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Nutritional, antioxidant, angiotensin-converting-enzyme and carbohydrate-hydrolyzing-enzyme inhibitory activities of underutilized leafy vegetable: African wild lettuce (*Lactuca taraxacifolia* Willd)

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Abstract

Background: African wild lettuce (*Lactuca taraxacifolia* Willd.) is an underutilised indigenous leafy vegetable containing essential nutrients and medicinal properties. Hence, this study aimed to determine the chemical composition, antioxidant activities, α-amylase, α-glucosidase and angiotensin converting enzyme inhibitory potentials of wild lettuce leaves powder samples.

Methods: Freshly harvested Wild Lettuce leaves were processed into whole leafy powder (WLF), extracted powder (WLE), residue (WLR) and leaf protein isolate (WPI). Chemical composition, antioxidant activities, α-amylase, α-glucosidase and angiotensin-converting enzyme inhibitory potentials of the powder samples were determined.

Results: Crude protein of Wild Lettuce leaves ranged from 23.27 to 46.57 and crude fiber from 4.17–37.37 g/100 g. Phosphorous was the most abundant element, while zinc had the lowest concentration. The samples essential amino acids, protein efficiency ratio, essential amino acid index and biological values were 39.83–50.65 mg/100 g protein. 2.79–3.51, 77.03–92.36% and 72.26–88.97%, respectively. Saponin, tannin, oxalate, phytate, terpennoids, flavonoid and phenol in the leafy vegetable samples were within tolerable levels. The African wild lettuce leaf protein isolate (WPI) had higher DPPH antioxidant activity (91.88%), percentage inhibitory properties on α-amylase (26.11%), α-glucosidase (64.24%) and angiotensin-1-converting enzyme (97.53%) than WLF (18.28, 25.44, 55.41 and 67.56), WLE (70.85, 24.97, 62.53 and 93.27) and WLR (53.07, 24.68, 50.03 and 85.28) respectively.

Conclusion: African wild lettuce leaf samples, particularly protein isolate, contain essential nutrients, antioxidant activities and ability to inhibit angiotensin-1-converting, α-amylase and α-glucosidase enzymes Therefore, the leafy vegetable samples, particularly WPI, may be suitable as antioxidant, antidiabetic and antihypertensive agent.

Keywords: Bio-efficacy, Phytonutrient, Antidiabetic activities, Antihypertensive potential

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Introduction

Medicinal plants utilization are becoming more popular in developed and developing counties due to the increasing inefficacy of many synthetics drugs used for the treatment chronic diseases like hypertension, diabetes [1, 2]. Leafy vegetables are able to produce a large number of diverse bioactive compounds like phytochemicals with ability to act as antioxidant and to prevent free radical damage [3]. Study has shown that many plants are rich source of natural antioxidants like vitamins A, C, E, and phenolic compounds such as flavonoids, tannins, and lignins, found in plants, all act as antioxidants [3]. The consumption of leafy vegetables is linked with several health benefits, because of medicinal properties and high nutritional value [4].

In Nigeria wild leafy vegetables are underutilized which is attributed to poor knowledge of their health benefits. African wild lettuce leaf (*Lactuca taraxacifolia* Willd) is one of the underutilized wild vegetables in Nigeria. It is consumed as food and for its medicinal benefits [5]. It is also reported to be rich in vitamins, minerals, proteins, essential fatty acids, dietary fibre and flavonoids [6–8].

African wild lettuce is an annual West Tropical Africa herb commonly known as wild lettuce, and it is a tropical plant, which is part of the Asteraceae family and known as African Lettuce. There are some common names of the plant; that is, in Ghana it is called agblòke, Hausa; *namijindayi*, Yoruba; *efo yanrin*, and Sierra Leone-*Kissi; bekuhoa-pomboe*. In different parts of Africa, including Nigeria, the leaves are used to make soup, salad or eaten as spinach [9]. It is consumed in Congo as green vegetables [10]. The leaves are used traditionally for preparing meals and serve as medicine in Ghana [6, 7]. Evidence-based reports indicate that *Launaea taraxacifolia* leaves possess antioxidant activity [11], protective effects against DNA and kidney damage [12] antimicrobial and antiarthritic effects [13] and hypolipidaemic properties [14, 15]. In Nigerian folkloric medicine, the plant is used for the treatment of epilepsy [16]. Hence, the present study aimed at evaluating the nutritional, antioxidant, angiotensin converting-enzyme and carbohydrate-hydrolyzing-enzyme inhibitory activities of underutilized leafy vegetable: African wild lettuce (*Lactuca taraxacifolia* Willd).

Materials and methods

Source of wild lettuce powder

Fresh wild lettuce leaves were sourced from Ilara - Mokin, Ondo State of geographical coordinates of 7.3497° N 5.1067° E, and the leaves were authenticated in the Department of Crop Soil and Pest Management, Federal University of Technology, Akure, Nigeria.

Experimental sample preparations

Preparation of African wild lettuce leaf powder sample

Freshly harvested African wild lettuce leaves were processed into powder. The leaves were sorted to remove unwanted materials like stones, pebbles and other foreign leaves, chop manually, washed with double distilled water and drained. The chopped leaves were oven dried at 60 °C for 20 h using a hot-air oven (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK), milled with a laboratory blender (Model KM 901D; Kenwood Electronic, Hertfordshire, UK) and passed through a 60 mm mesh sieve (British Standard) to obtain African wild lettuce leaf powder. The powder was packed in a plastic container, sealed and stored at room temperature (~ 27 °C) until analysis.

Preparation of wild lettuce leaf ethanolic extracts and residue

African wild lettuce leaf powder (500 g) was extracted exhaustively via marceration for 72 h with 2.8 L of 50% absolute ethanol. The mixture was filtered using muslin cloth and Whatman No.1 filter paper; and the filtrate was concentrated using rotary evaporator (Model 349/2, Corning Limited) at 40 °C for 36 h, and thereafter, it was freeze-dried to obtain wild lettuce leaf extract (WLE) and the residue obtained from the above filtrate was oven dried to obtain wild lettuce residue (WLR). The powder samples were packed in a plastic container, sealed and stored at room temperature (~ 27 °C) until required for use.

