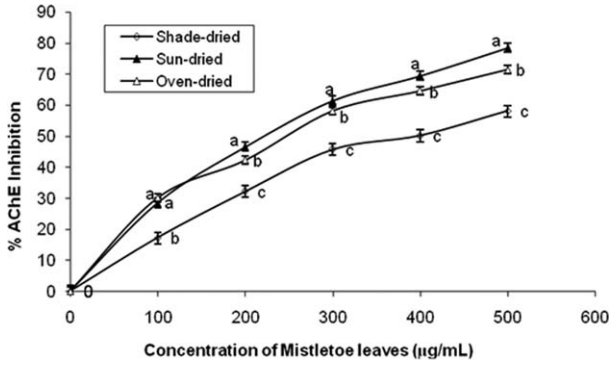

FIGURE 1 Effects of drying methods on kolanut host tree mistletoe leaves acetylcholinesterase inhibitory activity in rats' brain

TABLE 1 EC_{50} values of the effects of drying methods on kolanut host tree African mistletoe leaves cholinesterases activity in rats' brain and ACE inhibitory activity in rats' kidney and lungs

	EC_{50} for enzymes (AChE, BChE, and ACE) inhibition (μg/mL)			
			ACE inhibition	
Sample	AChE	BChE	Kidney	Lungs
Oven-dried	268.2 ± 3.3^{b}	279.9 ± 2.8^{b}	282.2 ± 2.1^{b}	374.5 ± 3.2^{b}
Sun-dried	241.1 ± 2.1^{a}	244.4 ± 2.7^{a}	260.7 ± 1.6^{a}	341.3 ± 4.1^{a}
Shade-dried	390.2 ± 4.2^{c}	346.5 ± 4.3^{c}	354.1 ± 2.0^{c}	420.2 ± 5.2^{c}

Abbreviations (ACE = angiotensin-I converting enzyme; AChE = acetylcholinesterase; BChE = butyrylcholinesterase).
Notes. Values represent mean ± *SD* ($n = 3$). Values with the same superscript alphabet on the same column are not significantly ($p < .05$) different.

Previously, Oboh, Agunloye, Akinyemi, Ademiluyi, and Adefegha (2013) reported that caffeic acid exhibited neuromodulatory potential. Similar use of African mistletoe (*Loranthus bengwensis* L.) is in the treatment/management of hypertension as reported by Obatomi et al. (1996). Hence, the ACE inhibitory activity of the mistletoe leaves observed in this study could be attributed to their phenolic content. Furthermore, it had been reported that the ACE inhibitory property of plants and plant foods is a function of the type and amount of its constituent phenolic compounds (Kwon, Apostolidis, & Shetty, 2008). Therefore, this cholinesterases and ACE inhibitory activities of the extract indicate a neuromodulatory and anti-hypertensive property and could provide the biochemical basis for its use as neuromodulatory and antihypertensive agent in traditional medicine.

3.2 | Effect of drying methods on the antioxidative properties of African mistletoe leaves

A significant increase ($p < .05$) in the MDA content was observed as shown following the incubation of rat heart in presence of 250 μM Fe^{2+} (Figure 5) and 7 μM SNP (Figure 6). All dried leaf extracts inhibited MDA production in heart cells in a dose-dependent (0–157.2 μg/mL) manner and the EC_{50} is presented in Table 2. Sun drying resulted in highest inhibition but there was no significant ($p > .05$) difference between the other two (shade and oven dried). The same trend was observed when SNP was the pro-oxidant (Table 2). Furthermore, rat's kidney homogenate was separately challenged with 250 μM Fe^{2+} and 7 μM SNP and this resulted to significant increase ($p < .05$) in MDA content (Figures 7 and 8). However, the presence of the mistletoe extracts resulted in the inhibition of MDA production in a dose-dependent (0–155.6 μg/mL) pattern.

Figure 10 shows that the sun-dried extract had the highest NO free radical scavenging ability followed by the oven-dried while the shade-dried sample showed the least as revealed by the EC_{50} values in Table 3.

Furthermore, the DPPH free radical scavenging abilities of aqueous extracts revealed they all scavenged DPPH radicals in dose-dependent pattern (0–833.3 μg/mL) as presented in Table 3. The sun-dried extract had 596.7 μg/mL that was the highest DPPH free radical scavenging ability while oven dried had 653.6 μg/mL and shade dried (833.3 μg/mL) showed the least. There was agreement between the NO and DPPH free radical scavenging ability, with sun-dried extracts having the highest radical scavenging ability for both. The radical scavenging ability

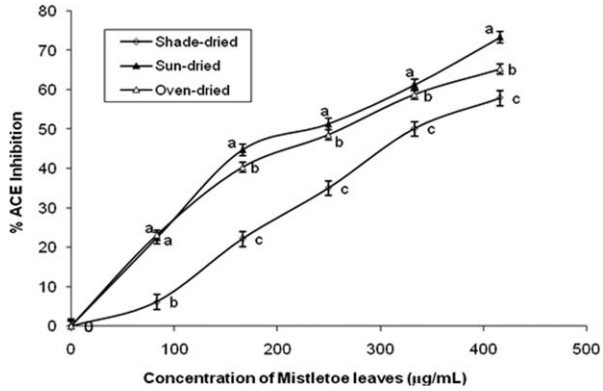

FIGURE 3 Effects of drying methods on angiotensin-I converting enzyme inhibitory activity of mistletoe leaves harvested from kolanut host tree in rat kidney

