

Antioxidant and Anticholinesterase Potential of Two Nigerian Bitter Yams Using a Simulated Gastrointestinal Digestion Model and Conventional Extraction

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ABSTRACT: The purpose of this study was to evaluate the antioxidant and anticholinesterase activities of yellow and white bitter yams from South Western Nigeria using methanolic extraction and simulated gastrointestinal digestion models. The phenolic compounds in the bitter yam varieties were evaluated by high performance liquid chromatography with a diode array detector (HPLC-DAD). The total phenolic content of the bitter yams was measured by the Folin-Ciocalteu method, reductive potential by assessing the ability of the bitter yam to reduce FeCl₃ solution, and the antioxidant activities were determined by the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•]) scavenging activity, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS^{•+}) scavenging activity, nitric oxide radical (NO[•]) scavenging ability, hydroxyl radical scavenging ability, and ability to inhibit Fe²⁺-induced lipid oxidation. The HPLC-DAD analysis revealed the presence of some phenolic compounds in the studied bitter yam varieties, with varying degree of quantitative changes after cooking. The antioxidant indices (total phenolic content, total flavonoid content, reducing power, DPPH[•] scavenging activity, ABTS^{•+} scavenging activity, and NO[•] scavenging activity) were higher in the simulated gastrointestinal digestion model compared to the methanolic extract, with the *in vitro* digested cooked white bitter yam ranking higher. Similarly, the *in vitro* digested yams had a higher inhibitory action against lipid oxidation compared to the methanolic extracts, with the cooked white bitter yam ranking high. The methanolic extracts and *in vitro* enzyme digests showed no acetylcholinesterase inhibitory abilities, while methanolic extracts and the *in vitro* enzyme digest displayed some level of butyrylcholinesterase inhibitory activities. Therefore the studied bitter yams could be considered as possible health supplements.

Keywords: antioxidant activity, phenolic compounds, anticholinesterase, *in vitro* digestion, bitter yam

INTRODUCTION

A number of studies have demonstrated the use of dietary components in the control of free radical mediated diseases, such as cancer and cardiovascular disease (1). The consumption of tubers, fruits, legumes, vegetables, and whole grains results in a reduced risk of developing these diseases. The use of dietary components in the control of chronic diseases could be ascribed to the presence of natural antioxidants in these foods, such as ascorbic acid, tocopherols, carotenoids, and phenolic compounds (2), besides other bioactive compounds. Among the compounds with antioxidant activity, polyphenols are important. These are found in a great variety of foods, such as apples, mulberries, cherries, grapes, raspberries, citric fruits, onions, spinach, peppers, oat, wheat, black tea,

wine, chocolate, and among others (3). Polyphenols have demonstrated higher *in vitro* antioxidant capacity than other antioxidants, such as ascorbic acid and α -tocopherol (4).

Generally, yams are important in the diet of most Africans, the Caribbean, and South Pacific, where it has been reported to represent 12% of the food intake (5). *Dioscorea dumetorum* is one of the varieties of yams and is of importance in a developing country like Nigeria. *D. dumetorum* is one of the first four varieties of yams that are indigenous to Africa and Nigeria (6-9). Bitter yam (*D. dumetorum*), being one of the most produced and consumed tuber crops in Africa, the Caribbean, and South Pacific, has an important role in the maintenance of good health (10,11).

The antioxidant compounds in plant foods have been

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extracted with different solvent and measured by several methods. Nevertheless, most of the extraction methods employed are “non-physiological” because they involve the use of chemical methods such as organic solvents and water (12-17). Thus, this extraction may be partial, which can lead to misinterpretation of their actual biological availability and activity (18). Though such investigations have clearly demonstrated that several plant foods have antioxidant capacity, the information does not automatically relate to the available antioxidants when the cooked food is degraded by the enzymes and juices of the gastro-intestinal tract (GIT). It has also been suggested that antioxidant activity from chemical extracts of the food material might misjudge the actual antioxidant capacity in the digestive tract (19); hence, the measurement of actual antioxidant capacity becomes imperative (20).

The cholinergic hypothesis of Alzheimer’s disease (AD) holds that the degeneration of neurons in the basal forebrain and the associated loss of cholinergic neurotransmission in the cerebral cortex and hippocampus contribute significantly to cognitive deterioration in AD (21). The loss of cholinergic neurons in AD leads to a reduction in the synthesis of the neurotransmitter acetylcholine (ACh), which has been associated with cognitive functions. This hypothesis has prompted the search for ways to increase ACh in AD patients. There is a need to inhibit the activity of cholinesterases (ChE) in order to increase the concentration of ACh needed for cognitive functions. Extracts from some plants have been documented to have ChE inhibitory activities (22,23).

Therefore the present investigation sought to evaluate the antioxidant potential of the phenolic content of two varieties of bitter yam and assess their anti-cholinesterase action using simulated *in vitro* enzyme digestion and methanolic extraction models, with a view to establishing the nutraceutical potential of bitter yam after its passage through the GIT.

MATERIALS AND METHODS

Chemicals

5,5'-Dithiobis(2-nitrobenzoic acid), acetylthiocholine iodide, butyrylcholine iodide, 2-deoxy-D-ribose, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt, 2,2-diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid (TCA), thiobarbituric acid (TBA), hydrogen peroxide (H₂O₂), Folin-Ciocalteu’s phenol reagent, and other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The chemicals used were of analytical grade.

