Food Sci. Biotechnol. 25(5): 1243-1249 (2016)
DOI 10.1007/s10068-016-0197-1

Food Science and Biotechnology

Polyphenolic compositions and *in vitro* angiotensin-I-converting enzyme inhibitory properties of common green leafy vegetables: A comparative study

Ganiyu Oboh[1,*], Ayodele Jacob Akinyemi[1,2], Blessing Adeleye[1], Sunday Idowu Oyeleye[1,4], Opeyemi Babatunde Ogunsuyi[1,4], Ayokunle Olubode Ademosun[1], Adedayo Oluwaseun Ademiluyi[1], and Aline Augusti Boligon[3]

[1]*Functional Foods and Nutraceutical Unit, Department of Biochemistry, Federal University of Technology, Akure, P.M.B., 704, Akure 340001, Nigeria*
[2]*Department of Biochemistry, Afe Babalola University, Ado-Ekiti, Nigeria P.M.B. 5454, Nigeria*
[3]*Phytochemical Research Laboratory, Department of Industrial Pharmacy, Federal University of Santa Maria, Build 26, room 1115, Santa Maria, CEP 97105-900, Brazil*
[4]*Department of Biomedical Technology, Federal University of Technology, Akure, P.M.B., 704, Akure 340001, Nigeria*

Received January 16, 2016
Revised April 19, 2016
Accepted June 30, 2016
Published online October 31, 2016

*Corresponding Author
Tel/Fax: +2348035123539
E-mail: goboh2001@yahoo.com

pISSN 1226-7708
eISSN 2092-6456

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Abstract This study compared the phenolic compositions of common green leafy vegetable extracts from *Vernonia amygdalina* (VA), *Telfairia occidentalis* (TO), *Talinium triangulare* (TT), and *Amaranthus hybridus* (AH) and their effects on the angiotensin-I-converting enzyme (ACE) and cisplatin-induced malonylaldehyde (MDA) production in an isolated rat kidney homogenate. HPLC confirmed the presence of phenolic compounds in the extracts. Furthermore, all extracts inhibited ACE activity dose-dependently; however, the extract from VA exhibited the highest ACE activity while TT exhibited the least. Incubation of the kidney homogenate with 1 mM cisplatin caused an increase in MDA production; however, all the extracts inhibited the level of MDA produced. Nevertheless, VA extract exhibited the highest inhibition. These activities of the vegetable extracts could be attributed to their phenolic compositions and may suggest some possible mechanism of the actions. However, VA appeared to be the most potent among the vegetables tested.

Keywords: vegetables, polyphenolic, angiotensin-I-converting enzyme, oxidative stress, renal damage

Introduction

The renin-angiotensin system is a key factor in the pathophysiology of hypertension and kidney dysfunction (1,2). Renin catalyzes the conversion of angiotensinogen to active angiotensin-I, which could be converted to angiotensin-II by the action of angiotensin-I-converting enzyme (ACE). Angiotensin-II is a potent vasoconstrictor and its production deactivates bradykinin (vasodilator), which plays a vital role in hypertension and kidney diseases. Hence, the regulation of angiotensin-II formation could be of great physiological relevance in the management of hypertension and oxidative stress-induced renal injury. Therefore, reduction in the level of angiotensin-II production via inhibition of ACE activity could be an effective strategy for the prevention and management of hypertension and kidney damage (1,2). Although several ACE inhibitors such as captopril, enalapril, lisinopril, and temocapril are already in use, reports have shown that they also inflict several side effects. Consequently, bioactive compounds such as phenolic acids and flavonoids from plant materials, including fruits and vegetables, have been reported to act as excellent ACE inhibitors (1,2).

Chronic kidney disease (CKD), a condition in which the kidneys are damaged, has been reported to be one of the leading health challenges among the adult population throughout the world because of increasing incidences of hypertension (3,4). Furthermore, CKD can be induced by oxidative stress because of the continuous excessive production of free radicals such as reactive oxygen and nitrogen species in the body either during normal cellular metabolism and/or because of exposure to environmental factors such as tobacco smoke, alcohol, radiation, and drugs (5). These radicals or their metabolites are capable of inducing oxidative stress and damage to biomolecules such as lipids, proteins, and DNA. This could lead to the development and progression of several chronic diseases, including CKD and other oxidative stress-induced metabolic disorders such as hypertension (1,2). Thus, combating oxidative stress in the body could be one of the therapeutic ways of ensuring holistic treatment and management of hypertension and CKD.