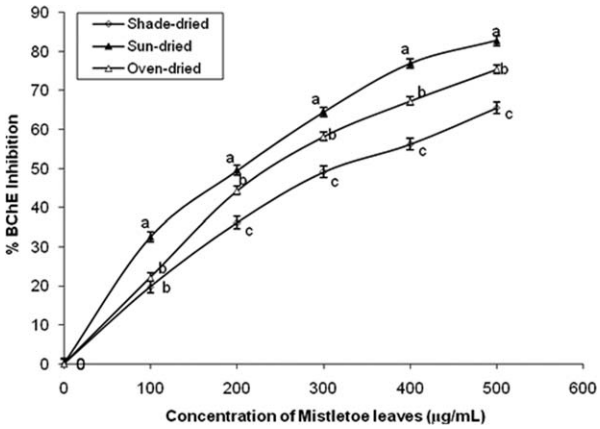

FIGURE 2 Effects of drying methods on kolanut host tree mistletoe leaves butyrylcholinesterase inhibitory activity in rats' brain

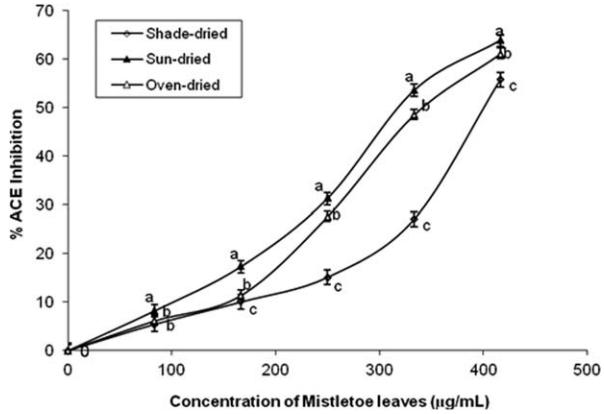

FIGURE 4 Effects of drying methods on angiotensin-I converting enzyme inhibitory activity of mistletoe leaves harvested from kolanut host tree in rat lungs

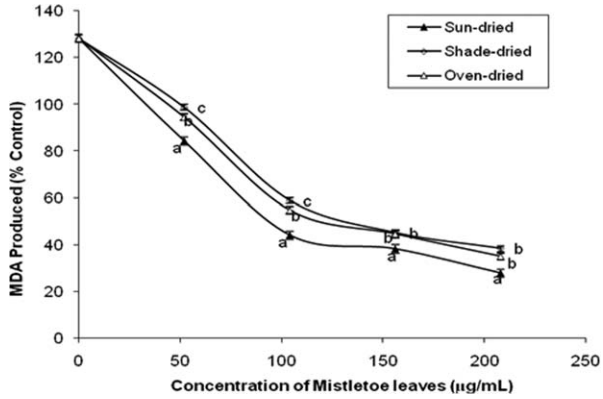

FIGURE 5 Effects of some drying methods on the inhibition of Fe^{2+}-induced lipid peroxidation in rat heart by mistletoe leaves harvested from kolanut host tree

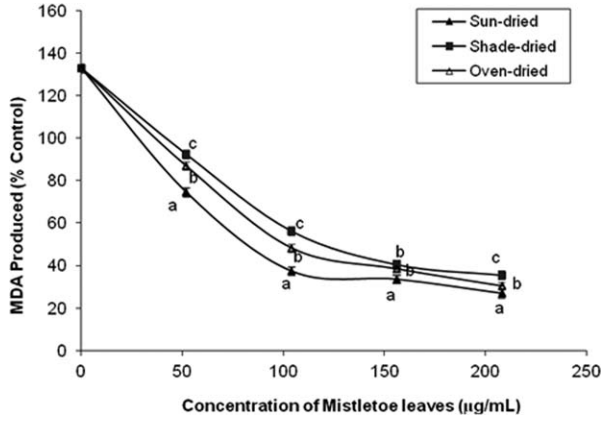

FIGURE 6 Effects of some drying methods on the inhibition of sodium nitroprusside-induced lipid peroxidation in rat heart by mistletoe leaves harvested from kolanut host tree

of the mistletoe extracts also correlated with total phenol and flavonoid contents. These findings agreed with earlier reports of Ademiluyi and Oboh (2012) as well as Chu, Sun, Wu, and Liu (2002) that plant antioxidant properties correlates with their phenolic contents.