Preparation of wild lettuce leaf protein isolate

The wild lettuce leaf protein isolate was prepared according to the process described by Dawodu and Abdulsalam [17] with minor modifications. Wild lettuce leaf powder sample was dispersed in distilled water (water: powder ratio, 1:20 (w/v)), adjusted the pH 11.0 with 1 N NaOH to solubilize the proteins, continuously stirred and maintained at 37 °C for 2 h. The slurry was centrifuged at 10, 000 g for 30 min at 4 °C. The residue was discarded, while the supernatant was adjusted to the isoelectric point (pH 4.0) with 1 N HCl to precipitate the proteins. The precipitated protein was washed three times and subsequently centrifuged at 10,000 g for 30 min at 4 °C. The precipitate protein was re-dissolved in water, neutralized to pH 7 with 0.1 N NaOH at room temperature, and then freeze-dried. The wild lettuce leaf protein isolate was stored for further analysis.

Determination of chemical composition of wild lettuce leaf samples

Proximate composition of African wild lettuce leaf powder samples was determined using standard procedures [18]. Moisture content was determined in a hot-air

circulating oven (Galenkamp). Ash was determined by incineration (550 °C) of known weights of samples in a muffle furnace (size 3, Hotbox oven, Gallencamp) [18]. Crude fat was determined by exhaustively extracting a known weight of sample in petroleum ether (boiling point, 40 to 60 °C) using a Tecator Soxhlet [Model 2043(20430001), Slandegarupgade, Hilleroed, Denmark]. Protein content (N × 6.25) was determined by the micro-Kjeldahl method (Method No 978.04). Crude fiber was determined after digesting a known weight of fat-free sample in refluxing 1.25% sulfuric acid and 1.25% sodium hydroxide. Carbohydrate content was determined by addition of all percent of moisture, fat, crude protein, ash and crude fibre subtracted from 100%, giving the amount of nitrogen free extract otherwise known as carbohydrate.

Calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu) and zinc (Zn) were determined using an Atomic Absorption Spectrophotometer (Model 703 Perkin Elmes, Norwalk, CT). Sodium (Na) and potassium (K) in the samples were determined using a flame emission photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK) with NaCl and KCl as standards. Phosphorus was determined using the Vanodo-molybdate method.

Amino acids analysis was conducted using the method described by Jeong and Shim [19] with slight modifications. Each of the samples (0.1 g) was placed in heatproof screw cap test tubes and dissolved in 5 mL of 6 N HCl with vortexing (TSS2, IKA, Korea) for 30 s. The tubes were flushed with N gas for 1 min and then sealed. The samples were thereafter incubated in a hot-air oven (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK) at 110 °C for 24 h. After the reaction was completed, the supernatant was removed using a glass filter. To remove HCl, the supernatant was evaporated in a vacuum rotary evaporator (HS-2005S, Jisico Co, Ltd., Korea) and the concentrate dissolved in 3 mL of sodium citrate buffer (pH 2.2). Finally, the solution was filtered with 0.45 lm PTFE filters (Sigma-Aldrich, Seoul, South Korea) and analyzed using an auto amino acid analyzer *(Technicon Instruments Corporation, New York)* at 570 nm.

The tryptophan content was determined in a separate analysis. The weighed samples were placed in polypropylene tubes and after the addition of the internal standard (norleucine), they were hydrolysed in 4.67 mol/L KOH containing 1% (w/v) thiodiglycol for 18 h at 110 °C. After hydrolysis, KOH was neutralised with 2.4 mol/L perchloric acid, and the supernatant was adjusted to pH 3.0 with acetic acid. A 20 μL aliquot of the hydrolysed sample was subjected to derivatization as described above. The solution of amino acid standard was supplemented with tryptophan. Tryptophan analysis was performed using a Waters C_{18} reversed phase column (3.9 × 150 mm) (Waters Milford, MA) and the solvents and gradient conditions were as described by Hariharan et al. [20]. Use of this elution protocol was necessary in order to adequately separate tryptophan from ornithine, which results from the alkaline hydrolysis of arginine.

Protein quality indices of African wild lettuce powder samples

Protein quality of wild lettuce leaf powder was determined on the basis of the amino acid profiles. The Essential Amino Acid Index (EAAI) was calculated using the method of Labuda et al. [21].

The Protein Efficiency Ratio (PER) was estimated according to the regression equations developed by Alsmeyer et al. [22] cited by Mune-Mune et al. [23].

Phytochemicals composition of African wild lettuce leaf samples

The total flavonoid (TF) content was determined by the Aluminium Chloride colorimetric assay as modified by Nsor-Atindana et al. [24]. Tannin concentration in samples was determined by a standard procedure [18]. Phenols concentration was determined as described by Singleton et al. [25]. Total saponin content was determined by the method described by Obadoni and Ochuko [26]. Phytate was determined using the spectrophotometric method of Lolas and Markakis [27]. Phytic acid was extracted from each 3 g of powder samples with 3% trichloroacetic acid by shaking at room temperature followed by high speed centrifugation as described by Wheeler and Ferrel [28]. Oxalate was determined by Anonymous [18] method. Total phenolic content was determined by Folin Ciocalteu reagent method [29] with some modifications.

Antioxidant activity of African wild lettuce leaf powder samples

Antioxidant potentials of the African wild lettuce powder samples were evaluated using DPPH radical scavenging, ABTS, OH free radicals, Iron chelation and FRAP assays.

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) assay was performed as described by Benzie and Strain [30] with some modifications. A 30 ml freshly prepared FRAP reagent was warmed to 37 ℃ and a reagent blank reading was taken at 593 nm; 100–150 μL of each sample was then added and volume made up to a total volume of 1 ml with distilled water. Absorbance (A) readings were taken with a Jenway Vis Spectrophotometer 6305 after 0 s and 4 min. Thereafter, and the change in absorbance (*A*593 nm) between the final reading selected and the blank reading was calculated for each sample and related

to *A*593 nm of a Fe^2 standard solution tested in parallel. The 4 min readings were selected for calculation of FRAP values.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay

This was determined as per protocol described by Pownall et al. [31] with slight modification. The stock solution of DPPH reagent was prepared by adding 10 mL of the stock (0.6 mM) to 45 mL of methanol. For each extract, 1 mL methanol DPPH was added to 30–200 μL extract (at different concentrations). Absorbance was determined after 30 min at 517 nm using a spectrophotometer (Jenway 6305). The percentage inhibition activity on DPPH radical was calculated.

Ferrous ion-chelating activity

The chelating activity of the extracts and proteins on Fe^{2+} was measured according to the method of El and Karakaya [32], with some modifications. Sample aliquots (200 μL) were mixed with 740 μL of deionized water and 20 μL of 2 mM FeCl2 solution, and incubated for 30 min at room temperature. After incubation, 200 μL of 5 mM ferrozine was added and the mixture was incubated for an additional 10 min in the same conditions. The absorbance was determined at 562 nm. Distilled water was used as control instead of the sample. Metal-chelating capacity was calculated.