Cisplatin (CP), an antineoplastic drug, is used in the treatment of various forms of cancer. Though effective, it also induces some side effects such as kidney dysfunction via inhibition of antioxidant enzyme activities and initiation of lipid peroxidation. This could result in oxidative stress in the renal tissue (1,6). These deleterious effects may be linked to the fact that kidneys, which are the major route of excretion, can bio-accumulate CP to a greater degree, which could induce CKD. Nowadays, the need for new therapeutic modalities for CKD treatment and oxidative stress management has become a major research focus. Hence, inhibition of ACE has been suggested to be a good therapeutic approach for the management and prevention of CKD, and dietary phytochemicals have promising potential in similar applications (1,7).

From time immemorial, green leafy vegetables have been utilized either for culinary purposes or as spices in human diets. Also, the use of their infusion or extracts to treat or manage several human ailments in traditional medicine is a major practice in folklore (8). Interestingly, a report has logically linked the nutraceutical values of consuming vegetable-rich foods and/or extracts for the management of several human diseases to their antioxidative properties and constituent phytochemicals, such as vitamins C, α-tocopherol, β-carotene, and polyphenols (9).

In Nigeria, green leafy vegetables form a major part of the local dishes. Notable among them are *Vernonia amygdalina* (VA), *Telfaria occidentalis* (TO), *Talinium triangulare* (TT), and *Amaranthus hybridus* (AH). VA, which is generally known as bitter leaf because of its bitterness. These vegetables are cultivated majorly for consumption and various pharmacological purposes (10). TO is another vegetable that is very desirable not only for its nutritive value but also for its medicinal properties. According to Oboh *et al.* (11) and Akang *et al.* (12), leaves are rich in vitamins, minerals, protein, fiber, and phytochemicals such as polyphenols. The pharmacological properties of leaves include anxiolytic, sedative, and antidiabetic (10-13). A study reported that according to folklore, *TT* can increase stamina and prevent hepatic ailments and cancer. It also exhibits immuno-stimulatory and immunomodulatory properties (14). Studies on its pharmacological, pharmacognostic, and phytochemical components have also been reported. AH contains polyphenols, steroids, terpenoids, saponins, betalains, and a large amount of squalene (15). These vegetables are commonly consumed in most rural areas and villages across Nigeria mainly because of their cheapness, accessibility, and palatability. According to the report of Oboh and Rocha (9), consumption of these green leafy vegetables could alleviate the risk of CKD; however, their efficacy in managing/treating this ailment could be different. On the basis of the abovementioned findings, this study aims to determine and compare the phenolic constituents and biological properties of common green leafy vegetables extracts from VA Del. (bitter leaf), TO Hook f. (Ugwu leaf), TT (water leaf), and AH (African spinach) via their effects on ACE activity and cisplatin-induced lipid peroxidation in an isolated rat kidney homogenate.

Materials and Methods

Chemicals and reagents A rabbit's lung ACE (EC 3.4.15.1) and the substrate [hippuryl-L-histidyl-L-leucine (HHL)] were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cisplatin was purchased from Korea United Pharm. Inc. (Seoul, Korea). All other chemicals and reagents were of analytical grade, and glass-distilled water was used. HPLC with diode array detection (HPLC-DAD) was performed via a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan) equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, a SPD-M20A diode array detector, and the LC solution 1.22 SP1 software. A Jenway ultraviolet-visible (UV-Vis) spectro-photometer (Model 6305; Bibby Scientific Limited, Stone, UK) was used to measure the absorbance.

Sample collection and preparation of extracts Green leafy vegetables, namely VA, TO, TT, and AH were freshly harvested from a local farmland at Ilara-Mokin, Akure metropolis, Nigeria. Authentication of the vegetables was conducted by A. A. Sorungbe, Department of Biology, Federal University of Technology, Akure, Nigeria, and voucher numbers FUTA/BIO/201, FUTA/BIO/202, FUTA/BIO/203, and FUTA/BIO/204 were assigned to VA, TO, TT, and AH, respectively. The leaves were separated from the stems, washed twice with distilled water, air-dried, and pulverized using a laboratory blender. Each powdered sample (10 g) was extracted with 100 mL of distilled water for 16 h in an orbital shaker. Thereafter, the homogenate was filtered using a muslin cloth and further centrifuged at 360x *g* for 10 min to obtain a clear supernatant, which was freeze-dried into powder using a freeze-drier. Each powdered sample (1 g) of dried extract was redissolved in 100 mL of distilled water and stored at 4°C for subsequent analysis. The freeze-dried extracts were used for the HPLC-DAD analysis.

