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Original Article

Blanching alters the phenolic constituents and *in vitro* antioxidant and anticholinesterases properties of fireweed (*Crassocephalum crepidioides*)

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الملخص

أهداف البحث: يحقق هذا البحث في تأثير الإبيضاض على توزيع الفينول وخصائص مضادات الأكسدة ومضادات الكولينستراز في المختبر لمستخلصات ميثانوليك- حمض الهيدروكلوريك المستخرجة من العشبة.

طرق البحث: تم تحضير المستخلصات الغنية بالفينول باستخراج أوراق العشبة المجففة المبيضة وغير المبيضة مع مزيج ثابت ١ م من حمض الهيدروكلوريك وميثانول (١:١). تم تحليل المكونات الفينولية باستخدام كروماتوجرافيا عالية الأداء مقرونة مع كاشف المجموعة ثنائي الصمام. علاوة على ذلك، تم تحديد مضادات الأكسدة مخبريا ((خصائص جذور الكسح وقدرات الحديد الخالبة وكبت الحديد التي يسببها بيروكسيد الدهون في دماغ الفأر المعزول] وتم تحديد تأثير كبت مستخلصات اسيتايل كولينستراز وبيوتريكولينستراز مخبريا.

النتائج: كشفت التحاليل المخبرية عن أربعة من الأحماض الفينولية وثلاثة من مركبات الفلافونويدز المتوفرة بكثرة في المستخلصات غير المبيضة من العشبة. وأظهرت النتائج أيضا أن المستخلصات غير المبيضة من العشبة لها خصائص عالية كمضادات أكسدة وخصائص اسيتايل كولينستراز وبيوتريكولينستراز مثبطة بالمقارنة بالعشبة المبيضة.

الاستنتاجات: الخصائص العالية كمضادات للأكسدة ومضادات الكولينستراز لمستخلصات العشبة غير المبيضة ممكن أن ترتبط بمكوناتها العالية من الفينول بالمقارنة بالعشبة المبيضة. عليه، يؤدي الإبيضاض إلى نقص الخصائص كمضادات للأكسدة ومضادات الكولينستراز للعشبة والبرهان على ذلك انخفاض خسارة محتويات الفينول من العشبة المبيضة.

الكلمات المفتاحية: عشبة؛ مضادات الأكسدة؛ مضادات الكولينستراز؛ الفينوليكس؛ تبييض

Abstract

Objective: This research investigated the effect of blanching on the phenolic distribution and *in vitro* antioxidant and anticholinesterase properties of the HCl-methanolic extracts from fireweed.

Methods: Phenolic-rich extracts were prepared by extracting the powdered blanched and unblanched fireweed leaves with a mixture of absolute 1 M HCl and methanol (1:1 v/v). The phenolic constituents were analysed using high-performance chromatography coupled to a diode array detector (HPLC-DAD). Furthermore, the extracts' *in vitro* antioxidant [1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azinobis(3-ethylbenzo-thiazoline-6-sulfonate (ABTS) radical scavenging and Fe^{2+} chelating abilities and inhibition of Fe^{2+}-induced lipid peroxidation in isolated rat brain] properties and inhibitory effects against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were determined *in vitro*.

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Results: The HPLC-DAD analysis detected four phenolic acids (gallic, chlorogenic, caffeic and ellagic acids) and three flavonoids (catechin, rutin and quercetin) that were abundant in the unblanched fireweed extract. The findings further showed that the unblanched fireweed extract had higher antioxidant and AChE and BChE inhibitory properties compared to the blanched fireweed extract.

Conclusion: The increased antioxidant and anticholinesterase properties of the unblanched fireweed extract could be linked to its higher concentrations of the phenolic constituents compared to the blanched firewood. Therefore, blanching leads to a decrease in the antioxidant and anticholinesterase properties of fireweed, as evidenced by a reduction/loss in the polyphenolic content from the blanched fireweed.