2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS)

The scavenging activity was determined according to the method described by Re et al. [33] with slight modifications. ABTS solution, prepared 12 h before use, contained 7 mM ABTS and 2.5 mM potassium persulfate. The obtained solution was then diluted with 200 mM phosphate buffer pH 7.4 to an absorbance of 0.7 ± 0.02 at 734 nm, and 4 mL of diluted solution was mixed with 40 mL of extract solution 0.1 mg/mL. Absorbance at 734 nm was read after 10 min using water instead of sample as a control.

Hydroxyl (OH) free radicals scavenging activity

Hydroxyl radical scavenging assay: Hydroxyl radical scavenging activity of the extracts was determined according to the method reported by Klein et al. [34]. The reaction mixture contained 1.0 ml of different concentration of extracts (2–10 mg/ml), 1.0 ml of iron-EDTA solution (0.13% ferrous ammonium sulphate 0.26% EDTA), 0.5 ml of 0.018% EDTA, 1.0 ml of DMSO (0.85% in 0.1 M phosphate buffer pH 7.4) and 0.5 ml of 0.22% ascorbic acid. The tubes were capped tightly and heated in a water bath at 80–90 °C for 15 min, the reaction was terminated by adding 1.0 ml of ice-cold TCA (17.5%). To the above reaction mixture 3.0 ml of Nash reagent (75.0 g of ammonium acetate, 3.0 ml of glacial acetic acid and 2.0 ml of acetyl acetone were mixed and distilled water was added to a total volume of 1 L) was added and incubated at room temperature for 15 min for colour development. The intensity of the yellow colour formed was measured at 412 nm against a reagent blank. Ascorbic acid and gallic acid were used as standards.

Determination of angiotensin converting enzyme (ACE) inhibition activity of African wild lettuce leaf samples

The ACE-inhibitory activity was determined in vitro using spectrophotometric method described by Zhang et al. [35] with slight modifications [36]. This method was based on the liberation of hippuric acid from hippuryl-L-histidyl-L-leucine (Hip-His-Leu) catalyzed by ACE. For the assay, 42.5 μL of the sample solution (2 mg/mL) was pre-incubated at 37 °C for 5 min with 10 μL ACE (0.6 mU/mL) enzyme. The mixture, 20 μL of the substrate (5 mM HHL in 10 μM zinc chloride containing 100 mM sodium trizma base and 300 mM NaCl at pH 8.3) was added and incubated (37 °C for 60 min). After incubation, 12.5 μL of 5 M HCl. was added to terminate the reaction. The ACE-inhibition (%) was determined by HPLC system with a 486 tunable UV detector. The average value from three determinations at each concentration was used to calculate the ACE-inhibition (%) rate as follows:

$$\text{ACE–inhibition}\ (\%) = \left[\frac{(\text{A228a–A228b})}{\text{A228a–A228c}}\right] * 100$$

where A is the absorbance (Abs) of HA generated in the presence of ACE-inhibitor, B the Abs of HA generated without ACE-inhibitor and C the Abs of HA generated without ACE (corresponding to HHL autolysis in the course of enzymatic assay). Captopril was used as a positive control for ACE-inhibition.

Alpha-amylase inhibition assay of African wild lettuce leaf powder samples

The activity of *α*- amylase was carried out according to the method of Mao and Kinsella [37], based on the starch-iodine colour changes with minor modifications. Soluble starch solution (1%) was used as substrate. The starch solution was prepared by adding 1 g of soluble potato starch in 10 mL water and then boiled for 2 min. After cooling, water was added to reach a final volume of 100 mL. *α*-amylase solution (0.1 mL of 15 *μ*g/mL in 0.1 M acetate buffer at pH 7.2 containing 0.0032 M sodium chloride) was added to a mixture of 3 mL of 1% soluble starch solution and 2 mL of acetate buffer (0.1 M, pH 7.2) pre-equilibrated at 30 °C in a water bath. Substrate and *α*- amylase blank determinations were undertaken under the same conditions. At zero time (*t*=

0 min) and at the end of the incubation period (t = 60), 0.1 mL of reaction mixture was withdrawn from each tube after mixing and transferred into 10 mL of an iodine solution (0.254 g iodine and 4.0 g potassium iodide in 1 L). After mixing, the absorbance of the starch-iodine mixture was measured immediately at room temperature at 565 nm using a spectrophotometer. The absorbance of the starch blank was subtracted from the sample reading. One unit of amylase activity was arbitrarily defined as follows: $(Ao - At/Ao) * 100$, where Ao and At are absorbance of the iodine complex of the starch digest at zero time and after 60 min of hydrolysis. Specific activity of α-amylase was defined as unit/mg protein/60 min. Percentage inhibition was calculated using the equation below. The different extracts and proteins were diluted as appropriate to establish dose-dependent effects and for calculation IC_{50} values, that is, the concentration of α-glucosidase inhibitor to inhibit 50% of its activity.

Alpha-glucosidase inhibition assay of African wild lettuce leaf powder samples

α-glucosidase inhibitory activity was performed following the modified method of Pistia-Brueggeman and Hollingsworth [38]. In test-tubes, a reaction mixture containing 500 μL of phosphate buffer (50 mM, pH 6.9), 100 μL of α-glucosidase (1 U/mL), and 200 μL of plant extract of varying concentrations was pre-incubated for 5 min at 37 °C and then 200 μL of 1 mM PNPG (4-nitrophenyl- α-D-glucopyranoside) substrate was added to the mixture. After further incubation at 37 °C for 30 min, the reaction was stopped by the addition of 500 μL of sodium carbonate (0.1 M). Enzyme, inhibitor, and substrate solutions were all prepared using the same buffer. The yellow colour produced (due to pnitrophenol formation) was quantitated by colorimetric analysis and reading the absorbance at 405 nm. Each experiment was performed in triplicate, along with appropriate blanks. The percentage inhibition was calculated.

Statistical analysis

The data were subjected to a one-way analysis of variance (ANOVA) and the significant difference between mean values was determined by Duncan's multiple range test ($p < 0.05$) using SPSS (Statistical Package for the Social Sciences) version 13.0. (SPSS Inc., Illinois, USA). Values expressed were mean of triplicate determinations ±standard error of mean (SEM).