ACE Inhibition assay The ability of the extracts to inhibit ACE activity was assayed using the spectrophotometric method described by Cushman and Cheung (16). Different concentrations of the extract (0-100 μL) and 50 μL ACE (EC 3.4.15.1) solution (4 mU/mL) were incubated at 37°C for 15 min. Thereafter, 150 μL of 8.33 mM hippuryl-L-histidyl-L-leucine (HHL) in a 125 mM Tris-HCl buffer (pH 8.3) was added to the reaction mixture and incubated at 37°C for 30 min. The reaction was stopped by adding 250 μL of 1 M HCl. The hippuric acid produced was extracted using 1.5 mL ethyl acetate. Then, the extracted acid was centrifuged. The ethyl acetate layer (1 mL) was transferred to a clean tube and evaporated to dryness. The residue was reconstituted with 1 mL distilled water, and the absorbance was measured at 228 nm. The ACE inhibitory activity of the extract was calculated and expressed as follows:

$$\% \text{ Inhibition}=[(Abs_{control}-Abs_{samples})/Abs_{control}]\times 100 \quad (1)$$

where $Abs_{control}$ is the absorbance without the extract and $Abs_{samples}$

is the absorbance with extract.

Handling of the experimental animals Ten adult male Wistar strain albino rats weighing 200-210 g were purchased from the animal breeding colony of the Animal Production and Health Department, Federal University of Technology, Akure. They were handled in accordance with the outlined criteria, i.e., the Guide for the Care and Use of Laboratory Animals, prepared by the National Academy of Science, published by the National Institute of Health, United State of America (17). The handling and use of animals for this study was approved by the institution's ethical committee with reference no.: FUTA/SOS/1413. The rats were kept for 2 weeks to acclimatize, during which they were maintained at room temperature on a 12 h light/12 h dark cycle with access to standard animal feed and water ad libitum before the commencement of the work. All institutional and national guidelines for the care and use of laboratory animals were followed.

Lipid peroxidation and thiobarbituric acid reactions assay CP-induced lipid peroxidation in an isolated rat kidney homogenate was conducted according to the method of Ohkawa *et al.* (18). The mixture containing 100 μL of the kidney homogenate was mixed with the mixture containing 30 μL of 0.1 M Tris-HCl buffer (pH 7.4), 0-300 μL of extract, 30 μL of 1 mM CP solution, and 300 μL of distilled water. The mixture was incubated at 37°C for 1 h and allowed to cool. Thereafter, 300 μL of 8.1% sodium dodecyl sulfate (SDS) and 500 μL of acetic acid/HCl (pH 3.4) solution were added. Furthermore, 500 μL of 0.8% thiobarbituric acid (TBA) was added, and the mixture was boiled for 1 h. The absorbance of thiobarbituric acid reactive species (TBARS) was measured at 532 nm and calculated using malondialdehyde as the control.

Determination of hydroxyl radical scavenging ability The method of Halliwell and Gutteridge (19) was used to determine the ability of the extracts to scavenge hydroxyl radicals produced from CP/H_2O_2-induced deoxyribose. The extract (0-100 μL) was added to a mixture of 100 μL of 20 mM deoxyribose, 400 μL of 0.1 M phosphate buffer, 40 μL of 20 mM H_2O_2, 40 μL of 1 mM CP solution and 800 μL of distilled water. The reaction mixture was incubated at 37°C for 30 min and was then stopped by adding 500 μL of 2.8% trichloroacetic acid (TCA). Thereafter, 400 μL of 0.8% TBA solution was added and subsequently incubated in boiling water for 20 min. The absorbance was measure at 532 nm and the hydroxyl (OH) radical scavenging ability was subsequently calculated using Eq. (1).