Sample treatment and preparation

White and yellow bitter yams were obtained from Igbar-

Odo market in Ado Ekiti, Nigeria, and the authentication of the samples was carried out at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria. The tubers were washed and divided into various portions. One portion was peeled, washed, and chipped with a chipping machine and subsequently sun dried for 10 days. The second portion was peeled and cooked by boiling in distilled water for 90 min using a gas cooker on medium heat. The cooked samples were chipped with the chipping machine and were spread thinly on a dark nylon and sun dried for 15 days. The dried yam chips were milled into powder and stored in air-tight containers for analysis.

Preparation of methanolic extracts

Methanolic extraction was done using a modified method of Bhandari and Kawabata (24). Five grams of bitter yam flour was soaked in 80 mL methanol and kept overnight. The suspension was filtered through Whatman No.1 filter paper, and the filtrate was diluted to make up to 100 mL with methanol. Sample solutions were stored at 4°C in amber bottles and served as the stock solution for subsequent analyses.

In vitro enzymatic procedure

The *in vitro* digestion using sequential enzymatic steps was based on a slightly modified method reported by Deigado-Andrade et al. (25). Two grams of the milled bitter yam sample was weighed and dissolved in 40 mL of distilled water. 300 µL of α -amylase (32.5 mg of α -amylase was dissolved in 25 mL of 1 mM CaCl₂ at pH 7) was added to the tubes. The tubes were incubated in a shaking water bath at 37°C for 10 min and at 80 strokes/min. After 10 min, the pH was adjusted to 2 using concentrated HCl. After 30 min incubation in a shaking water bath at 37°C, 2 mg pepsin which was dissolved in 1 mL of 0.05 M HCl was added to the tubes. The tubes were then incubated in a shaking water bath at 37°C for 10 min and at 80 strokes/min. After further 20 min of shaking the tubes, the pH was adjusted to 6 using NaOH. Then 10 mL of pancreatin (3 g of pancreatin was dissolved in 20 mL distilled water) was added, and the tubes were incubated in a shaking water bath at 37°C for 20 min. The pH was adjusted to 7.5 using NaOH (simulating pH conditions in the small intestine). Then the tubes were incubated for 10 min in a shaking water bath at 37°C. The digested sample was incubated at 100°C for 4 min to inactivate the enzymes, and the digested sample was then centrifuged for 60 min at 3,200 g, and then the soluble fraction was kept in the refrigerator for antioxidant, anticholinesterase, and lipid peroxidation analyses. The insoluble fraction was discarded. An undigested control was used for the *in vitro* digestion without the enzymes to serve as the enzyme control.

Quantification of phenolic compounds by high performance liquid chromatography with a diode array detector (HPLC-DAD)

Reverse phase chromatographic analyses were carried out under gradient conditions using a C₁₈ column (4.6 mm×150 mm) packed with 5 µm diameter particles; the mobile phase was water containing 1% formic acid (A) and acetonitrile (B), and the composition gradient was: 17% of B until 10 min and changed to obtain 20, 30, 50, 60, 70, 20, and 10% B at 20, 30, 40, 50, 60, 70, and 80 min, respectively, following the method described by Kamdem et al. (26), with slight modifications. Yellow bitter yam (raw/cooked), white bitter yam (raw/cooked) samples, and mobile phase were filtered through a 0.45 µm membrane filter (Millipore, Billerica, MA, USA) and then degassed by ultrasonic bath prior to use, the yam extracts were analyzed at a concentration of 20 mg/mL. The flow rate was 0.7 mL/min, injection volume 40 µL, and the wavelengths were 254 nm for gallic acid, 280 nm for catechin and epicatechin, 327 nm for chlorogenic, caffeic, and ellagic acids, and 365 nm for quercetin, quercitrin, rutin, and kaempferol.

All the samples and mobile phase were filtered through a 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.025~0.350 mg/mL for kaempferol, quercetin, quercitrin, rutin, catechin, and epicatechin and 0.030~0.450 mg/mL for gallic, ellagic, caffeic, and chlorogenic acids. Chromatography peaks were confirmed by comparing their retention times with those of reference standards and by DAD spectra (200 to 500 nm). All chromatography operations were carried out at ambient temperature and in triplicate. 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. 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 (27).

Total phenolic content (TPC)

The total phenolic content of methanolic extracts, the *in vitro* enzyme digested samples, and the enzyme control was determined by the Folin-Ciocalteu assay as described by Waterman and Mole (28). Briefly, 500 µL of Folin reagent was added and mixed with a solution containing 100 µL of the extract and 2 mL of distilled water. Then 1.5 mL of 7.5% Na₂CO₃ was added to the solution, and the volume was made up to 10 mL with distilled water. The mixture was left to stand for 2 h after addition of the Na₂CO₃, and the absorbance of the mixture was measured at 760 nm using a Lambda EZ150 spectrophotometer (Perkin Elmer, Waltham, MA, USA). The standard

used was tannic acid, and the result was expressed as mg tannic acid equivalents per gram of sample.