Results

Nutrient composition of wild lettuce leaf powder samples

The proximate composition of *L. taraxacifolia* leaf samples is presented in Table 1. The crude protein content of the leaf samples ranged from 23.28% in whole leaf powder (WLF) to 46.57% in leaf protein isolate (WPI). Crude fibre content of wild lettuce leaf samples ranged between 10.59 in WLE and 37.97 g/100 g in WLR, while that of total ash content varied from 9.41% in WPI to 15.26% in WLR.

The mineral compositions of wild lettuce powder samples are presented in Table 2. The mineral composition of wild lettuce leaf powder samples, that is, WLF, WLE, WLR and WPI, contained magnesium, sodium, potassium, calcium, phosphorus, manganese, iron, zinc and copper in appreciable amount. However, phosphorous was found to be in most abundant of the elements, with values ranged between 9.27 ± 0.02 in WPI and 15.43 ± 0.02 mg/100 g WLR, while copper (Cu) had the lowest value, which ranged from 0.006 to 0.009 mg/100 g. The Na/K molar ratios of the leaf samples ranged between 0.59 in WLR to 0.63 in WLE; while Ca/P molar ratios ranged from 0.12 in WLF to 0.58 in WLE.

Amino acid profiles of wild lettuce leafy vegetable powder samples are presented in Table 3. Total amino acid composition of the leafy vegetable samples ranged from 96.43 g/100 g protein in WLE to 99.06 g/100 g of protein in WLF, while total essential amino acids ranged from 39.83 g/100 g protein in WLR to 50.65 g/100 g protein in WPI. For the non-essential amino acids, the values ranged from 47.66 g/100 g protein in WPI to 58.76 g/100 g protein in WLR. The range values of protein efficiency ratio (PER), essential amino acid index (EAAI) and biological values (BV) of raw (WLF), residue (WLR), extract (WLE) and protein isolate (WPI) of wild lettuce leaf powder samples were 2.79–3.51, 77.03–92.36% and 72.26–88.97%, respectively. The argine/lysine ratios varied from 0.94 to 1.95, and the value was higher in WPI than in WLF, WLR and WLE powder samples. For the branched chain amino acids (valine, leucine and isoleucine), the values ranged between 17.42 and 19.21 g/100 g protein; and with higher value in WPI than that of WLF, WLR and WLE, respectively.

The phytochemical composition of wild lettuce leaf samples is presented in Table 4. The whole leaf powder sample (WLF) had highest concentration of saponin, tannin, oxalate, phytate and terpennoids, while leaf protein isolate (WPI) had the lowest concentration with the following range values 59.09–199.09, 4.23–57.37, 7.20–24.45, 11.95–19.86 and 51.86–70.21 ($mg{\cdot}g^{-1}$) respectively. However, leaf protein isolate (WPI) had the highest concentration in flavonoid (13.43 mg/g) and phenol (28.89 mg/g), while WLF had the lowest values (4.48 mg/g, 19.47 mg/g). Also, Table 4 shows the relationship between the phytate and bioavailability of calcium, zinc and iron in the wild lettuce leaf samples. The Phytate: calcium, Phytate:zinc and phytate:iron molar ratios of wild lettuce leaf samples ranged 0.15–0.75, 16.35–34.93 and 2.04–3.59, respectively; while that of phytate:calcium:zinc molar ratio was between 0.50 and 4.37.

Table 1 Proximate composition (g/100 g DW) of African wild lettuce leaves

Component	WLF	WLE	WLR	WPI
Protein	23.28 ± 0.04^{cb}	24.17 ± 0.02^{b}	7.89 ± 0.02^{d}	46.57 ± 0.08^{a}
Fiber	17.31 ± 2.02^{b}	10.59 ± 0.96^{c}	37.37 ± 2.98^{a}	4.17 ± 0.02^{d}
Fat	3.95 ± 0.08^{a}	3.64 ± 0.36^{a}	3.97 ± 0.04^{a}	1.95 ± 0.57^{b}
Ash	14.05 ± 0.56^{b}	10.03 ± 0.47^{c}	15.26 ± 0.51^{a}	9.41 ± 0.52^{d}
Moisture	4.44 ± 0.55^{b}	6.03 ± 0.55^{a}	7.05 ± 0.55^{a}	4.15 ± 0.52^{b}
Carbohydrate	37.07 ± 3.15^{a}	45.94 ± 4.08^{a}	46.45 ± 2.19^{a}	34.07 ± 3.17^{a}

[a]*WLF* Wild lettuce powder, *WLE* Wild lettuce extracts, *WLR* Wild lettuce residue, *WPI* Wild lettuce protein isolate
[b]Means followed by different letters are significantly different ($p < 0.05$) according to Duncan post-hoc test; means of three replicates ± standard deviation

Antioxidant activities of African wild lettuce powder samples

The antioxidant activities of wild lettuce powder samples are presented in Table 5. The ability of the wild lettuce leaf powder samples to scavenge free radical against DPPH, ABTS, OH free radical scavenging and Fe^{2+}chelation ranged as follows: 18.28% in WLF to 91.88% in WPI, 169.64 mg/g in WLR to 383.92 mg/g in WPI, 60% in WLR to 80.25% in WPI and 5.84 in WLR to 36.64% in WPI, respectively. Its noteworthy that the DPPH, ABTS, OH and Fe^{2+} chelation show highest activity in WPI compared with other samples (i.e. WPI > WLE > WLF > WLR) respectively.

α-amylase and α-glucosidase enzyme inhibitory potential of wild lettuce leaf samples

Alpha-amylase and α-glucosidase enzyme inhibitory potential of wild lettuce leaf samples are presented in Fig. 1. The result showed that wild lettuce protein isolate (WPI) (64.7%) exhibited highest α-glucosidase enzyme inhibitory potential, while wild lettuce residue (WLR) had the least activity (50.82%). For the α-amylase inhibitory activities, the WPI (protein isolate) and WLE (extract) showed high inhibition on α-amylase enzyme with the percentage of inhibition of 26.21% and 25.49%, respectively.

Angiotensin converting enzyme inhibition wild lettuce powder samples

The angiotensin converting enzyme (ACE) inhibitory potential of African wild lettuce (whole leave powder, extract, residue and protein isolate) samples is shown in Fig. 2. The result showed that African wild lettuce samples, except WLF, had higher percentage of inhibition on angiotensin converting enzyme than Captopril (a synthetic antihypertensive agent). However, WPI, a protein isolate sample, (97.5%) had higher percentage of angiotensin converting enzyme inhibitory activities when compared with WLE (93.3%), WLR (85.3%) and WLF (67.6%) samples.