Determination of free-radical scavenging ability The hydrogen atom/electron donating ability of the extracts was computed from the bleaching property of the purple methanol solution of DPPH. The stable free-radical scavenging ability was evaluated as described by Gyamfi *et al.* (20). Appropriately diluted extracts (1 mL) were mixed with 1 mL of 0.4 mM methanolic solution containing a DPPH radical. The mixture was left in the dark for 30 min, and the absorbance was measured at 516 nm. The DPPH radical scavenging ability was subsequently calculated using Eq. (1).

Determination of ferric reducing antioxidant power In brief, 500 μL of the extract was added to a mixture of 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6), and 2,500 μL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, followed by the addition of 2.5 mL of 10% TCA. The mixture was centrifuged at 4,000× *g* for 10 min. Thereafter, 5 mL of the supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride (21). The absorbance was measured at 700 nm, and the ferric reducing antioxidant property (FRAP) was subsequently calculated as the ascorbic acid equivalent (AAE) using ascorbic acid as the control.

Determination of vitamin content Vitamin C content of the extract was determined using the method of Benderitter *et al.* (22). The absorbance was measured at 520 nm, and the vitamin C content was subsequently calculated and presented as the ascorbic acid equivalent.

Determination of total phenol and total flavonoid contents The total phenol content of the extract was determined using the method reported by Singleton *et al.* (23), and the total phenolic content was subsequently calculated as the gallic acid equivalent (GAE) using gallic acid as the standard. The total flavonoid content of the extract was determined using the method used by Meda *et al.* (24) and calculated using quercetin as the standard.

Quantification of phenolic compounds via high-performance liquid chromatography-diode-array Detector (HPLC-DAD) The quantification of the phenolic compounds in the extracts was conducted according to the method described by Adedayo *et al.* (25) using HPLC–DAD. The quantifications were conducted by integrating the peaks using the external standard method at 254 nm for gallic and ellagic acids; at 280 nm for catechin; at 327 nm for caffeic acid, chlorogenic acid, and *p*-coumaric acid; and at 366 nm for Vitexin, orientin, quercetin, quercitrin, apigenin, rutin, and luteolin. The chromatographic peaks were confirmed by comparing retention time of the sample's phenolic compounds with those of the reference standards via DAD spectra (200-600 nm). All chromatographic operations were conducted at ambient temperature and in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated on the basis of the standard deviation of the responses and the slope using three independent analytical curves, as defined by Adedayo *et al.* (25).

Data analysis The results of the three experiments were pooled and expressed as mean±standard deviation (SD). One-way analysis of variance (ANOVA) was used to analyze the mean, and the post hoc treatment was performed using the Duncan multiple test. Significance was accepted at $p \leq 0.05$. IC_{50} (extract concentration causing 50%

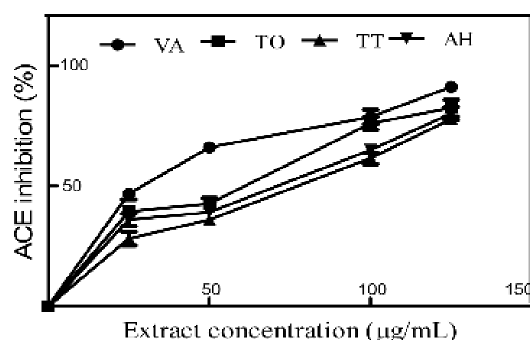

Fig. 1. Percentage inhibition of ACE activity by the extracts. VA, TO, TT, and AH denote *Vernonia amygdalina*, *Telfairia occidentalis*, *Talinium triangulare*, and *Amaranthus hybridua*, respectively.

Table 1. IC_{50} values (μg/mL) of angiotensin-1 converting enzyme (ACE) activity inhibitory, hydroxyl (OH) and DPPH radical scavenging abilities of the aqueous extracts of *Vernonia amygdalina* (VA), *Telfairia occidentalis* (TO), *Talinium triangulare* (TT) and *Amaranthus hybridus* (AH) vegetables

Vegetable extract	ACE activity	OH radical scavenging activity	DPPH radical scavenging activity
VA	28.46±1.6[1)a2)]	45.35±1.1[a]	29.58±1.3[a]
TO	44.59±1.4[b]	57.06±1.4[b]	37.72±1.4[b]
TT	63.13±1.2[d]	76.64±1.9[c]	54.91±0.19[c]
AH	53.44±1.1[c]	58.01±2.0[b]	36.38±2.0[b]