Keywords: Anticholinesterases; Antioxidants; Blanching; Fireweed; Phenolics

Introduction

Diverse neurodegenerative diseases affect the central nervous system, including Alzheimer's disease (AD) and Parkinson's disease (PD).[1] The prominent causative factors of neurodegenerative diseases are oxidative stress, protein degradation and aggregation, mitochondrial dysfunction and neuro-inflammatory processes.[2] The pernicious decrease in memory initiates AD and progresses to deterioration in cognitive and adaptive functions.[3] Neurodegenerative diseases such as AD and PD can be initiated by the oxidation of nucleic acids, lipids and other vital component of the cell by free radicals. Antioxidant compounds are highly important in improving health by reducing the risk of degenerative/chronic diseases. Dietary antioxidants are able to trap these free radicals, thereby halting the degeneration of biomolecules.[4]

The inhibition of cholinesterases [acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)] is also a therapeutic strategy used in the management of AD and other related neurodegenerative disease.[5,6] In AD, cholinesterase (ChE) inhibitors, such as galantamine and rivastigmine, are employed to restore the acetylcholine levels and positively influence the AD patient.[7] Today, most of these synthetic drugs used in the treatment/management of AD are selective AChE inhibitors. AChE and BChE have been reported to act simultaneously in the catabolism of acetylcholine (ACh) in the synaptic gap of AD brains, thereby terminating its neurotransmitter action.[7,8] Moreover, these AChE inhibitors come with several side effects, such as nausea, vomiting, hepatotoxicity, dyspepsia, myalgia, and anorexia dizziness.[8–10] Therefore, a naturally sourced co-inhibitor of this enzyme with little or no side effects is desirable for the holistic treatment/management of AD.

Fireweed [*Crassocephalum crepidiodes* S. MOORE (Family: Asteraceae), popularly called 'Ebolo' by the Yoruba speaking people of Nigeria] is a prominent succulent herb in Africa, and its leaves and stems are consumed as a vegetable and for medicinal purposes.[11] Fireweed has been reported to contain some phytochemicals, including tannin, dihydroisocoumarins, pyrrolizidine, alkaloids (jacobine and jacoline) and monoterpenes (myrcene, limonene and α-copaene).[11,12] In Nigeria, fireweed is mostly treated with hot water, a process otherwise known as blanching, before preparation in soups or sauces, to purposely increase its acceptability and palatability. However, to date, there is little or no information on the effect of the blanching process on the medicinal/nutraceutical values of this vegetable. This study sought to evaluate the effect of the blanching process on the phenolic constituents, antioxidative properties and inhibition of cholinergic enzymes (AChE and BChE) of the HCl-methanolic extract of fireweed leaves.

Materials and Methods

Chemicals

Chemicals, such as acetylthiocholine and butyrylthiocholine iodide, thiobarbituric acid (TBA) and 5,5′-dithiobis(2-nitrobenzoic acid (DTNB), were purchased from Sigma-Aldrich and ChemieGmH (Steinheim, Germany); acetic acid was procured from BDH Chemical Ltd., (Poole, England). Unless otherwise stated, all other chemicals and reagents are of analytical grade and the water was glass distilled. A JENWAY UV-visible spectrophotometer (Model 6305; Jenway, Barlo world Scientific, Dunmow, United Kingdom) was used to measure absorbance throughout the study.

Sample collection and preparation

Fresh *C. crepidioides* leaves were obtained from a local farmland in the Akure metropolis, Nigeria. The sample was authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria. The inedible part was removed and thoroughly washed with distilled water to remove any dirt, chopped into small pieces using a table knife and immediately transferred into a vessel containing tap water to prevent oxidation. Then, the samples were randomly divided into two (2) portions. One portion was blanched for 10 min at 80 °C, while the other portion was not. The blanched portion was then drained. Both portions were dried in hot air at 40 °C and then milled into a powder using a laboratory blender. One gram of each powdered sample was extracted with 100 mL of methanol and 1 N HCl (1:1 w/v). The filtrate was then evaporated to dryness using a rotary evaporator. The flask containing the extracts was thoroughly washed with distilled water and then frieze dried with the aid of a freeze drier. One hundred milligrams of each dried extract were then reconstituted in one hundred millilitres of water and stored in a

refrigerator for subsequent analysis. The freeze-dried samples were used for the GC-FID analysis.