Total flavonoid content (TFC)

The total flavonoid content of the extracts/digests was determined using a slightly modified method reported by Meda et al. (29). Briefly, 0.5 mL of appropriately diluted sample was mixed with 0.5 mL methanol, 50 µL of 10% AlCl₃, 50 µL of 1 mol/L C₂H₃KO₂, and 1.4 mL water, and incubated at room temperature for 30 min. Thereafter, the absorbance of each reaction mixture was subsequently measured at 415 nm. The total flavonoid was calculated using quercetin as a standard by making use of a seven point standard curve (0~40 µg/mL), the total flavonoid content of samples was determined in triplicates, and the results were expressed as mg quercetin equivalents per gram of sample.

Ferric reducing antioxidant power

The reducing powers of the extracts/digests were determined by assessing the ability of each extract to reduce the FeCl₃ solution as described by Oyaizu (30). Briefly, an appropriate dilution of each extract (1 mL) was mixed with 1 mL of 200 mM sodium phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide. Each mixture was incubated at 50°C for 20 min and then 1 mL of 10% trichloroacetic acid was added. The mixture was centrifuged at 650 rpm for 10 min. Then 2 mL of the supernatant was mixed with 2 mL of distilled water and 0.4 mL of 0.1% FeCl₃. The absorbance was measured at 700 nm. The ferric reducing antioxidant power was determined in triplicate and expressed as mg ascorbic acid equivalents/g of sample.

ABTS antiradical assay

Antioxidant activities of the extracts/digests were determined using the ABTS antiradical assay (31). The ABTS radical cation (ABTS^{•+}) (stock solution) was prepared by mixing equal volumes of 8 mM ABTS and 3 mM K₂S₂O₈ (both prepared using distilled water) in a volumetric flask, which was wrapped with foil paper and allowed to react for a minimum of 12 h in a dark place. The working solution was prepared by adding 2.5 mL of the stock solution with 7.5 mL phosphate buffer (pH 7.4). A range of 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) standard solutions (100~1,000 µM) were prepared in methanol. The working solution (2.9 mL) was added to the methanolic extracts/enzyme digest (0.1 mL) or Trolox standard (0.1 mL) in a test tube and mixed with a vortex. The test tubes were allowed to stand for exactly 30 min. The absorbance of the standards and samples were measured at 734 nm with a Lambda EZ150 spectrophotometer (Perkin Elmer). The results were determined in triplicates and expressed as µM Trolox equiv-

alents/g sample, on a dry weight basis.

DPPH antiradical assay

The DPPH assay was performed according to the method of Brand-Williams et al. (32), with slight modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and then stored at -20°C until needed. The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1.1 units at 515 nm using a spectrophotometer. Phenol extracts/digests (300 μL) were allowed to react with 2,700 μL of the DPPH solution for 6 h in the dark. Then the absorbance was measured at 515 nm. Results were determined in triplicates and expressed in μM Trolox equivalents/g sample. Additional dilution was conducted only if the DPPH absorbance value measured was over the linear range of the standard curve.

Nitric oxide radical (NO^{\cdot}) scavenging ability

The NO^{\cdot} scavenging capacities of the extracts/digests were measured by the Griess reaction (33). Sodium nitroprusside (2.7 mL, 10 mM) in phosphate buffered saline was added to 0.3 mL extracts/digests and incubated at 25°C for 150 min. Then 0.5 mL of the incubated aliquot was added to 0.5 mL of Griess reagent [1% (w/v) sulfanilamide, 2% (v/v) H_3PO_4 , and 0.1% (w/v) naphthylethylene diamine hydrochloride (prepared in an amber bottle and kept away from light)]. The absorbance was measured at 546 nm. Ascorbic acid was used as the reference standard. The NO^{\cdot} scavenging activity of the extracts, the enzyme digest, and ascorbic acid was subsequently calculated.

Hydroxyl radical ($\cdot\text{OH}$) scavenging ability

The ability of the extracts and digests to prevent $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge (34). Briefly, freshly prepared extracts/digests (20~100 μL) were added to a reaction mixture containing 120 μL , 20 mM deoxyribose, 400 μL of 0.1 M phosphate buffer (pH 7.4), 40 μL of 20 mM H_2O_2 , and 40 μL of 500 mM FeSO_4 , and the volume was made to 800 μL with distilled water. The reaction mixture was incubated at 37°C for 30 min and stopped by the addition of 0.5 mL of 2.8% TCA. This was followed by the addition of 0.4 mL of 0.6% TBA solution. The reaction tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm with a spectrophotometer, and the percentage radical inhibition which was determined in triplicates was subsequently calculated.

Lipid peroxidation using brain and liver homogenates

The ability of the methanolic extracts and the *in vitro* di-

gested samples to inhibit lipid peroxidation was tested using a modified method of Ohkawa et al. (35). Adult male albino rats weighing 150~170 g were obtained from the teaching and research farm of The Federal University of Technology, Akure, Nigeria and were treated according to the standard guidelines of the care and use of experimental animal resources. This was also approved by the ethical committee of the Federal University of Technology, Akure, Nigeria on the use of animals (Approval/Ethic Number: FUTA/BCH/FPT/ 001). The rats were allowed to acclimatize for a week before the experiment. A 100 μL of liver and brain homogenate obtained from the male albino rats were incubated with (or without for the blank) 50 μL of freshly prepared 0.071 mM FeSO_4 , 30 μL of 100 mM Tris-HCl (pH 7.4), and *in vitro* digested samples (0~100 μL) together with an appropriate volume of deionized water to give a total volume of 300 μL were then incubated at 37°C for 1 h. The color reaction was carried out by adding 300 μL of 8.1% sodium dodecyl sulfate, 500 μL of 0.15% acetic acid solution (pH 3.4), and 500 μL of 0.6% thiobarbituric acid, respectively. The absorbance was read after cooling the tubes at 532 nm. For the control, the homogenate was peroxidized with 0.071 mM FeSO_4 without the methanolic extract and the *in vitro* enzyme digested sample. A blank containing the other reagents except FeSO_4 , homogenate and the extracts was also prepared.

Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities

AChE and BuChE inhibitory activities were measured by the spectrophotometric method developed by Ellman et al. (36), with slight modifications, having acetylcholine iodide and butyrylcholine iodide as substrates respectively. The rate of thiocholine production is determined by the continuous reaction of the thiol with 5,5-dithiobis-2-nitrobenzoate (DTNB) ion to produce the yellow anion of 5-thio-2-nitrobenzoic acid. Briefly, 1 mL of 10 mM DTNB dissolved in 10 mM sodium phosphate buffer (pH 7.0) was added to 0.6 mL of distilled water. The rats were allowed to acclimatize for a week before the experiment. A 0.1 mL of brain homogenate (enzyme source) and 0.1 mL of the digested sample and the methanolic extract were then added to the mixture and incubated for 2 min at 25°C before 0.2 mL 8 mM acetylcholine iodide (substrate) was added.

The absorbance of the mixture was read at 412 nm at intervals of 30 s for 5 min immediately after the substrate was added. For the control, 0.1 mL of brain homogenate (enzyme source) was added to 1 mL of 10 mM DTNB dissolved in 10 mM sodium phosphate buffer (pH 7.0) and 0.7 mL of distilled water. The mixture was incubated at 25°C for 2 min before 0.2 mL 8 mM of acetylcholine iodide was added, and the absorbance was

measured immediately. A 1 mL of distilled water and 1 mL of 10 mM DTNB was used as blank. The procedure was repeated using 8 mM butyrylcholine iodide as the substrate. The results were expressed in $\mu\text{M min}^{-1} \text{mg protein}^{-1}$ using a molar extinction coefficient $13.6 \times 10^3 \text{ M}^{-1} \text{cm}^{-1}$.

Statistical analysis

All the analyses were conducted in triplicates. Results were computed using Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA) and followed by analysis of variance (ANOVA) Duncan's multiple range test to compare the means that showed a significant variation by using SPSS 11.09 for Windows (IBM SPSS, Inc., Armonk, NY, USA). The significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Profiles of phenolic compounds

Over the years, the exploration of natural products has been on the rise leading to the identification and improvement of plant products that are beneficial to mankind. Phytochemicals are natural bioactive compounds found in plant foods that work with nutrients and dietary

fiber to protect against diseases by helping to slow down the ageing process and reduce the risk of many diseases, including cancer, heart disease, stroke, high blood pressure, cataracts, urinary tract infections, and osteoporosis (37). *D. dumetorum* is one of the many plant foods that has been identified as a multifunctional versatile plant with enormous economic, nutritional, and health potentials (38).

Phenolic compounds are commonly found in plants and have been reported to have several biological activities (39-41). Studies have focused on the biological activities of phenolic compounds, which have potential antioxidants and free radical scavenging abilities (42). The HPLC-DAD quantification of phenolic compounds in white and yellow bitter yams revealed the presence of phenolic acids and flavonoids. It was observed that the most abundant phenolic compounds in processed and raw white and yellow bitter yams were quercetin and quercitrin while gallic acid, chlorogenic acid, epicatechin, kaempferol, rutin, caffeic acid, catechin, and ellagic acid were present in moderate amounts (Fig. 1, Table 1). The quantitative estimates revealed some variation in the levels of each of the phenolic compounds in the two studied varieties, with the white variety having the highest phenolic compounds from a quantitative point of view. The quantitative differences in the identified phe-