[1)]Values represent mean±standard deviation of triplicate readings.
[2)]Values with the same superscript letter along the same column are not significantly different (p>0.05)

antioxidant activity) was determined via the nonlinear regression analysis using the GraphPad Prism version 5.00 for Windows (GraphPad Software, Inc., La Jolla, CA, USA). The Pearson correlation coefficients (r) were calculated using the statistical package for social science (SPSS) version 21.0 (International Business Machines (IBM) Corporation, Armonk, New York, USA). The number of samples treated was four (n=4), and all the correlations were significant at the 0.01 level (two-tailed).

Results and Discussion

Dose-dependent (0-125 μg/mL) inhibition of ACE activity exhibited by the extracts is presented in Fig. 1, and their IC_{50} values are listed in Table 1 (Lower IC_{50} values mean stronger enzyme or radical inhibition). The extract from VA (IC_{50}=28.46 μg/mL) exhibited the highest inhibitory ability, followed by the extract from TO (IC_{50}=44.59 μg/mL). The extract from TT (IC_{50}=63.13 μg/mL) exhibited the least ACE inhibitory activity. ACE inhibitors have been the preferred agents for providing protection against the progression of kidney diseases (2). In this study, the extracts of the studied vegetables dose-dependently inhibited ACE activity. The ability of the aqueous extracts from the studied vegetables to inhibit ACE activity could indicate their antihypertensive and renoprotective potentials and could also be the reason behind their use in folklore for the management of hypertension and renal diseases. Furthermore, the inhibition of ACE activity by extracts could be linked to the synergistic effects of the present characterized phenolic compounds such as caffeic acid, gallic acid, quercetin, and rutin. Phenolic compounds such as rutin, apigenin, luteolin quercetin, gallic, and caffeic acids have been reported to inhibit ACE activity (2). This could also be buttressed by the reports of Oboh *et al*. (1) and Ademiluyi *et al.* (2) that phenolic-rich extracts from natural products inhibit ACE activity. However, the highest ACE inhibitory ability achieved by the extract from VA could be attributed to its highest total flavonoid content (Table 2). This is consistent with the report of Guerrero *et al.* (26), which states that flavonoids and/or flavonoid-rich extracts exhibit higher ACE inhibitory activity, a factor that could be linked to the interaction with the ACE active site.

The strong correlation (Table 3) between the phenolic contents and the ACE inhibitory properties of the extracts suggests that these phyto-constituents are largely responsible for the observed enzyme inhibitory effects. Several reports have established the link between the inhibition of ACE activity and the management/treatment of renal injury (1,2). ACE activity leads to the catalytic production of angiotensin-II, a vasoconstrictor capable of causing pressure-induced renal injury by inducing systemic and glomerular hypertension and/or ischemia-induced renal injury, which is secondary to intrarenal vasoconstriction and decrease of renal blood flow (1,2). Angiotensin-II is also involved in the induction of local inflammation, vascular and mesangial cell proliferation, and hypertrophy (27). Inhibition of ACE activity has been shown to reduce glomerular filtration rate via efferent arteriole dilation, thereby preventing CKD (28). Thus, the observed inhibition of ACE activity by the studied extracts could suggest a possible mechanism of action for their renoprotective potential in the prevention and management of renal dysfunction or diseases.

The antioxidant activity of the extracts was assayed using three methods: inhibition of CP-induced lipid peroxidation, OH, and DPPH radicals scavenging abilities (Fig. 2A-2C). The results show that all the extracts inhibited CP-induced lipid peroxidation in a concentration-dependent manner (75-300 μg/mL); however, the extract from VA had the highest inhibitory effect on CP-induced lipid peroxidation. All the extracts also scavenged radicals (OH and DPPH) at different concentrations, but the extract from VA had the highest OH (IC_{50}=45.35 μg/mL) and DPPH (IC_{50}=29.58 μg/mL) radical scavenging abilities, whereas that from TT had the least (OH, IC_{50}=76.64 μg/mL; DPPH, IC_{50}=54.91 μg/mL) radical scavenging abilities. The relationship between angiotensin-II and oxidative stress has been well established because the formation of angiotensin-II induces not only hypertension but also oxidative stress (1,2). The reactive species produced are capable of causing lipid peroxidation and, ultimately, oxidative