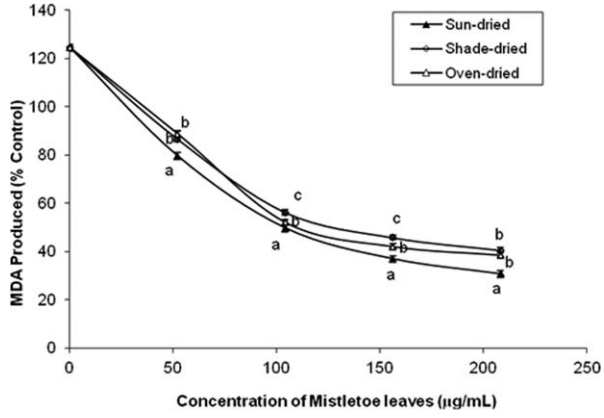

FIGURE 7 Effects of some drying methods on the inhibition of Fe^{2+}-induced lipid peroxidation in rat kidney by mistletoe leaves harvested from kolanut host tree

TABLE 2 EC_{50} values of the effects of some drying methods on the inhibition of SNP and Fe^{2+}-induced lipid peroxidation in rat kidney and heart by African mistletoe leaves from kolanut host tree

Tissue →	EC_{50} for inhibition of SNP and Fe^{2+}-induced lipid peroxidation (µg/mL)			
	Kidney		Heart	
Pro-oxidants →	(SNP)	(Fe^{2+})	(SNP)	(Fe^{2+})
Sample				
Oven-dried	126.5 ± 1.8^{b}	150.7 ± 1.2^{b}	142.3 ± 0.6^{b}	151.7 ± 2.0^{b}
Sun-dried	115.5 ± 1.2^{a}	139.6 ± 1.1^{a}	133.3 ± 1.4^{a}	138.3 ± 1.7^{a}
Shade-dried	133.6 ± 1.5^{c}	155.6 ± 1.6^{c}	150.2 ± 1.3^{c}	157.2 ± 1.7^{b}

Abbreviation (SNP = sodium nitroprusside).
Notes. Values represent mean ± *SD* ($n = 3$). Values with the same superscript alphabet on the same column are not significantly ($p < .05$) different.

The inhibition of lipid peroxidation induced by the pro-oxidants (Fe^{2+} and SNP) in both rat's heart and kidney homogenates by the extracts is an indication of potent antioxidant capacity. However, the effect of drying methods on this property suggests dependence on the phenolic constituents. This was in agreement with a previous report by Oboh et al. (2016) that showed a strong correlation between the antioxidant properties of plants and their phenolic constituents. Inhibition of lipid peroxidation has been identified as an antioxidant mechanism of action (Oboh et al., 2007). As revealed in Figures 9–11, the extracts scavenged OH, NO and DPPH radicals in dose dependent manner confirming the antioxidative potential of the extracts. Hydroxyl radical is known to be extremely dangerous to cells (Sikka & Hellstrom, 2002) and it is assumed to play a key role in disease progression (Gazzaruso et al., 2008; Ma et al., 2008). Similarly, NO radical is implicated in some cytotoxic and neurotic processes and could contribute to some extent to the production of plaque in affected organ/tissues. NO and OH radicals are generally produced because of aggravation or inflammation and reducing their bioavailability because of endothelial and nerve damage they may cause is vital to forestall oxidative stress

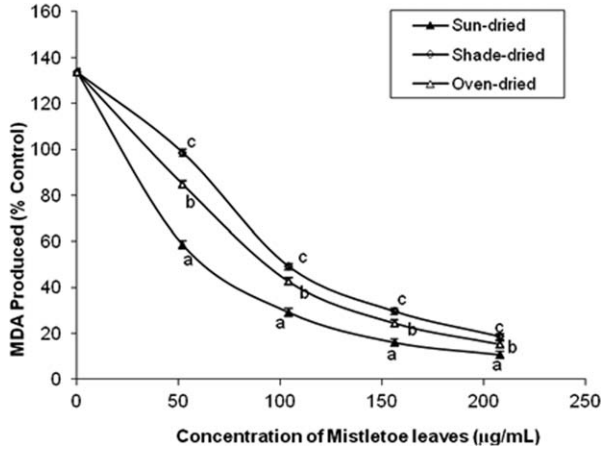

FIGURE 8 Effects of some drying methods on the inhibition of sodium nitroprusside-induced lipid peroxidation in rat kidney by mistletoe leaves harvested from kolanut host tree

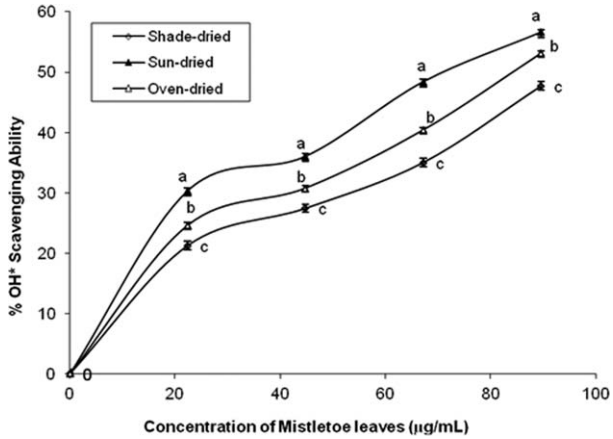

FIGURE 9 Effects of drying methods on the OH^- radical scavenging ability of mistletoe leaves harvested from kolanut host tree.