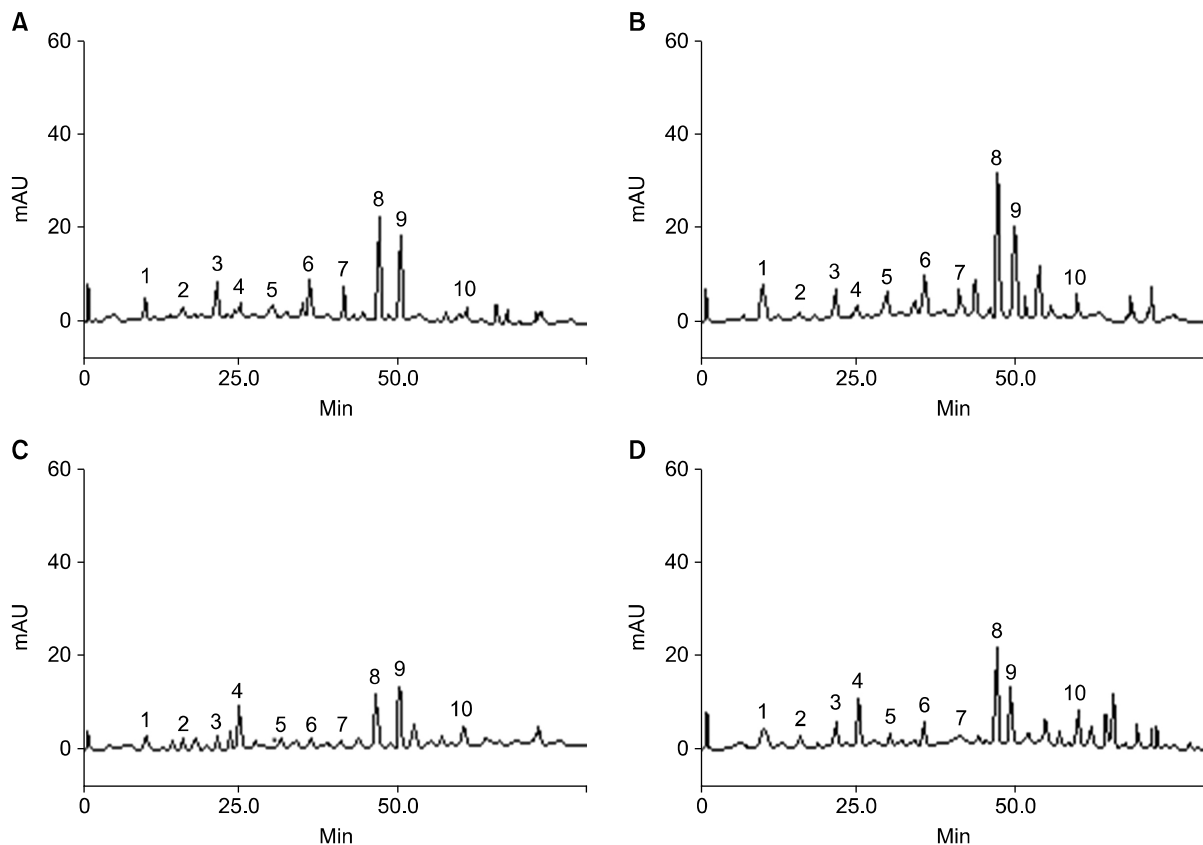


Fig. 1. Representative high performance liquid chromatography profile of white bitter yam [raw (A) and cooked (B)] and yellow bitter yam [raw (C) and cooked (D)]. Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), epicatechin (peak 6), rutin (peak 7), quercitrin (peak 8), quercetin (peak 9), and kaempferol (peak 10).

Table 1. Phenolic acids and flavonoid composition of raw and cooked bitter yam extracts

Compounds	Yellow bitter yam		White bitter yam		LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
	Raw (mg/g)	Cooked (mg/g)	Raw (mg/g)	Cooked (mg/g)		
Gallic acid	1.06 \pm 0.01	1.73 \pm 0.02	1.12 \pm 0.01	3.19 \pm 0.03	0.013	0.045
Catechin	0.98 \pm 0.03	0.95 \pm 0.01	0.85 \pm 0.01	0.72 \pm 0.02	0.021	0.078
Chlorogenic acid	1.09 \pm 0.01	1.68 \pm 0.01	2.93 \pm 0.02	1.65 \pm 0.01	0.030	0.102
Caffeic acid	3.15 \pm 0.02	3.08 \pm 0.01	0.87 \pm 0.01	0.81 \pm 0.02	0.019	0.062
Ellagic acid	0.94 \pm 0.01	0.91 \pm 0.03	0.91 \pm 0.01	1.68 \pm 0.01	0.027	0.089
Epicatechin	0.97 \pm 0.01	1.57 \pm 0.01	3.08 \pm 0.03	3.07 \pm 0.01	0.008	0.025
Rutin	0.93 \pm 0.01	0.92 \pm 0.02	2.97 \pm 0.02	1.60 \pm 0.02	0.020	0.067
Quercitrin	3.67 \pm 0.02	6.14 \pm 0.01	9.45 \pm 0.01	12.35 \pm 0.03	0.033	0.115
Quercetin	4.82 \pm 0.01	4.27 \pm 0.03	7.89 \pm 0.03	8.11 \pm 0.01	0.015	0.059
Kaempferol	1.57 \pm 0.03	2.89 \pm 0.02	0.81 \pm 0.01	1.62 \pm 0.03	0.028	0.096

Results are expressed as mean \pm SD of three determinations.

nolic compounds of the two varieties of bitter yam may be due to differences in the cultivar of the studied bitter yams (43,44). The results showed that heat treatment due to processing (boiling) did not show a particular trend on the levels of the phenolic compounds in both varieties of bitter yam; some of the quantified phenolic compounds increased after cooking while others were reduced after cooking. However, overall, cooking brought about a marked increase in the levels of quantified phenolic compounds (45,46).