Quantification of the phenolic compounds by HPLC-DAD

The reverse phase chromatographic analyses of the extracts were performed under gradient conditions using a Phenomenex C_{18} column (4.6 mm × 250 mm) packed with 5 μm diameter particles. The mobile phase was water containing 1% phosphoric acid (A) and acetonitrile (B), and the composition gradient was 13% of B for 10 min and changed to 20%, 30%, 50%, 60%, 70%, 20% and 10% B at 20, 30, 40, 50, 60, 70 and 80 min, respectively, using the method described by Silva et al.,[13] with slight modifications. The blanched and unblanched extracts were analysed at a concentration of 15 mg/mL. The flow rate was 0.6 mL/min, the injection volume was 40 μL and the wavelengths were 271 nm for gallic and ellagic acids; 280 nm for catechin; 325 nm for chlorogenic and caffeic acids; and 366 nm for quercetin and rutin. The samples and mobile phase were filtered through a 0.45 μm membrane filter (Millipore) and then degassed in an ultrasonic bath prior to use. Stock solutions of the reference standards were prepared in the HPLC mobile phase at a concentration range of 0.030–0.250 mg/mL. The chromatography peaks were confirmed by comparing their retention times with those of the reference standards and the DAD spectra (200 to 600 nm). All chromatography operations were performed at ambient temperature and in triplicate.

Determination of the LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves. The LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve as defined by Abbas et al.[14]

Free radical scavenging assay

The free radical scavenging ability of the extracts against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was evaluated as described by Gyamfi et al.[15] Briefly, 1 mL of extracts was mixed with 1 mL of a 0.4 mM methanolic solution containing the DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was then measured at 516 nm. The DPPH free radical scavenging ability was subsequently calculated.

Total antioxidant capacity

The total antioxidant capacity of the extracts was determined by the ability of the extracts to scavenge the ABTS radical using the method described by Re et al.[16] The ABTS radical was generated by reacting a 7 mM ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM, final concentration) in the dark for 16 h and adjusting the Abs at 734 nm to 0.700 with ethanol. Then, two hundred microliters of the extracts were added to 2.0 mL of the ABTS radical solution. After 15 min, the absorbance was measured at 734 nm. The total antioxidant capacity of the extract was subsequently calculated by normalizing to the trolox standard.

Iron (Fe^{2+}) chelation assay

The extracts' ability to chelate Fe^{2+} was determined using the method of Puntel et al.,[17] with some modifications. A 500 μmol L^{-1} solution of freshly prepared Fe_2SO_4 (150 μL) was added to the mixture containing 168 μL of 0.1 mol L^{-1} Tris-HCl (pH 7.4), 218 μL saline and the extracts (0–100 μL). The mixture was incubated for 5 min and then 13 μL of 0.25% 1,10-phenanthroline (w/v) was added. The absorbance was measured at 510 nm in a spectrophotometer. The percentage of chelated Fe^{2+} was subsequently calculated.

Animal handling

The handling and use of animals for this study was approved by the institution's ethical committee for the use of animals in laboratory experiments (FUTA/SOS/1413). For this study, male albino rats weighing 200–210 g were purchased from the animal breading colony of the animal production and health department, Federal University of Technology, Akure. They were maintained at room temperature on a 12 h light/12 h dark cycle, with access to food and water. The rats were acclimatized for 10–14 days under this condition before the experiments commenced.