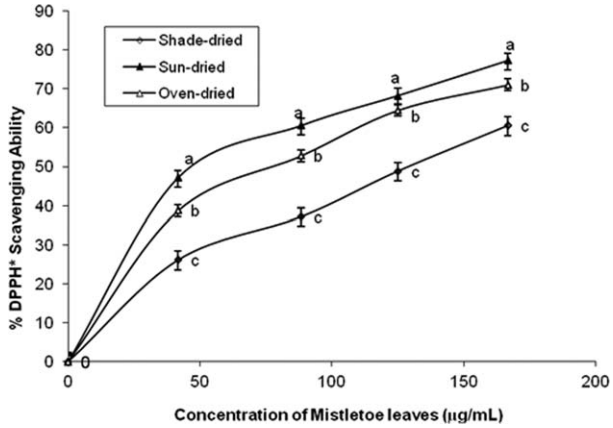

FIGURE 11 Effects of drying methods on the DPPH radical scavenging ability of aqueous extract of mistletoe leaves harvested from kolanut host tree

and eventual cell death (Stefan & Schmieder, 2003). Alterations in the endogenous free radical scavenging defense mechanisms could lead to ineffective scavenging of ROS, resulting in oxidative damage, tissue injury and possible progression to AD, hypertension, and other degenerative diseases. Hence, steady supply of dietary antioxidants to augment the endogenous antioxidant defense mechanisms has been proposed as a means to control free radical-mediated oxidative stress in AD and hypertension (Adefegha, Oboh, Omojokun, & Adefegha, 2016).

Figure 12 also revealed that all the extracts chelated Fe^{2+} [sun dried (361.5 µg/mL), oven dried (385.8 µg/mL), and shade dried (519.8 µg/mL)]. Furthermore, drying methods altered the Fe^{2+} chelating property. The high Fe^{2+} chelating property of the sun-dried extract also correlated with its phenolic content and radical scavenging abilities.

3.3 | Effect of drying methods on total phenolic, total flavonoid, vitamin C contents, and the phenolic phytoconstituents of African mistletoe leaves

Table 4 shows the results of total phenol, flavonoid, and vitamin C contents of the different extracts that sun dried (8.1 mg GAE/100 g dried sample) had the highest total phenol content followed by oven dried (7.7 mg GAE/100 g dried sample) while shade dried (6.2 mg GAE/100 g dried sample) had the least. Total flavonoid content followed the same trend with sun dried (3.1 mg QUE/100 g dried sample) having the highest followed by oven dried (2.9 mg QUE/100 g dried sample) while shade dried (2.6 mg QUE/100 g dried sample) had the least. For the vitamin C content, sun-dried mistletoe leaves (10.1 mg AAE/100 g dried sample) had the highest content followed by oven dried (9.6 mg AAE/100 g dried sample) while shade-dried sample (8.2 mg AAE/100 g dried sample) had the least. Previous studies by Kiremire, Musinguzi, Kikafunda, and Lukwago (2010) reported that sun drying resulted in great loss of vitamin C and β-carotene, this occurred when ultra violet light oxidized vitamin C to a less stable dehydro-ascorbic acid which maybe oxidized to other compounds. The higher vitamin C content in the sun-dried sample in this study negates the claim of Kiremire et al. (2010), we thus suggested that low Vitamin C content in the shade-dried sample may be as a result of oxidation reactions occurring over the prolonged drying period.

Table 5 depicted the chromatographic analysis of constituent phenolic compounds in the sun-dried, oven-dried, and shade-dried extracts. Caffeic acid, protocatechuic acid, *p*-coumaric acid, *o*-coumaric acid, vanillic acid, *p*-hydroxybenzoic acid, ferulic acid, gentisic acid, gallic

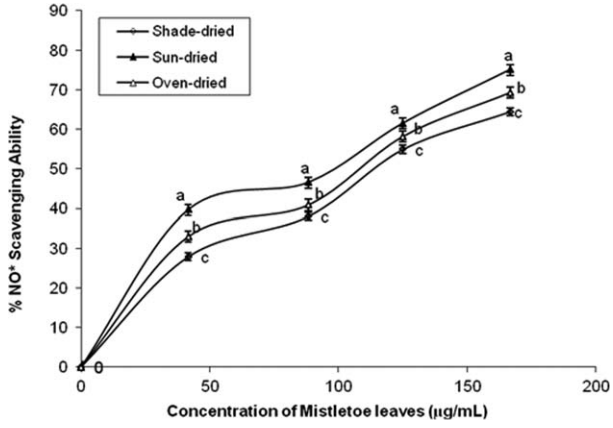

FIGURE 10 Effects of drying methods on the NO radical scavenging ability of mistletoe leaves harvested from kolanut host tree

TABLE 3 Effects of drying methods on the OH^-, NO, DPPH radical scavenging, and Fe^{2+} chelating ability of African mistletoe leaves harvested from kolanut host tree

	EC_{50} for scavenging and chelating abilities (µg/mL)			
Sample	OH*	NO*	DPPH*	Fe^{2+} chelation
Oven-dried	80.7 ± 1.8^b	110.1 ± 2.3^b	101.1 ± 4.0^b	86.8 ± 2.2^b
Sun-dried	71.9 ± 1.8^a	101.2 ± 1.8^a	92.3 ± 3.5^a	79.5 ± 1.9^a
Shade-dried	93.3 ± 2.2^c	121.9 ± 2.2^c	128.8 ± 5.2^c	116.8 ± 3.8^c

Abbreviations (DPPH = 1,1-diphenyl-2 picrylhdrazyl; NO = nitric oxide).
Notes. Values represent mean ± *SD* ($n = 3$). Values with the same superscript alphabet on the same column are not significantly ($p < .05$) different.