Antioxidant properties

Phenolic compounds have attracted much interest recently because *in vitro* studies suggest that they have a variety of beneficial biological properties such as anti-inflammatory, anti-tumor, and anti-microbial activities (47-50). Studies have attributed antioxidant properties to the presence of phenols and flavonoids (51,52). The *in vitro* enzyme digested white and yellow bitter yams showed significantly ($P \leq 0.05$) higher TPC than the methanolic extracts and the undigested control (Table 2). Thus, the maximum amounts of total phenolics were released dur-

ing the *in vitro* digestion process as a result of the activity of the enzymes (α -amylase, pepsin, and pancreatin) of the GIT. This observation is in agreement with a previous report (53) that suggested that digestion might be a determinant factor in the release of nutritionally relevant compounds from the food matrix. In addition, our results are also in agreement with the report of Bhatt and Patel (54), who concluded that solvent extraction methods do not represent the natural conditions occurring *in vivo*, in which phenolic compounds are subjected to a number of physical and chemical changes during their GIT transit and are released from the food matrix. The phenolic content of the processed (cooked) white and yellow bitter yams using solvent extraction and *in vitro* enzyme digestion treatments was also determined to evaluate the effect of cooking on the release of polyphenols during solvent extraction and enzyme digestion. The results revealed that *in vitro* digested cooked white and yellow bitter yams had higher TPC than their raw counterparts, conversely, a higher phenolic content was recorded in the methanolic extracts of raw white and yellow bitter yams compared to their cooked counterparts.

Table 2. The total phenolic, total flavonoid content and ferric reducing power of raw and cooked bitter yam extracts

Properties	Sample	White bitter yam		Yellow bitter yam	
		Raw	Cooked	Raw	Cooked
TPC (mg TAE/g dry sample)	AME	14.71 \pm 1.09 ^d	9.90 \pm 0.95 ^c	14.62 \pm 0.96 ^d	6.72 \pm 0.31 ^a
	ED	18.97 \pm 0.63 ^e	20.34 \pm 1.30 ^f	14.88 \pm 0.68 ^d	18.58 \pm 0.97 ^e
	UC	9.32 \pm 2.85 ^c	9.93 \pm 4.42 ^c	7.42 \pm 0.97 ^b	6.03 \pm 3.71 ^a
TFC (mg QE/g dry sample)	AME	8.45 \pm 0.30 ^c	6.18 \pm 0.29 ^{bc}	6.56 \pm 0.05 ^{bc}	5.84 \pm 0.31 ^b
	ED	10.64 \pm 0.14 ^e	14.82 \pm 0.36 ^{fg}	11.00 \pm 0.10 ^e	13.46 \pm 0.08 ^f
	UC	5.04 \pm 0.07 ^b	7.17 \pm 0.37 ^{bc}	9.17 \pm 0.13 ^d	3.37 \pm 0.08 ^a
Reducing power (mg AAE/g dry sample)	AME	35.83 \pm 1.11 ^c	33.85 \pm 1.67 ^{bc}	33.34 \pm 0.51 ^{bc}	29.36 \pm 0.30 ^{ab}
	ED	37.75 \pm 0.23 ^{cd}	55.84 \pm 0.60 ^f	38.22 \pm 1.29 ^{cd}	50.96 \pm 0.36 ^e
	UC	29.78 \pm 0.17 ^{ab}	36.20 \pm 0.16 ^c	36.88 \pm 0.12 ^c	27.12 \pm 0.18 ^a

Values represent mean \pm SD of triplicate experiments.

Values in same properties followed by different letters (a-g) are significantly different ($P < 0.05$).

TPC, total phenolic content; TAE, tannic acid equivalent; QE, quercetin equivalent; AAE, ascorbic acid equivalent; TFC, total flavonoid content; AME, aqueous-methanolic extract; ED, enzyme digested; UC, undigested control.

The increase in the TPC of the *in vitro* digested cooked samples compared with those digested in the raw form may be due to alterations in the chemical structure and composition as a result of heat during boiling (45). Thus, cooking might have enhanced the breakdown of the insoluble fiber matrix of both bitter yam varieties thereby making its polyphenols more accessible for further breakdown by the enzymes of the GIT (53). *In vitro* digested white bitter yam had higher TPC compared to the *in vitro* digested yellow bitter yam. This could be ascribed to the higher level of most of the identified phenolic compounds in white bitter yam than in yellow bitter yam as revealed by the HPLC-DAD analyses.

The pharmacological effects of flavonoids have been correlated with their antioxidant activities (55). Similarly, TFC of the studied bitter yams revealed a higher value for the *in vitro* digested samples compared with the methanol extracts and the control for the enzyme digest. It was also observed that the *in vitro* digested cooked white and yellow bitter yams had higher TFC than their raw counterparts, while a higher TFC was recorded in the methanolic extracts of raw white and yellow bitter yams compared to their cooked counterparts. The observed higher flavonoid content in the *in vitro* digested white and yellow bitter yams compared to their raw counterparts might be due to the breakdown of the insoluble fiber matrix of both bitter yam varieties thereby making its flavonoids more accessible for further breakdown by the enzymes of the GIT (53).

The reducing capacity of a compound may serve as an important indicator of its potential antioxidants activity (56). The reducing activity of a compound generally depends on the presence of reductases, which had exhibited antioxidant potential by breaking the free radical chain,

donating a hydrogen atom. The reducing potential of the studied bitter yams is shown in Table 2. The results also revealed a higher reducing potential for the *in vitro* enzyme digested white and yellow bitter yams compared with their respective methanolic extracts. This observation is in agreement with previous reports (53,54). Similar to what was observed in the total flavonoid estimation, the *in vitro* digested cooked white and yellow bitter yams had higher reducing potential compared to the *in vitro* digested raw white and yellow bitter yams (53). The results also revealed a higher reductive potential in the methanolic extracts of raw bitter yams for both white and yellow varieties compared to their cooked counterparts.