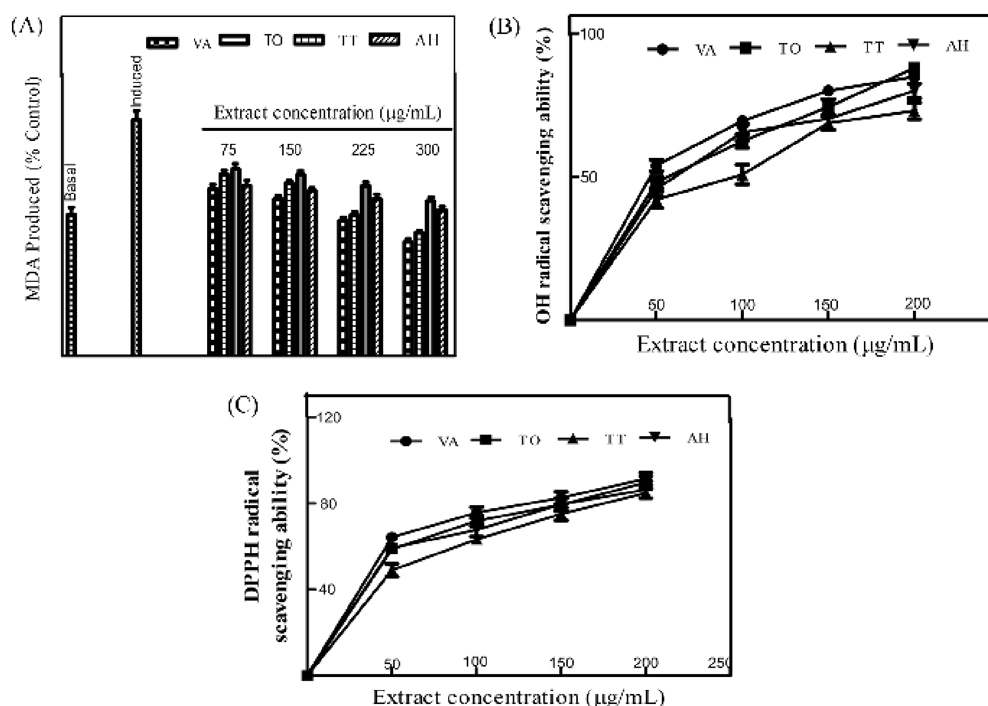

Fig. 2. (A) Inhibition of cisplatin-induced lipid peroxidation in the isolated rat kidney homogenate by the extracts; (B) Percentage hydroxyl (OH) radical scavenging ability of the extracts; (C) Percentage DPPH radical scavenging ability of the extracts. VA, TO, TT, and AH denote *Vernonia amygdalina*, *Telfairia occidentalis*, *Talinium triangulare*, and *Amaranthus hybridua*, respectively.

damage to the kidney if not checked. Furthermore, CP, which is an effective anticancer agent, has been also reported to cause kidney damage because of its ability to induce radical production, lipid peroxidation, and tissue/cellular oxidation combating enzymes in the renal tissue (6). The inhibition of CP-induced lipid peroxidation by the extracts indicates their richness in biologically active phytochemicals, which are capable of protecting the kidneys from oxidative damage and renal dysfunction (1,2,29). The protective ability of the extracts against CP-induced lipid peroxidation, as shown in this study, could be another possible mechanism in which the studied vegetables could prevent hypertension and oxidative stress-induced kidney damage arising from this drug or other toxic compounds capable of inducing kidney damage with a similar mechanism.

The ability of the extracts to scavenge hydroxyl (OH) and DPPH radicals in a dose-dependent manner could also be a mechanism behind their antioxidant properties and therefore behind the inhibition of CP-induced lipid peroxidation. Considering the Pearson correlation coefficient (Table 3), the observed free-radical scavenging ability of the extracts could be largely associated with the presence of polyphenols and vitamin C. These phytochemicals have been reported as electron or hydrogen atom donors. This is a critical factor for characterizing antioxidant activity that involves scavenging of free radicals. Antioxidant agents exhibit their protective abilities either by preventing the production and/or neutralizing free radicals produced in the body (1,2,30). The radical scavenging ability of the extracts therefore supports the usefulness of these vegetables as excellent sources of antioxidants for the prevention/management of radical-mediated hypertension and kidney damage. Interestingly, the extract from VA had the highest hydroxyl and DPPH radical scavenging ability, which may account for it being the most potent extract for preventing lipid peroxidation compared with other vegetable extracts tested. This is consistent with the report of Fasakin *et al.* (31), where polyphenolic fractions from the leaves of VA had higher radical scavenging potential compared with that of the leaves of