Discussion

Nutrient composition of wild lettuce leaf powder samples

Leafy vegetables are good sources of food and medicine. Nutritionally, green leafy vegetables provide essential nutrients like protein, fibers and minerals to the less privileged in developing countries who cannot afford animal-

Table 2 Mineral composition of African wild lettuce leaves

Element or ratio	WLF	WLE	WLR	WPI
Cu	0.01 ± 0.00^{b}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
Fe	0.47 ± 0.02^{d}	0.51 ± 0.02^{b}	0.63 ± 0.00^{a}	0.49 ± 0.03^{c}
Zn	0.06 ± 0.00^{c}	0.08 ± 0.01^{a}	0.06 ± 0.01^{c}	0.07 ± 0.01^{b}
Mn	1.35 ± 0.03^{d}	2.01 ± 0.02^{a}	1.92 ± 0.02^{b}	1.72 ± 0.02^{c}
K	8.93 ± 0.22^{b}	11.99 ± 0.32^{c}	13.09 ± 2.02^{d}	6.61 ± 0.12^{a}
Na	5.36 ± 0.11^{b}	7.56 ± 0.11^{a}	7.78 ± 0.02^{a}	4.04 ± 0.03^{b}
Mg	0.74 ± 0.03^{d}	2.54 ± 0.12^{b}	3.10 ± 0.11^{a}	1.86 ± 0.11^{c}
Ca	1.61 ± 0.04^{c}	6.15 ± 0.32^{b}	6.31 ± 0.03^{a}	1.22 ± 0.03^{d}
P	13.91 ± 0.02^{b}	10.60 ± 1.02^{c}	15.43 ± 3.23^{a}	9.27 ± 0.33^{d}
Ca/P	0.12 ± 0.11^{a}	0.58 ± 0.03^{a}	0.41 ± 0.02^{a}	0.13 ± 0.03^{a}
Na/K	0.6 ± 0.01^{a}	0.63 ± 0.02^{a}	0.59 ± 0.03^{a}	0.61 ± 0.01^{a}
Zn/Cu	6.0 ± 0.03^{c}	8.0 ± 1.16^{a}	6.0 ± 0.02^{c}	7.0 ± 0.02^{b}

[a]*WLF* Wild lettuce powder, *WLE* Wild lettuce extracts, *WLR* Wild lettuce residue, *WPI* Wild lettuce protein isolate
[b]Means followed by different letters are significantly different ($p < 0.05$) according to Duncan post-hoc test; means of three replicates ± standard deviation

Table 3 Amino acid profile (g/100 g protein) of African wild lettuce leaves

Amino acid	WLF	WLR	WLE	WPI	Adult	Children
Methionine	1.53 ± 0.01^{d}	1.89 ± 0.23b	1.65 ± 0.01^{c}	12.88 ± 1.02^{a}	1.5	2.7
Valine	6.96 ± 0.21^{a}	6.45 ± 0.22^{b}	6.93 ± 0.33^{a}	6.07 ± 0.21^{c}	2.6	3.8
Phenylalanine	5.38 ± 0.13^{c}	5.57 ± 0.02^{b}	4.89 ± 0.14^{d}	5.79 ± 0.33^{a}	2.5	6.9
Isoleucine	4.53 ± 0.01^{c}	4.87 ± 0.41^{b}	4.87 ± 0.11^{b}	5.29 ± 0.03^{a}	2.5	6.9
Leucine	5.93 ± 0.02^{a}	7.68 ± 0.05^{a}	6.53 ± 0.02^{a}	7.85 ± 0.11^{a}	–	1.0
Threonine	4.74 ± 0.22^{b}	3.94 ± 0.03^{d}	4.86 ± 0.05^{a}	4.17 ± 0.03^{c}	3.9	7.3
Histidine	1.97 ± 0.00^{b}	1.6 ± 0.11^{c}	5.69 ± 0.13^{a}	1.46 ± 0.00^{d}	–	1.0
Tryptophan	6.11 ± 1.11^{a}	6.09 ± 0.42^{a}	4.16 ± 0.01^{c}	5.60 ± 0.01^{b}	1.5	3.7
Lysine	6.45 ± 0.31^{b}	3.34 ± 0.02^{c}	7.06 ± 0.00^{a}	3.0 ± 0.02^{d}	0.4	1.25
ΣEAAs	41.63 ± 4.12^{b}	39.83 ± 2.02^{d}	40.95 ± 5.02^{c}	50.65 ± 3.22^{a}	3.4	6.4
Non-Essential amino Acids						
Glycine	6.48 ± 0.22^{a}	6.14 ± 0.03^{b}	5.77 ± 0.11^{c}	4.25 ± 0.12^{d}	–	–
Alanine	6.70 ± 0.00^{b}	4.71 ± 0.22^{d}	5.78 ± 0.02^{c}	6.74 ± 0.13^{a}	–	–
Serine	4.48 ± 0.04^{c}	5.33 ± 0.00^{a}	4.29 ± 0.03^{d}	4.95 ± 0.02^{b}	–	–
Proline	6.40 ± 0.01^{b}	6.09 ± 0.21^{c}	6.72 ± 0.33^{a}	5.22 ± 0.00^{d}	–	–
Arginine	6.33 ± 0.032^{b}	5.27 ± 0.02^{d}	6.62 ± 0.04^{a}	5.86 ± 0.03^{c}	–	–
Tyrosine	1.97 ± 0.04^{c}	4.88 ± 0.03^{b}	1.39 ± 0.01^{d}	5.29 ± 0.21^{a}	–	–
Cystine	1.36 ± 0.00^{b}	1.39 ± 0.22^{b}	1.04 ± 0.01^{c}	1.67 ± 0.22^{a}	–	–
Aspartate	9.09 ± 0.11^{b}	6.99 ± 0.42^{c}	9.54 ± 0.03^{a}	6.24 ± 0.04^{d}	–	–
Glutamate	12.65 ± 0.32^{c}	13.08 ± 0.05^{a}	12.94 ± 1.02^{b}	2.15 ± 0.01^{d}	–	–
ΣNEAAs	57.43 ± 4.42^{b}	58.76 ± 2.03^{a}	55.48 ± 4.02^{c}	47.66 ± 3.03^{d}	–	–
Protein Quality Indices						
TAA	99.06 ± 6.02^{a}	98.59 ± 3.02^{b}	96.43 ± 4.02^{c}	98.31 ± 2.02^{b}		
TEAA/TNEAA	0.72 ± 0.00^{b}	0.68 ± 0.22^{c}	0.73 ± 0.12^{b}	1.06 ± 0.02^{a}	–	
ΣSAA (meth+cys)	2.89 ± 0.01^{c}	3.28 ± 0.11^{b}	2.69 ± 0.02^{d}	14.55 ± 0.12^{a}	–	
ΣArAA (phy + tyr)	7.35 ± 0.23^{c}	10.45 ± 2.11^{b}	6.28 ± 0.32^{d}	11.08 ± 0.52^{a}	–	
Arg/Lys	0.98 ± 0.02^{c}	1.58 ± 0.03^{b}	0.94 ± 0.11^{d}	1.95 ± 0.02^{a}	–	
BCCAs	17.42 ± 3.02^{c}	19.00 ± 1.22^{a}	18.33 ± 2.02^{b}	19.21 ± 2.02^{a}	–	
BV (%)	77.04	72.26	85.75	88.97	70	
PER (g/100 g)	3.00	2.79	3.21	3.51	–	
EAAI (%)	81.41	77.03	89.40	92.36	–	