Most plant foods are rich sources of free radical scavenging molecules and other metabolites, which are rich in antioxidant activity (57). The result of the radical scavenging potentials (Table 3) of the *in vitro* enzyme digested white and yellow bitter yam samples displayed a higher radical scavenging activities (DPPH[·], ABTS^{·+}, and NO[·] scavenging activities) compared to the methanolic extracts, while the [·]OH scavenging activities of the studied bitter yams revealed a higher antiradical action for the methanol extracts compared with the *in vitro* enzyme digestion. It has been established that organic extraction methods do not represent the natural conditions occurring *in vivo* in which phenolic compounds are subjected to a number of physical and chemical changes during their GIT transit (54). Similarly, the antiradical actions (DPPH[·], ABTS^{·+}, and NO[·] scavenging activities) of the *in vitro* digested cooked white and yellow bitter yams were higher compared to their raw counterparts, while that of the methanolic extracts were higher in the raw bitter yams.

Table 3. ABTS^{·+}, DPPH[·], NO[·], and [·]OH scavenging ability of raw and cooked bitter yam extracts

Properties	Sample	White bitter yam		Yellow bitter yam	
		Raw	Cooked	Raw	Cooked
ABTS ^{·+} scavenging ability (μ M TE/g dry sample)	AME	1,414.00 \pm 7.25 ^b	1,272.67 \pm 9.45 ^g	1,142.67 \pm 19.63 ^f	810.67 \pm 5.77 ^e
	ED	2,190.63 \pm 6.25 ⁱ	2,223.96 \pm 4.77 ^j	2,190.63 \pm 6.2 ⁱ	2,219.79 \pm 1.80 ^j
	UC	417.67 \pm 3.61 ^d	312.25 \pm 4.25 ^b	374.50 \pm 3.13 ^c	285.11 \pm 3.13 ^a
DPPH [·] scavenging ability (μ M TE/g dry sample)	AME	677.95 \pm 3.08 ^h	172.31 \pm 0.78 ^b	245.13 \pm 1.11 ^c	99.49 \pm 0.47 ^a
	ED	1,134.66 \pm 0.62 ^k	1,209.06 \pm 0.73 ^l	556.09 \pm 0.82 ^f	644.23 \pm 1.30 ^g
	UC	945.51 \pm 0.41 ^j	870.19 \pm 0.48 ⁱ	500.00 \pm 0.17 ^e	471.15 \pm 0.46 ^d
% NO [·] scavenging ability	AME	58.15 \pm 0.01 ^c	46.54 \pm 0.01 ^b	44.32 \pm 0.01 ^{ab}	41.11 \pm 0.00 ^a
	ED	82.01 \pm 0.23 ^f	84.75 \pm 0.08 ^g	82.01 \pm 0.02 ^f	84.27 \pm 0.01 ^g
	UC	82.78 \pm 0.22 ^f	75.55 \pm 0.26 ^d	79.88 \pm 0.09 ^{de}	79.43 \pm 0.24 ^{de}
% [·] OH scavenging ability (0.005 mg/mL)	AME	74.68 \pm 0.07 ^g	86.54 \pm 0.01 ^h	87.06 \pm 0.01 ^h	89.00 \pm 0.01 ^{hi}
	ED	20.53 \pm 0.01 ^{ab}	36.55 \pm 0.21 ^d	19.13 \pm 0.06 ^a	27.18 \pm 0.23 ^c
	UC	37.71 \pm 0.27 ^d	44.64 \pm 0.26 ^e	49.35 \pm 0.25 ^{ef}	48.11 \pm 0.24 ^{ef}

Values represent mean \pm SD of triplicate experiments.

Values in same properties followed by different letters (a-l) are significantly different ($P < 0.05$).

ABTS^{·+}, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation; DPPH[·], 2,2-diphenyl-1-picrylhydrazyl radical; NO[·], nitric oxide radical; [·]OH, hydroxyl radical; TE, Trolox equivalent; AME, aqueous-methanolic extract; ED, enzyme digested; UC, undigested control.

Table 4. The inhibitory effect of raw and cooked bitter yam extracts (0.036 mg/mL) on iron II sulphate induced lipid oxidation in albino rat's brain and liver homogenate (%)

Sample	Sample	White bitter yam		Yellow bitter yam	
		Raw	Cooked	Raw	Cooked
Brain	AME	60.19±0.97 ^e	59.03±0.82 ^e	38.36±1.43 ^{ab}	36.42±1.22 ^a
	ED	61.41±1.10 ^e	63.71±0.93 ^f	64.61±0.97 ^f	67.13±0.93 ^g
	UC	53.39±0.96 ^{cd}	50.00±1.21 ^c	35.28±1.21 ^a	37.50±0.95 ^{ab}
Liver	AME	64.92±0.99 ^f	54.20±1.43 ^b	67.32±0.86 ^g	66.57±0.98 ^g
	ED	58.21±1.41 ^{cd}	65.47±1.43 ^f	66.37±1.27 ^g	69.53±1.34 ^h
	UC	47.07±1.21 ^a	64.51±1.11 ^f	61.27±1.42 ^e	56.32±1.32 ^c

Values represent mean±SD of triplicate experiments.