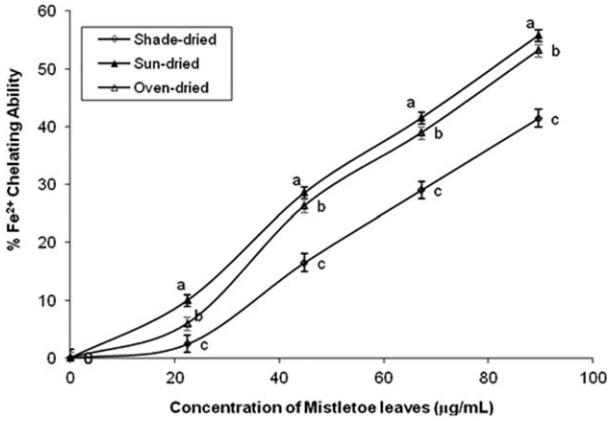

FIGURE 12 Effects of drying methods on the iron chelating ability of mistletoe leaves harvested from kolanut host tree

acid, syringic acid, quercetin, apigenin, gingerol, kaempferol, luteolin, isorhamnetin, myricetin, rosmarinic acid, chlorogenic acid, and gossypetin were the predominant phenolic compounds in all dried mistletoe leaves. The sun-dried extracts had the highest values followed by oven dried while shade dried had the least of all the phenolic compounds with caffeic acid being the most abundant.

Ogunmefun et al. (2013) reported that mistletoe, being a parasitic plant, would share some similarities to the host plant in terms of their phytoconstituents which may influence their biological activities. Also, findings by Moustapha, Gutiérrez-Avella, Fuentes-Ordaz, Castañeda-Moreno, and Martínez (2011) have reported that the phytoconstituents of mistletoes is a direct function of the host plant. As observed in this study, gossypetin, chlorogenic, and rosmarinic acid identified in the extract were totally absent in almond host tree mistletoe leaves. Furthermore, the substantial quantity of gossypetin identified in the sun dried (0.76 mg/100g) extract as compared with the oven dried (0.47 mg/100g) and shade dried (0.09 mg/100g) may be attributed to the potential of gossypetin to withstand UV radiation-induced phenolic degradation as previously reported by Harborne (1981) in the flowers of *Coronilla valentine*. This may also have accounted for its higher Vitamin C content and a generally higher biological activity elicited by the sun-dried sample. Also, as a result of prolonged drying time which occurred at ambient temperature, there is longer retention of moisture content in shade-dried samples compared to the two other drying methods, this may cause deterioration to the leaves and in turn could induce rapid degradation of the phenolic compounds in the shade-dried samples hence the reason for its low phenolic content and biological activity. Thus, we affirm that each plant needs a particular drying method that soothes it best so as to retain its overall biological activity and because of similarities in the secondary metabolites of a typical plant family, a particular drying method might be the best to be used for all plants in that family. We also suggest further evaluation of other drying methods that remove moisture content at low temperatures with the use of irradiation or vacuum pump different from the traditional methods used in this study stands a challenging prospective that should be explored. In addition, we suggest a comprehensive in vivo experiment be carried out to fully substantiate this claim.

TABLE 4 Total phenol, flavonoid, and vitamin C content of aqueous extract of three differently dried mistletoe leaves from kolanut tree

Sample	Total phenol (mg GAE/100 g dried sample)	Total flavonoid (mg QUE/100 g dried sample)	Vitamin C (mg AAE/100 g dried sample)
Shade-dried	6.2 ± 0.1^{c}	2.6 ± 0.1^{c}	8.2 ± 0.2^{c}
Sun-dried	8.1 ± 0.1^{a}	3.1 ± 0.1^{a}	10.1 ± 0.1^{a}
Oven-dried	7.7 ± 0.1^{b}	2.9 ± 0.1^{b}	9.6 ± 0.2^{b}

Notes. Values represent mean ± *SD* ($n = 3$). Values with the same superscript alphabet on the same column are not significantly ($p < .05$) different.

TABLE 5 Effect of some drying methods on the high performance liquid chromatography characterization of phenolic constituents of mistletoe (*Loranthus begwensis* L.) leaves harvested from kolanut host tree