Lipid peroxidation and thiobarbituric acid reaction assays

The male albino rats were decapitated under mild diethyl ether anaesthesia and the brain tissue was rapidly isolated, placed on cold ice and weighed. The brains were subsequently homogenized in cold saline (0.9%) with approximately 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 supernatant (S.I) was kept for the lipid peroxidation assay using the method of Ohkawa et al.,[18] with slight modifications. Briefly, 100 μL of the supernatant was mixed with 30 μL of 0.1 M Tris-HCl buffer (pH 7.4), the extract (0–100 μL) and 30 μL of a freshly prepared 250 μM Fe_2SO_4 solution. The volume was increased to 300 μL with water before incubation at 37 °C for 1 h. The colour was developed by adding 300 μL of 8.1% SDS (Sodium dodecyl sulphate) to the mixture, followed by the addition of 600 μL of acetic acid/HCl (pH 3.4) and 600 μL of 0.8% TBA (Thiobarbituric acid). This mixture was incubated at 100 °C for 1 h and the absorbance of the TBARS (Thiobarbituric acid reactive species) produced was measured at 532 nm and calculated as the percent of MDA (Malondialdehyde) produced (% Control) using the MDA standard curve.

Acetylcholinesterase (AChE) and butyrylcholinesterase inhibition assays

AChE inhibition was assessed by the method described by Ellman et al.[19] The AChE activity was determined in a reaction mixture containing 200 μL of a solution of AChE (EC 3.1.1.7) in 0.1 M phosphate buffer, pH 8.0, 100 μL of

a solution of 5, 5′-dithiobis (2-nitrobenzoic) acid (DTNB, 3.3 mM in 0.1 M phosphate-buffered saline, pH 7.0, containing 6 mM $NaHCO_3$), the extracts, and 500 µL of phosphate buffer, pH 8.0. After incubation for 20 min at 25 °C, one hundred microliters of a 0.05 mM acetylthiocholine iodide solution was added as the substrate, and the AChE activity was measured from the absorbance changes at 412 nm for 3 min at room temperature using a UV spectrophotometer. One hundred microliters of butyrylthiocholine iodide were used as a substrate to assay the butyrylcholinesterase activity, and all other reagents and conditions were the same. The AChE and BChE inhibitory activities were expressed as the percent inhibition.

Data analysis

The results of three replicates were pooled and expressed as the means ± standard deviation (S.D.). The student's *t*-test, one-way analysis of variance (ANOVA), and least significance difference (LSD) test were performed.[20] The effective concentration needed to scavenge/inhibit 50% of the radical/enzyme activity under the described assay conditions (IC_{50}) was calculated after plotting the radical/enzyme inhibition (%) against concentration of the extracts. This was determined by non-linear regression analysis. Significance was accepted at $P \leq 0.05$.

Results

HPLC fingerprinting of the HCl-methanolic extracts revealed the presence of seven phenolic compound in the extracts, which were gallic acid (t_R = 10.17 min; peak 1), catechin (t_R = 15.83 min; peak 2), chlorogenic acid (t_R = 19.02 min; peak 3), caffeic acid (t_R = 21.97 min; peak 4), ellagic acid (t_R = 28.15 min; peak 5), rutin (t_R = 36.21 min; peak 6) and quercetin (t_R = 40.09 min; peak 7) (Figure 1 and Table 1). The dry weight concentration of the individual phenolic constituents was (blanched and unblanched): gallic acid, 1.68 and 1.72 mg; catechin, 0.53 and 0.49 mg; chlorogenic acid, 3.27 and 3.08 mg; caffeic acid, 1.65 and 3.10 mg; ellagic acid, 4.09 and 5.27 mg; rutin, 0.97 and 7.31 mg; and quercetin, 6.13 and 7.28 mg per g, respectively. The unblanched fireweed extract exhibited particularly high phenolic concentrations.

Table 1: Components of the fireweed (*C. crepidioides*) extracts.

Compounds	Blanched extract	Unblanched extract	LOD	LOQ
	mg/g	mg/g	µg/mL	µg/mL
Gallic acid	1.68 ± 0.01[a]	1.72 ± 0.03[a]	0.018	0.061
Catechin	0.53 ± 0.02[b]	0.49 ± 0.01[b]	0.009	0.029
Chlorogenic acid	3.27 ± 0.01[c]	3.08 ± 0.02[c]	0.015	0.050
Caffeic acid	1.65 ± 0.01[a]	3.10 ± 0.01[c]	0.029	0.093
Ellagic acid	4.09 ± 0.03[d]	5.27 ± 0.01[d]	0.031	0.102
Rutin	0.97 ± 0.02[e]	7.31 ± 0.02[e]	0.027	0.088
Quercetin	6.13 ± 0.01[f]	7.28 ± 0.03[e]	0.008	0.027

The results are expressed as the means ± standard deviations (SD) of three determinations. The averages followed by different letters were significantly different by Turkey test at $P < 0.05$.