[a]*WLF* Wild lettuce powder, *WLE* Wild lettuce extracts, *WLR* Wild lettuce residue, *WPI* Wild lettuce protein isolate
[b]Means followed by different letters are significantly different ($p < 0.05$) according to Duncan post-hoc test; means of three replicates ± standard deviation
Recommended daily allowance (RDA) of essential amino acids ((mg/100 g b.w)) for Adult and Children (< 5 yrs.) [39]. Total essential amino acids (ΣEAA), Total non-essential amino acids (ΣNEAA), Total sulphur amino acid (ΣSAA), Total Aromatic amino acids (ΣArAA), Limiting amino acid (LAA), Abundant amino acid (AAA), Branched chain amino acids (ΣBCAAs = Leu., Isoleu., Valine)

based foods. The African wild lettuce leafy vegetable that is being considered in this present study contain appreciable amount of protein, minerals and fibers. The protein content in this leafy vegetable was comparable with or higher than what reported for water Spinach, *Momordica foetida* leaf, and balsam apple leaf [40]. However, the crude protein of wild lettuce leaf samples, expect for WPI, was found to be lower when compared to 27.51% in *moringa olefera* leaf [41], 29.78% *Piper guineeses* and 31.0% in *Talinum triangulare* [42]. This implies that regular intake of wild lettuce leafy vegetable may provide between 41 and 95% of recommended dietary allowance (RDA) of protein for both children and adults. Hence, preventing cases of protein deficiency among vulnerable children. The fiber content in wild lettuce leaf samples, particularly WLF & WLR, was comparatively similar to what obtained for *Moringa oleifera* leaf (19.25%) [43] and balsam apple leaf (29.00%) [40]. Scientific studies have reported that regular consumption leafy vegetable provides fibres, which are highly beneficial in terms of aiding digestion, reducing serum cholesterol level, and also preventing risks of coronary heart diseases and

Table 4 Phytochemical compositions ($mg{\cdot}g^{-1}$) of African wild lettuce leaf samples

Sample	WLF	WLE	WLR	WPI
Saponin	199.09 ± 1.29^{a}	68.18 ± 1.29^{c}	150.00 ± 1.29^{b}	59.09 ± 1.29^{d}
Tannin	57.37 ± 4.27^{a}	5.43 ± 0.85^{c}	27.17 ± 0.00^{b}	4.23 ± 0.85^{c}
Oxalate	24.45 ± 0.19^{d}	11.03 ± 0.32^{b}	20.84 ± 0.91^{c}	7.20 ± 0.11^{a}
Flavonoid	4.48 ± 0.19^{b}	0.42 ± 0.00^{d}	2.77 ± 0.32^{c}	13.43 ± 0.19^{a}
Phytate	19.86 ± 0.19^{a}	14.914 ± 0.12^{b}	15.74 ± 0.12^{b}	11.95 ± 0.58^{c}
Terpenoids	70.21 ± 1.13^{a}	58.38 ± 0.56^{b}	67.15 ± 0.94^{a}	51.86 ± 1.88^{c}
Phenol	19.47 ± 0.04^{c}	27.08 ± 0.04^{b}	15.33 ± 0.08^{d}	28.89 ± 0.04^{a}
Phytate/mineral (Ca, Zn and Fe) molar ratios				
Phytate/calcium	0.75	0.15	0.15	0.59
Phytate/zinc	34.93	17.49	27.68	16.35
Phytate/iron	3.59	2.48	2.11	2.04
Phytate/Ca & Zn	1.40	2.69	4.37	0.50

[a]*WLF* Wild lettuce powder, *WLE* Wild lettuce extracts, *WLR* Wild lettuce residue, *WPI* Wild lettuce protein isolate
[b]Means followed by different letters are significantly different ($p < 0.05$) according to Duncan post-hoc test; means of three replicates ± standard deviation

hypertension [43, 44]. Total ash content of wild lettuce leafy vegetables in this present study was high, which indicate that the leaf is a good source of inorganic elements. Comparatively, the value of ash content in the leaf was higher than which reported for sweet potato leaf (1.8%) [45], *Moringa oleifera* (7.13%) [41], *Tribulus terrestris* leaf (5%), and water spinach leaf (10.83%) [46].

Total amino acid profile and essential amino acids of African wild lettuce are comparable to or higher than other indigenous leafy vegetables reported by other researchers [47–49]. This implies that regular consumption of wild lettuce leafy vegetable may provide essential amino acids require for physiological needs for both children and adults. Nutritionally, the percentage ratio of TEAA to TAA in the wild lettuce leaves sample were all above the values considered to be adequate for ideal protein for infants (39 g/d), children (26 g/d) and that of adults (11 g/d) [39]. Hence, the essential amino acids in wild lettuce leafy vegetable may be adequate to promote growth and development in children and body maintenance in adults. In addition, studies have reported that plasma concentrations of branched chain amino acids like valine, leucine and isoleucine, which are present in abundant in wild lettuce leafy vegetables, and are essential in blood glucose regulation [50–52]. Several studies have reported that branched chain amino acids are essential in management of type - 2 diabetes [50–52], hence, African wild lettuce leaf protein isolate sample (WPI) may be suitable as antidiabetic agent. The nutritional quality of wild lettuce leafy vegetables in terms of protein efficiency ratio, essential amino acid index and biological values are considerably high for an idea vegetable-based food samples. This observation further established that regular consumption of this vegetable may provide essential amino acids require for physiological and biochemical activities in children and adults. The ratio of arginine to lysine in wild lettuce leaf is considerably high, and this indicates that arginine concentration is high in the leaf; and this may be of health benefits, particularly in the management of high blood pressure. Scientific research had established that arginine plays important roles in the production of nitric oxide in the body, which helps in the relaxation of the arteries to enhance easy flow of blood, and thereby reduce risks of high blood pressure [53].