	Amount of phenolic compounds in mistletoe leaves (mg/100 g dry sample)		
Drying methods →	Shade-dried	Sun-dried	Oven-dried
Phenolics			
Protocatechuic acid	10.84 ± 0.62^{c}	22.76 ± 0.94^{a}	19.85 ± 0.55^{b}
p-Coumaric acid	28.00 ± 1.62^{c}	38.69 ± 0.65^{a}	36.11 ± 0.22^{b}
o-Coumaric acid	1.04 ± 0.02^{b}	1.21 ± 0.07^{a}	1.12 ± 0.12^{a}
Vanillic acid	0.39 ± 0.16^{b}	0.85 ± 0.03a	0.26 ± 0.08^{b}
p-Hydroxybenzoic acid	0.41 ± 0.03^{b}	0.59 ± 0.02^{a}	0.53 ± 0.05^{a}
Gentisic acid	16.10 ± 0.65^{c}	21.43 ± 0.82^{a}	18.79 ± 0.98^{b}
Gallic acid	0.03 ± 0.01^{b}	0.16 ± 0.01^{a}	0.15 ± 0.02^{a}
Caffeic acid	267.94 ± 5.82^{c}	295.57 ± 3.61^{a}	287.43 ± 4.66^{b}
Ferrulic acid	0.01 ± 0.01^{a}	0.03 ± 0.01^{a}	0.02 ± 0.01^{a}
Syringic acid	0.06 ± 0.01^{b}	0.10 ± 0.01^{a}	0.04 ± 0.02^{b}
Apigenin	9.22 ± 0.68^{b}	11.84 ± 0.82^{a}	11.53 ± 0.45^{a}
Kaemferol	9.55 ± 0.72^{b}	13.22 ± 0.88^{a}	10.07 ± 0.32^{b}
Luteolin	20.00 ± 0.65^{b}	23.27 ± 0.90^{a}	20.93 ± 0.61^{b}
Gingerol	0.08 ± 0.02^{c}	0.30 ± 0.02^{a}	0.23 ± 0.02^{b}
Quercetin	33.84 ± 1.00^{c}	44.22 ± 1.31^{a}	36.97 ± 0.96^{b}
Isorhamnetin	0.01 ± 0.01^{c}	0.73 ± 0.02^{a}	0.34 ± 0.01^{b}
Myricetin	4.01 ± 0.86^{c}	8.59 ± 0.26^{a}	7.13 ± 0.55^{b}
Gossypetin	0.09 ± 0.02^{c}	0.76 ± 0.12^{a}	0.47 ± 0.05^{b}
Chlorogenic acid	3.42 ± 0.04^{c}	4.44 ± 0.02^{a}	4.12 ± 0.08^{b}
Rosmarinic acid	3.74 ± 0.12^{b}	4.75 ± 0.11^{a}	4.61 ± 0.18^{a}

Notes. Values represent mean of triplicate analysis. Values with the same superscript alphabet on the same row are not significantly ($p < .05$) different.

4 | CONCLUSIONS

Inhibition of basic enzymes linked with AD (AChE and BChE) and hypertension (ACE) by extracts of dried mistletoe leaves from kolanut host tree could be part of the mechanism through which mistletoe leaves elicit its neuromodulatory and antihypertensive effect justifying its folkloric use in the management of neurological disorder and hypertension. However, the modes of drying employed to prolong the shelf-life of kolanut host tree African mistletoe leaves significantly altered the phenolic phytoconstituents and its overall bioactivity. Therefore, to retain appreciable quantity of the phytoconstituents, potent cholinesterases and ACE inhibitory activity of mistletoe harvested from kolanut tree, sun drying is the most effective conventional drying method advised as observed in this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest as far as this manuscript is concerned.

ORCID

Olasunkanmi S. Omojokun http://orcid.org/0000-0001-5897-624X

REFERENCES

Adefegha, S. A., Oboh, G., Omojokun, O. S., & Adefegha, O. M. (2016). Alterations of Na^+/K^+-ATPase, cholinergic and antioxidant enzymes activity by protocatechuic acid in cadmium-induced neurotoxicity and oxidative stress in Wistar rats. *Biomedicine and Pharmacotherapy*, *83*, 559–568.

Ademiluyi, A. O., & Oboh, G. (2012). Phenolic-rich extract from selected tropical underutilized legumes inhibit α-amylase, α-glucosidase and angiotensin I converting enzyme in vitro. *Journal of Basic Clinical Physiology and Phamacology*, *23*(1), 17–25.

Anglister, L., Etlin, A., Finkel, E., Durrant, A., & Lev-Tov, A. (2008). Cholinesterases in development and disease. *Chemico-Biological Interactions*, *175*(1–3), 92–100.

Belle, N. A. V., Dalmolin, G. D., Fonini, G., Rubim, M. A., & Rocha, J. B. T. (2004). Polyamines reduces lipids peroxidation induced by different pro-oxidant agents. *Brain Research*, *1008*, 245–251.

Benderitter, M., Maupoil, V., Vergely, C., Dalloz, F., Briot, F., & Rochette, L. (1998). Studies by electron paramagnetic resonance of the importance of iron in the hydroxyl scavenging properties of ascorbic acid in plasma: Effects of iron chelators. *Fundamental & Clinical Pharmacology*, *12*(5), 510–516.

Chu, Y., Sun, J., Wu, X., & Liu, R. H. (2002). Antioxidant and antiproliferative activity of common vegetables. *Journal of Agricultural and Food Chemistry*, *50*(23), 6910–6916.

Cummings, J. L., & Cole, G. (2002). Alzheimer disease. *Journal of American Medical Association*, *287*(18), 2335–2338.

Cushman, D. W., & Cheung, H. S. (1971). Spectrophotometric assay and properties of the Angiotensin I-converting enzyme of rabbit lung. *Biochemical Pharmacology*, *20*(7), 1637–1648.