The results of the DPPH radical scavenging assay of the blanched and unblanched fireweed extracts are presented in Figure 2 and the IC_{50} values in Table 2 (a lower IC_{50} value indicates stronger enzyme/radical inhibition). The result revealed that the extracts scavenged the DPPH radical in a concentration-dependent manner. However, the unblanched fireweed extract (IC_{50} = 0.91 mg/mL) exhibited significantly ($P < 0.05$) higher DPPH radical scavenging ability than that from blanched fireweed (IC_{50} = 1.64 mg/mL). The ABTS radical scavenging ability of the extracts is presented in Figure 3. Similarly, the unblanched fireweed extract (69.64 mmol TEAC/100 g) exhibited higher ($P < 0.05$) ABTS radical scavenging ability than that of the blanched extract (21.42 mmol TEAC/100 g). The result of the Fe^{2+} chelating assay (Figure 4) revealed that the extracts chelated Fe^{2+} in a concentration-dependent manner;

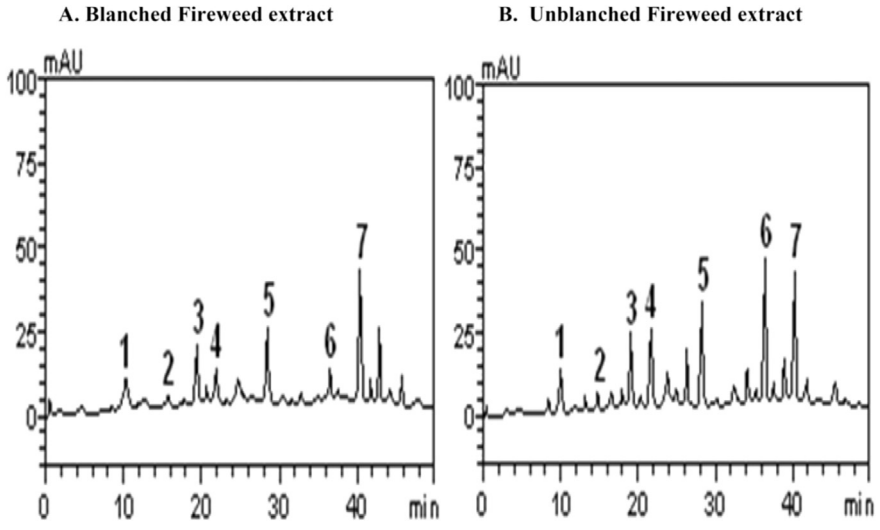

Figure 1: Representative high performance liquid chromatography profile of blanched (A) and unblanched (B) fireweed (*Crassocephalum crepidioides*) extracts. Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), rutin (peak 6) and quercetin (peak 7).

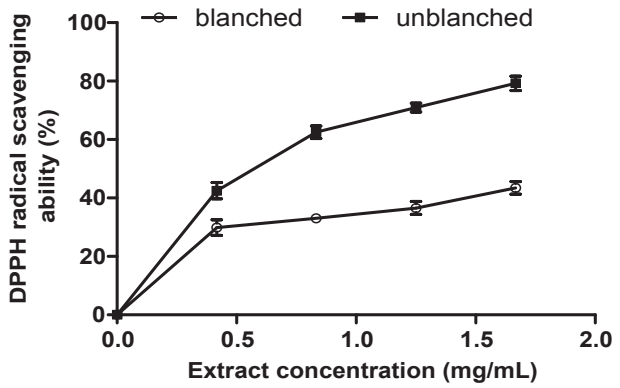

Figure 2: DPPH radical scavenging abilities of blanched and unblanched fireweed (*C. crepidioides*) extracts.

however, the unblanched fireweed extract (IC_{50} = 0.39 mg/mL) chelated significantly more ($P < 0.05$) Fe^{2+} than the blanched fireweed extract (IC_{50} = 0.52 mg/mL).