Values in same organs followed by different letters (a-h) are significantly different ($P<0.05$).

AME, aqueous-methanolic extract; ED, enzyme digested; UC, undigested control.

Lipid oxidation assay

Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage. The damage has been implicated in the patho-physiology of various human diseases such as atherosclerosis, diabetes and cancer (58). The initiation of the peroxidation sequence in membranes or polyunsaturated fatty acids is due to the abstraction of a hydrogen atom from the double bond in the fatty acids (58). The inhibitory action against lipid oxidation using rat brain and liver as the lipid rich source is shown in Table 4. The result of the inhibitory action against lipid oxidation also revealed a higher inhibitory action in the *in vitro* digested white and yellow bitter yams compared with the methanolic extracts. The result also showed a higher inhibitory action in the cooked *in vitro* digested studied bitter yams compared to their raw counterparts and a higher inhibitory action for the raw methanolic extracts of the studied bitter yams compared to their cooked counterparts. Phenolic compounds have been reported to prevent the decomposition of H₂O₂ into free radicals (59). Hence, the observed inhibition of lipid oxidation by the methanolic extracts, *in vitro* digested and undigested control samples of white and yellow bitter yam varieties may be due to the presence of some phenolic compounds.

Anticholinesterase action

Inhibition of cholinesterase is a promising approach for the treatment of AD and for possible therapeutic applications in the treatment of Parkinson's disease, ageing, and myasthenia gravis (60). In all cases, the therapeutic strategy is to increase the persistence of synaptic ACh by blocking its degradation, such that there is a net increase in cholinergic receptor activation. This overall strategy is based on a clinical condition wherein activation of cholinergic receptors is deficient. Thus, increasing the residence of acetylcholine molecules within synapses by inhibiting AChE at least partially counteract a deficiency in either the release of neurotransmitter or a reduction in cholinergic receptors/signalling. There is some evidence suggesting that BuChE activity may be involved in the pathogenesis of AD. This has led to the hypothesis that the use of non-selective cholinesterase inhibitors that inhibit both BuChE and AChE may be more beneficial to patients with AD than the use of selective cholinesterase inhibitors that inhibit AChE alone (61,62). Plant alkaloids are best known for inhibiting cholinesterase enzymes, however, recent reports have indicated new classes of cholinesterase-inhibiting phytochemicals such as coumarins, flavonols, terpenoids, and especially monoterpenes that are relevant antioxidant phytochemicals (63-66).

Table 5. Effect of raw and cooked bitter yam extracts on brain acetylcholinesterase and butyrylcholinesterase activity ($\mu\text{mol min}^{-1} \text{mg protein}^{-1}$)

Sample	Sample	Control	White bitter yam		Yellow bitter yam	
			Raw	Cooked	Raw	Cooked
AChE	AME	2.63±0.12 ^{cd}	3.17±0.13 ^e	2.63±0.08 ^{cd}	3.65±0.16 ^{ef}	2.75±0.21 ^{cd}
	ED	1.91±0.09 ^{bc}	2.81±0.14 ^{cd}	2.75±0.08 ^{cd}	2.45±0.08 ^c	2.57±0.13 ^c
	UC	1.91±0.15 ^{bc}	0.96±0.09 ^a	0.96±0.16 ^a	1.38±0.13 ^b	1.91±0.10 ^{bc}
BuChE	AME	4.24±0.09 ^f	3.17±0.12 ^d	3.35±0.11 ^{de}	1.49±0.13 ^{ab}	2.75±0.14 ^{cd}
	ED	2.09±0.12 ^{bc}	1.77±0.13 ^b	1.32±0.12 ^a	1.95±0.08 ^b	1.91±0.09 ^b
	UC	2.09±0.12 ^{bc}	2.39±0.09 ^c	3.35±0.11 ^e	3.16±0.12 ^d	3.52±0.10 ^{de}

Values represent mean±SD of triplicate experiments.

Values in same enzymes followed by different letters (a-f) are significantly different ($P<0.05$).

AChE, acetylcholinesterase; BuChE, butyrylcholinesterase; AME, aqueous-methanolic extract; ED, enzyme digested; UC, undigested control.

AChE and BuChE inhibitory action of the studied bitter yams is shown in Table 5. The results of this study show that the *in vitro* digested and methanolic extracts of both raw and cooked bitter yams showed no AChE inhibitory potential while the undigested control of raw and cooked bitter yams displayed some ability to inhibit AChE activity. The ability of the undigested control samples to inhibit AChE activity might be due to the presence of cholinesterase-inhibiting phytochemicals in the controls of enzyme digest of white and yellow bitter yams. However, the *in vitro* digested and methanolic extracts displayed the ability to inhibit BuChE activity, with the *in vitro* digested sample having the highest inhibitory action. It could be deduced from the antioxidant activities and BuChE inhibitory action that the studied white and yellow bitter yams could serve as a functional food. The observed biological action could be as a result of the phenolic constituents and other phytochemicals.

CONCLUSION

The results of this study revealed that the selected phenolic containing bitter yam varieties possess antioxidant potentials that would be better released after gastrointestinal enzyme digestion. The result also revealed that the methanolic extracts and the *in vitro* enzyme digests of the bitter yams could serve as a possible BuChE agent. Therefore, the studied bitter yams can be harnessed as natural products in the management of free radical mediated diseases.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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