Di Cesare, L. F., Forni, E., Viscardi, D., & Nani, R. C. (2003). Changes in the chemical composition of basil caused by different drying procedures. *Journal of Agricultural and Food Chemistry*, *51*(12), 3575–3581.

Gazzaruso, C., Solerte, S. B., Pujia, A., Coppola, A., Vezzoli, M., Salvucci, F., … Garzaniti, A. (2008). Erectile dysfunction as a predictor of cardiovascular events and death in diabetic patients with angiographically proven asymptomatic coronary artery disease. *Journal of the American College of Cardiology*, *51*(21), 2040–2044.

Giacobini, E. (2002). Long-term stabilizing effect of cholinesterase inhibitors in the therapy of Alzheimer's disease. *Journal of Neural Transmission*, *62*, 181–187.

Go, A. S., Mozaffarian, D., Roger, V. L., Benjamin, E. J., Berry, J. D., Borden, W. B., … Turner, M. B. (2013). Heart disease and stroke statistics—2013 update: A report from the American Heart Association. *Circulation*, *127*, e6–e245.

Gyamfi, M. A., Yonamine, M., & Aniya, Y. (1999). Free-radical scavenging action of medicinal herbs from Ghana: Thonningia sanguine on experimentally induced liver injuries. *General Pharmacology*, *32*(6), 661–667.

Halliwell, B., & Gutteridge, J. M. C. (1981). Formation of a thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: The role of superoxide and hydroxyl radicals. *FEBS Letters*, *128*, 347–352.

Harborne, J. (1981). Two gossypetin methyl ethers as ultraviolet patterning guides in the flowers of *Coronilla valentina*. *Phytochemistry*, *20*(5), 1117–1119.

Hossain, M., Barry-Ryan, C., Martin-Diana, A., & Brunton, N. (2010). Effect of drying methods on the antioxidant capacity of six Lamiaceae herbs. *Original Research Article in Food Chemistry*, *123*(1), 85–91.

Hung, P. V., & Tran, D. L. (2012). Effects of drying methods on bioactive compounds of vegetables and correlation between bioactive compounds and their antioxidants. *International Food Research Journal*, *19*(1), 327–332.

Jung, W.-K., Mendis, E., Je, J.-Y., Park, P.-J., Son, B. W., Kim, H. C., … Kim, S.-K. (2006). Angiotensin I converting enzyme inhibitory peptide from yellow fin sole (*Limanda aspera*) frame protein and its antihypertensive effect in spontaneously hypertensive rats. *Food Chemistry*, *94*(1), 26–32.

Kehoe, P. G., & Passmore, P. A. (2012). The renin–angiotensin system and antihypertensive drugs in Alzheimer's disease: Current standing of the angiotensin hypothesis? *Journal of Alzheimer's Disease*, *30*, 251–268.

Kelley, W. T. D., Coffey, D. L., & Mueller, T. C. (1994). Lipid chromatographic determination of phenolic acids in soil. *Journal of the Association of Analytical Chemistry*, *77*, 805–809.

Kienle, G. S., & Kiene, H. (2007). Complementary cancer therapy: A systematic review of prospective clinical trials on anthroposophic mistletoe extracts. *European Journal of Medical Research*, *12*, 1–17.

Kiremire, B. T., Musinguzi, E., Kikafunda, J. K., & Lukwago, F. B. (2010). Effects of vegetable drying techniques on nutrient content: A case study of south-western Uganda. *African Journal of Food, Agriculture, Nutrition and Development*, *10*(5), 2587–2600.

Kwon, Y. I., Apostolidis, E., & Shetty, K. (2008). *In vitro* studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. *Bioresearch Technology*, *99*(8), 2981–2988.

Ma, R. C., So, W. Y., Yang, X., Yu, L. W., Kong, A. P., Ko, G. T., … Tong, P. C. (2008). Erectile dysfunction predicts coronary heart disease in type 2 diabetes. *Journal of the American College of Cardiology*, *51*(21), 2045–2050.

Marcocci, L., Maguire, J. J., Droy-Lefaix, M. T., & Packer, L. (1994). The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. *Biochemical and Biophysical Research Communication*, *201*(2), 748–755.

Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline

contents in Burkina Faso honey, as well as their radical scavenging activity. *Food Chemistry*, *91*(3), 571–577.

Melzer, J., Iten, F., Hostanska, K., & Saller, R. (2009). Efficacy and safety of mistletoe preparations (*Viscum album*) for patients with cancer diseases. *Complementary Medicine Research*, *16*, 217–226.

Minotti, G., & Aust, S. D. (1987). An investigation into the mechanism of citrate Fe^{2+}-dependent lipid peroxidation. *Free Radical Biology and Medicine*, *3*(6), 379–387.

Moustapha, B., Gutiérrez-Avella, D. M., Fuentes-Ordaz, R., Castañeda-Moreno, R., & Martínez, M. (2011). Chemical constituents of the Mexican mistletoe (*Psittacanthus calyculatus*). *Molecules*, *16*(12), 9397–9403.