The extracts inhibited Fe^{2+}-induced lipid peroxidation (Figure 5 and Table 2), revealing that both extracts inhibited lipid peroxidation in a concentration-dependent manner. Compared to the blanched fireweed extract (IC_{50} = 1.20 mg/mL), the unblanched fireweed extract (IC_{50} = 1.11 mg/mL) exhibited higher inhibition of Fe^{2+}-induced lipid peroxidation. The effects of the blanched and unblanched fireweed extracts on cholinesterase activity were investigated *in vitro* and the results are presented in Figures 6 and 7 and the IC_{50} values are presented in Table 2. The results revealed that both extracts inhibited the AChE activity in a concentration-dependent manner. However, the unblanched fireweed extract (IC_{50} = 0.29 mg/mL) exhibited higher AChE inhibitory activity than the blanched fireweed extract (IC_{50} = 0.38 mg/mL). The result further revealed that the unblanched fireweed extract (IC_{50} = 0.24 mg/mL) exhibited higher BChE inhibitory activity than the blanched fireweed extract (IC_{50} = 0.28 mg/mL).

Discussion

As revealed by the HPLC analysis, the fireweed (blanched and unblanched) extracts possessed gallic acid, catechin, chlorogenic acid, caffeic acid, ellagic acid, rutin and quercetin. Quercetin, ellagic, chlorogenic and acid were dominant in the blanched extract, while rutin, quercetin, ellagic, chlorogenic and caffeic acid were the dominant phenolic

Table 2: IC_{50} values of the blanched and unblanched fireweed extracts' DPPH radical scavenging and Fe^{2+} chelating abilities, Fe^{2+}-induced lipid peroxidation, and AChE and BChE activities.

IC_{50} (mg/mL)	Blanched fireweed extract	Unblanched fireweed extract
DPPH scavenging ability	1.64 ± 0.06^a	0.91 ± 0.06^b
Fe^{2+}-induced lipid peroxidation	1.20 ± 0.04^a	1.11 ± 0.02^b
Fe^{2+} chelating ability	0.52 ± 0.03^a	0.39 ± 0.02^b
AChE	0.38 ± 0.02^a	0.29 ± 0.02^b
BChE	0.33 ± 0.03^a	0.24 ± 0.01^b

The values represent the means ± standard deviation of triplicate readings. The values on the same row with the same superscript letter are not significantly different ($P > 0.05$).

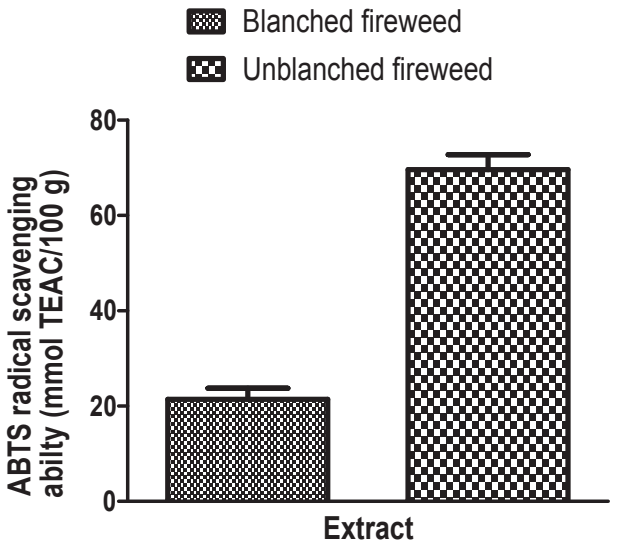

Figure 3: ABTS radical scavenging abilities of blanched and unblanched fireweed (*C. crepidioides*) extracts.