Table 5 Antioxidant activities of African wild lettuce leaf samples

Sample	WLF	WLE	WLR	WPI
Fe Chelation%	8.26 ± 0.09^{c}	34.43 ± 0.09^{b}	5.84 ± 0.09^{c}	36.64 ± 0.19^{a}
DPPH%	18.28 ± 0.04^{d}	70.85 ± 0.04^{b}	53.07 ± 0.04^{c}	91.88 ± 0.04^{a}
ABTS mg/g	205.36 ± 12.63^{b}	339.29 ± 25.21^{a}	169.64 ± 12.62^{b}	383.92 ± 12.62^{a}
FRAPS mg/g	47.46 ± 0.021^{c}	49.92 ± 0.04^{b}	44.17 ± 0.02^{d}	50.33 ± 0.02^{a}
OH %	74.00 ± 7.68^{ab}	60.00 ± 6.46^{b}	74.57 ± 7.67^{ab}	80.29 ± 2.82^{a}

[a]*WLF* Wild lettuce powder, *WLE* Wild lettuce extracts, *WLR* Wild lettuce residue, *WPI* Wild lettuce protein isolate
[b]Means followed by different letters are significantly different ($p < 0.05$) according to Duncan post-hoc test; means of three replicates ± standard deviation

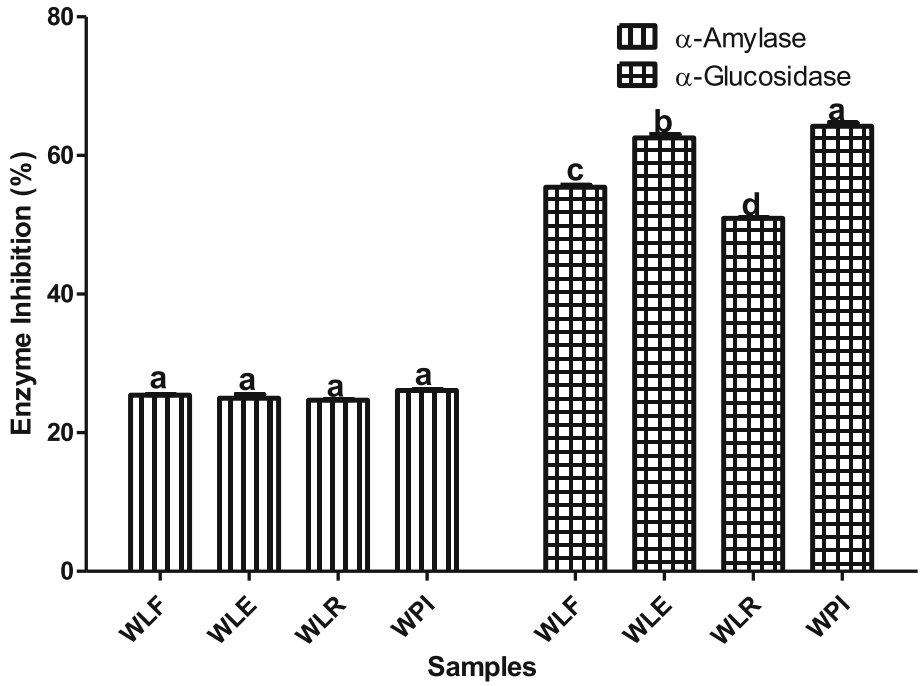

Fig. 1 α-amylase and α-glucosidase enzyme inhibitory activities of African wild lettuce leaf samples. Key: WLF; Whole wild lettuce powder, WLE; Wild lettuce extracts, WLR; Wild lettuce Residue, WPI; Wild lettuce protein isolate, Captopril (Antihypertensive synthetic agent)

The inorganic minerals like magnesium, sodium, potassium, calcium, phosphorus, manganese, iron, zinc and copper in wild lettuce leaf samples were present in appreciable amount. However, phosphorous was found to be in most abundant of all the elements that were determined, while copper (Cu) was observed to be lowest. The essential minerals like iron, zinc, magnesium, calcium and phosphorous are present in appreciable amount in wild lettuce leafy vegetable, and it is evident that these minerals play important roles such as carbohydrate metabolisms, electrolytes, heamoglobin, bone and teeth formation. For instance, studies have reported

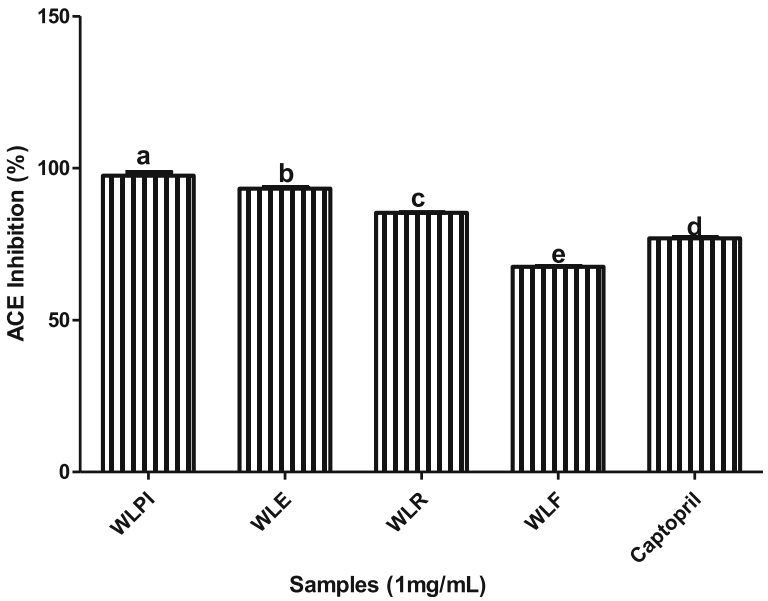

Fig. 2 Angiotensin converting enzyme inhibitory activities of wild lettuce powder. Key: WLF; Whole wild lettuce powder, WLE; Wild lettuce extracts, WLR; Wild lettuce Residue, WPI; Wild lettuce protein isolate, Captopril (Antihypertensive synthetic agent)