Obatomi, D. K., Aina, V. O., & Temple, V. J. (1996). Effect of African mistletoe extract on blood pressure in spontaneously hypertensive rat. *Journal of Pharmaceutical Biology*, *34*(2), 124–127.

Oboh, G., Ademosun, A. O., Ademiluyi, A. O., Omojokun, O. S., Nwanna, E. E., & Longe, K. O. (2014). *In vitro* studies on the antioxidant property and inhibition of α-amylase, α-glucosidase and angiotensin I-converting enzyme by polyphenol-rich extracts from cocoa (*Theobroma cacao*) bean. *Pathology Research International*, *2014*, 6. Article ID 549287. https://doi.org/10.1155/2014/549287.

Oboh, G., Agunloye, O. M., Akinyemi, A. J., Ademiluyi, A. O., & Adefegha, S. A. (2013). Comparative study on the inhibitory effect of caffeic and chlorogenic acids on key enzymes linked to Alzheimers' disease and some pro-oxidant induced oxidative stress in rats' brain *in-vitro*. *Neurochemical Research*, *38*(2), 413–419.

Oboh, G., Akinyemi, A. J., Omojokun, O. S., & Oyeleye, S. I. (2014). Anticholinesterase and antioxidative properties of aqueous extract of Kolanut (*Cola acuminata)* seed under *in vitro* condition. *International Journal of Alzhemier's Disease*, *2014*, 8. Article ID 498629. https://doi.org/10.1155/2014/498629.

Oboh, G., Omojokun, O. S., & Ademiluyi, A. O. (2016). Drying methods alter angiotensin-I converting enzyme inhibitory activity, antioxidant properties and phenolic constituents of African mistletoe (*Loranthus Bengwensis L.*) leaves. *Journal of Evidence-Based Complementary & Alternative Medicine*, *21*(4), 260–270.

Oboh, G., Puntel, R. L., & Rocha, J. B. T. (2007). Hot pepper (*Capsicum annum, Tepin and Capsicum chinese, Habanero*) prevents Fe^{2+} - induced lipid peroxidation in brain – In vitro. *Food Chemistry*, *102*(1), 178–185.

Ogunmefun, O. T., Fasola, T. R., Saba, A. B., & Oridupa, O. A. (2013). The ethnobotanical, phytochemical and mineral analyses of *Phragmanthera incana* (Klotzsch); species of mistletoe growing on three plant hosts in south-western Nigeria. *International Journal of Biomedical Science*, *9*(1), 33–40.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Annals of Biochemistry*, *95*(2), 351–358.

Perry, N. S. L., Houghton, P. J., Sampson, J., Theobald, A. E., Hart, S., Lis-Balchin, M., … Perry, E. K. (2001). *In-vitro* activity of *S. lavandulaefolia* (Spanish sage) relevant to treatment of Alzheimer's disease. *Journal of Pharmacy and Pharmacology*, *53*(10), 1347–1356.

Provan, G. J., Scobbie, L., & Chesson, A. (1994). Determination of phenolic acids in plant cell walls by microwave digestion. *Journal of the Science of Food and Agriculture*, *64*(1), 63–65.

Saliu, J. A., Ademiluyi, A. O., Akinyemi, A. J., & Oboh, G. (2012). Invitro antidiabetes and antihypertension properties of phenolic extracts from bitter leaf (*Vernonia amygdalina del.*). *Journal of Food Biochemistry*, *36*(5), 569–576.

Sikka, S. C., & Hellstrom, W. J. (2002). Role of oxidative stress and antioxidants in Peyronie's disease. *International Journal of Impotence Research*, *14*(5), 353–360.

Singleton, V. L., Orthofor, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrate and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, *299*, 152–178.

Skeggs, L. T., Kahn, J. R., & Shumway, N. P. (1956). The preparation and function of the hypertension-converting enzyme. *The Journal of Experimental Medicine*, *103*(3), 295–299.

Stefan, J., & Schmieder, R. E. (2003). Potential mechanisms of impaired endothelial function in arterial hypertension and hypercholesterolemia. *Current Hypertension Report*, *5*, 199–207.

Venskutonis, R. (1997). Effect of drying on the volatile constituents of thyme (*Thymus vulgaris L.*) and sage (*Salvia officinalis L.*). *Food Chemistry*, *59*(2), 219–227.

Watson, D. M. (2001). Mistletoe-A keystone resource in forests and woodlands worldwide. *Annual Review of Ecology and Systematics*, *32*(1), 219–249.

Yousif, A. L., Scaman, C. H., Durance, T. D., & Girard, B. (1999). Flavor volatiles and physical properties of vacuum-microwave and airdried sweet basil (*Ocimum basilicum L.*). *Journal of Agricultural and Food Chemistry*, *47*(11), 4777–4781.

Zar, J. H. (1984). *Biostatistical analysis*. Englewoods Cliffs, NJ: Prentice-Hall Inc. 620 p.

How to cite this article: Omojokun OS, Oboh G, Ademiluyi AO. Effects of drying on cholinesterases and angiotensin-I converting enzyme inhibitory potential and phenolic constituents of African mistletoe (*Loranthus bengwensis L*) leaves from kolanut host tree. *J Food Biochem*. 2018;e12510. https://doi.org/10.1111/jfbc.12510