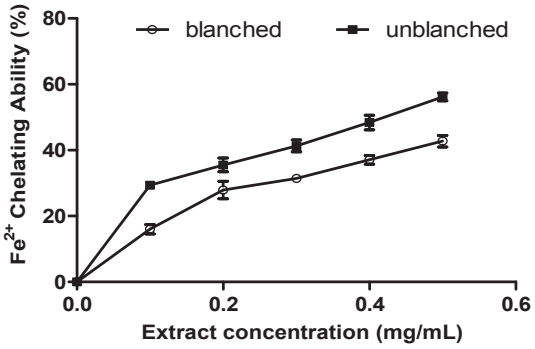

Figure 4: Fe^{2+} Chelating abilities of blanched and unblanched fireweed (*C. crepidioides*) extracts. Values represent mean ± standard deviation (n = 3).

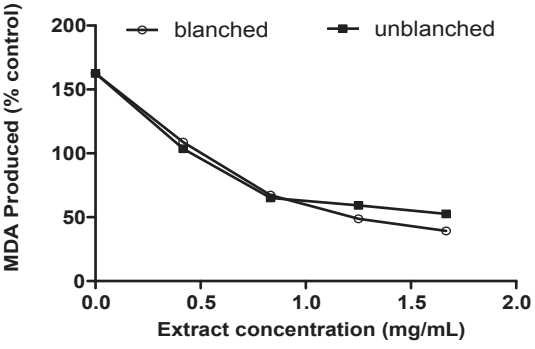

Figure 5: Inhibition of Fe^{2+}-induced lipid peroxidation by blanched and unblanched fireweed (*C. crepidioides*) extracts.

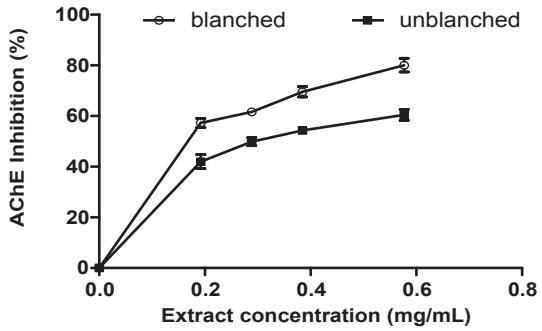

Figure 6: AChE inhibitory activities of blanched and unblanched fireweed (*C. crepidioides*) extracts.

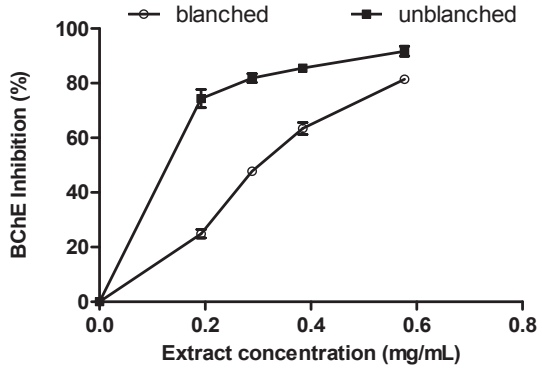

Figure 7: BChE inhibitory activities of blanched and unblanched fireweed (*C. crepidioides*) extracts.

compound in the unblanched extract. Quercetin is a prominent flavonoid in both the blanched and unblanched fireweed extracts and has been reported to possess anticholinesterase activity, thus making it an important phenolic compound in the management of neurodegenerative diseases such as AD.[21,22] It has also been shown to exhibit antioxidative potential due to its ability to alleviate oxidative damage.[23] Rutin is one of the phenolic compounds found in many fruits and vegetable, and has antioxidant and neuroprotective activities.[24–27] Several reports have shown that rutin could chelate Fe^{2+}, thereby preventing it from inducing the production of highly reactive free radicals such as the hydroxyl (OH) radical that may cause neuronal damage.[24,28–30] According to the reports of Oboh et al.[31] caffeic and chlorogenic acid have been shown to inhibit some enzymes that are linked to AD (AChE and BChE) and also exhibit radical scavenging abilities. It has been suggested that the sugar side chain of flavonoids may be important for neuroprotection.[32] However, the reduction of these compounds in the blanched fireweed extract could be the result of the heat treatment during blanching, as thermal processing is reported to cause a loss/reduction of some plant phytochemicals.[33,34]