that zinc, magnesium and calcium play important roles in the management of diabetes by serving as cofactors or components for enzyme involved in glucose metabolism, and thereby enhancing insulin action by activation of insulin receptor [54]. The Na/K molar ratio of wild lettuce leafy vegetable samples was lower than one (< 1.0) and this implies that this leafy vegetable contains appreciable amount of potassium compared to that of sodium. It has been scientifically established that foods with low Na/K molar ratios (< 1) are highly beneficial, particularly for hypertensive patients. Hence, regular consumption of wild lettuce leafy vegetable may be suitable to provide daily requirements of potassium for an adult that may be useful in the prevention and management of cardiovascular diseases [55]. The Ca/P molar ratio of wild lettuce samples was lower than recommended value (> 1.0). This indicates that this leafy vegetable was low in calcium when compared with other edible leafy vegetables like *Momordica balsamina* L. leaf (941 mg/100 g) [40], *Cassia siamea* leaf (17.95 mg/100 g) [56] and *Mucnuna flagellipes* (313.30 mg/100 g) [57]. Calcium is needed for growth and maintenance of bone, teeth and muscles in children and adults; thus, regular consumption of wild lettuce leafy vegetable may still provide a meaningful amount of dietary calcium needed for these purpose in man.

The phytochemicals in wild lettuce leaf are low and present within the tolerable level. The study established that flavonoids and polyphenols are present in abundant in the leaf, and these phytochemicals are of nutritional and health benefits. Scientific studies have established that dietary polyphenols have properties to inhibit carbohydrate digestion, glucose absorption in the intestine, and stimulate insulin secretion from the pancreatic β-cells [58–60]. Besides, evident has also established that polyphenol prevent formation of atherosclerotic plaques, and thereby reducing arterial stiffness and enhancing vasodilation of the arteries to ease blood flow [61]. The phytate/mineral molar ratios (an interrelationship between the phytate and bioavailability of minerals like calcium, zinc and iron) of the wild lettuce leaf samples are comparatively lower than the critical values [62–65]. Hence, the inhibitory effects of phytate on minerals (zinc, calcium and iron) in wild lettuce leafy vegetable are minimized. Ratios greater than the critical values have been associated with biochemical and/ or clinical evidence of minerals deficiencies [66]. Nutritionally, zinc and calcium are essential elements in human nutrition particularly in glucose metabolism by enhancing insulin production; hence, its bioavailability plays important roles in diabetes patients [54, 67, 68].

Antioxidant activities of African wild lettuce powder samples

The ability of wild lettuce leaf protein isolates to scavenge free radical against 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), OH free radical scavenging and Fe^{2+}chelation exhibited higher antioxidant and free radical scavenging activities when compared with other experimental samples, that is, whole leaf, extract and residue powder samples. This observation may be attributed to the potency of free bioactive peptides in the isolated protein to acts as antioxidant and scavenge free radicals. Comparatively, the antioxidant and free radical scavenging ability of wild lettuce leaf agreed with other study that reported on the antioxidant activities of leafy vegetables and their health benefits [69]. It is well established that antioxidant, that is, chemical substance that inhibit oxidation process by preventing the formation of free radicals play major roles in preventing chronic diseases like hypertension, diabetes, obesity and cancers [70–72].

α-amylase and α-glucosidase enzyme inhibitory potential of wild lettuce leaf samples

The wild lettuce leaf protein isolates sample exhibited higher α-amylase and α-glucosidase enzyme inhibitory activities when compared with other experimental samples (WLF, WLE & WLR) and Acarbose (a synthetic antidiabetic agent). The inhibitory activity of wild lettuce leafy vegetable on α-amylase and α-glucosidase enzymes observed in this study was comparable to or higher than which reported for *Cyperus esculentus* [73], *Adiantum caudatumand*, *Celosis argentea* [74] and *Cissus cornifolia* [75]. It is well established that enzyme inhibitory agents usually block the activities of both α-amylase and α-glucosidase enzymes in the small intestine, and thereby limits digestion of oligosaccharides and disaccharides to monosaccharides, necessary for gastrointestinal absorption [76]. Hence, postprandial blood glucose peaks may be attenuated by delayed glucose absorption. The main benefits attributable to α-amylase and α-glucosidase inhibitors are reductions in both postprandial glycaemic levels and postprandial blood glucose levels [77].

Angiotensin converting enzyme inhibition wild lettuce powder samples

The angiotensin converting enzyme (ACE) inhibitory potential of African wild lettuce leafy vegetable samples (whole leave powder, extract, residue and protein isolates) (Fig. 2) had activities to inhibit angiotensin-1 converting enzyme. However, protein isolate sample (WPI) had higher percentage of inhibition against angiotensin-1 converting enzyme when compared to leaf extract (WLE), leaf residues (WLR), whole leaf powder (WLF) samples and control sample (Captopril, a synthetic antihypertensive agent). The high percentage of enzymes inhibition observed for WPI and WLE samples could be attributed to free amino acid composition of the protein

isolate and phytochemicals such as saponin, flavonoid etc. in the extract samples. This finding was in line with the reports of Aluko [78], who reported that bioactive proteins exhibited strong inhibitory activities on ACE, which is responsible for the conversion of angiotensin I into angiotensin II. Also, studies have shown that bioactive phytochemicals such as flavonoid and saponin have inhibitory activities on angiotensin converting enzyme, hence, usually used as antihypertensive agents [79, 80].

Conclusion

The present study established African wild lettuce leafy vegetable contain appreciable amount of essential nutrients (protein and minerals), and that the bioactive compound in the leafy vegetable, i.e., bioactive protein and phytochemicals, exhibited high antioxidant activities and ability to inhibit activities of α-amylase, α-glucosidase and angiotensin-1-converting enzymes. Hence, regular consumption of wild lettuce leafy vegetables may be beneficial in preventing and managing oxidative stress and degenerative diseases like diabetes and hypertension.

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Authors' contributions

IJAROTIMI Oluwole Steve designed the research; while implementation of the research, data analyses was done by ADESANYA, Ibiyinka Helen. Manuscript preparation were done by and OLUWAJUYITAN Timilehin David and IJAROTIMI Oluwole Steve. The author(s) read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declared that there were no conflicts of interest for the study.

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