The dietary antioxidant potentials typified by the extracts' radical (DPPH* and ABTS*) scavenging and Fe^{2+} chelating abilities were also investigated. Free radicals and metal ions are involved in the pathophysiology of AD. Antioxidants prevent biological and chemical substances from oxidative damage induced by radicals and metal (Fe^{2+}, Cu^{2+} and Zn^{2+}) ions.[35] In this study, the extracts of the studied samples scavenged the DPPH and ABTS radicals and also chelated Fe^{2+}. However, the blanched fireweed extract had the lowest radical scavenging and Fe^{2+} chelating abilities. This could be attributed to the reduction of the phenolic constituents as a result of the blanching process.

Increased Fe^{2+} content in the human brain has been linked to a host of neurodegenerative diseases, including AD.[35,36] In the brain, the free form of Fe^{2+} produced reactive species that are capable of causing oxidative damage to membrane lipids and, ultimately, cell death via Fenton's reaction.[35] Hence, the removal of the free forms of Fe^{2+} could be another neuroprotective therapy in the management of oxidative stress-induced AD.[28] In this study, the incubation of a brain homogenate with a 250 μM Fe^{2+} solution caused an increase in the malondialdehyde (MDA) content, an oxidative stress biomarker (Figure 5). However, the addition of increasing concentrations of the extracts reduced the MDA levels. This finding correlate with our earlier report where plant extracts inhibited Fe^{2+}-induced lipid peroxidation in the brain homogenate *in vitro* and could be linked to the presence of phenolic compounds.[35,36] The reduced MDA inhibition exhibited by the blanched fireweed extract could be linked to the reduction in the amount of the phenolic constituents.

Several plants and their constituents are used in folk medicines for the management of AD by enhancing cognitive function. The observed inhibition of the AChE and BChE activities by the fireweed extracts is consistent with our earlier study of the effects of plant materials on ChEs.[35] The inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities has been accepted as an effective therapeutic strategy against AD.[37] In the brain, the inhibition of these enzymes prevents acetylcholine and butyrylcholine from being degraded and consequently increased the neurotransmitter concentrations at the synaptic cleft, which leads to increased communication between the nerve cells that use acetylcholine or butyrylcholine as a neuromessenger, thus temporarily improving or stabilizing the symptoms of Alzheimer's disease.[37,38] The cholinesterase inhibitor activity of plant polyphenolic compounds has been reported to be related to their structure and as a function of the number and position of the OH groups that form hydrogen bonds with specific amino acids at the enzymes' active sites.[39,40] This correlated with the result of this study, as the HCl-methanolic fireweed extracts were able to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Interestingly, the extracts exhibited a stronger inhibitory effect on BChE than on AChE, which could be an added advantage, as BChE is the predominant cholinesterase in the human brain at the late stage of AD.[41,42] Moreover, BChE inhibition could also decrease the production of plaques that are not only toxic but also increase the susceptibility of the brain to AD.[43,44] However, the reduced ChE inhibition by the blanched fireweed leaf extract could result from the reduction in the amount of the individual phenolic constituents present in the blanched extract. This could be a consequence of the reduced number and altered position of OH groups available to inhibit the enzymes, which could also be due to the effect

of thermal treatment on the polyphenolic compounds during blanching.

Conclusions

The blanched and unblanched fireweed leaf extracts exhibited antioxidant and anticholinesterase properties, which could result from the biological activities of the polyphenolic compounds. However, the reduction of these abilities in the blanched fireweed extract may result from the loss/reduction of the phenolics caused by the heat treatment during the blanching process. Therefore, this vegetable should be treated with moderate/mild heat during cooking to maximize the nutraceutical values and functional properties in the management/treatment of AD. Further in vivo and clinical studies should be performed.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

BCA, GO, IIE and SIO designed the experiments, interpreted the data and prepared the manuscript. SIO and IIE performed the bench experiments and data collection, and AAB and MLA performed the phenolic characterization experiments.

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