Table 2. Phenolics and vitamin contents, and ferric reducing antioxidant properties (FRAP) of the extracts from common leafy vegetables (unit: mg/100 g)

Samples	Total phenol (GAE)	Total flavonoid (QE)	Vitamin C (AAE)	FRAP (AAE)
VA[3)]	510.90±11.40[1)c2)]	130.39±10.09[c]	110.23±10.3[c]	64.10±3.1[c]
TO	506.40±10.56[c]	89.09±10.19[c]	108.18±10.1[c]	63.21±3.7[c]
TT	330.70±10.70[a]	55.95±10.23[a]	50.51±10.8[a]	50.20±3.3[a]
AH	460.90±10.98[b]	70.16±10.09[b]	70.01±10.9[b]	57.80±4.1[b]

[1)]Values represent mean±standard deviation of the triplicate readings.
[2)]Values with the same superscript in each column are not significantly different (p>0.05).
[3)]VA, *V. amygdalina*; TO, *T. occidentalis*; TT, *T. triangulare*; AH, *A. hybridus*

Gongronema latifolium, hence supporting the high value of VA in the management of hypertension and CKD. The FRAP values of the extracts indicate the corresponding concentration of electron-donating antioxidants. This could be a function of their constituent phenolics and vitamin C (32) deduced from the Pearson correlation coefficient. This shows a strong correlation between the FRAP of the extracts and their total phenol, total flavonoid, and vitamin C contents. The ferric reducing ability of the extracts could be beneficial in the prevention oxidative stress-induced hypertension and CKD.

Table 2 shows vitamin C and phenolics contents of the individual extracts. The vitamin C content, which was reported as the AAE, were as follows: VA=110.23 mg; TO=108.18 mg; TA=50.51 mg, and AH=70.01 mg per 100 g. VA and TO showed particularly high vitamin C content compared with the others. The result of the total phenol content of the vegetable extracts reported as the GAE were as follows VA=510.90 mg; TO=506.40 mg; TT=330.70 mg; AH=460.90 mg per 100 g. VA and TO extracts have the highest total phenol content when compared with the others. Furthermore, total flavonoid content reported as the quercetin equivalent (QE) were 130.39, 89.09, 55.95, and 70.16 mg per 100 g for VA, TO, TT, and AH extracts, respectively. Furthermore, the Pearson correlation coefficient revealed that there was a significant correlation (p=0.01) between the phytoconstituents (total phenol, total flavonoid, and vitamin C) and the ACE inhibitory as well as antioxidant properties (FRAP, DPPH, and OH free-radical scavenging abilities) of the extracts.

The HPLC-DAD identified and quantified 13 phenolic compounds (five phenolic acids and five flavonoids) in the studied vegetables (Table 4). The abundant phenolics detected in the VA extract were p-coumaric acid (9.38 mg), luteolin (6.45 mg), caffeic (5.17 mg/g), orientin (4.27 mg/g), and quercetin (4.31 mg/g). Seven phenolics were quantified in the extracts from TO and AH. Quercitrin (10.37 mg), rutin (4.65 mg), caffeic acid (3.27 mg), ellagic acid (3.18 mg), and luteolin (2.06 mg) dominated TO extract, whereas ellagic acid (10.48 mg), vitexin (3.81 mg), caffeic (3.65 mg) acid, and chlorogenic (2.73 mg) acid dominated the AH extract. Eight phenolic compounds were identified in the extract from TT, and orientin (3.91 mg), caffeic (3.04 mg) and ellagic (2.58 mg) acid, and apigenin (2.59 mg) were the dominant phenolics.

Table 3. Pearson correlation coefficients for total phenol, total flavonoid, Vitamin C contents, ACE inhibitory effects, FRAP, and free radicals (OH and DPPH) scavenging abilities of the extracts

	ACE[2)]	FRAP	OH	DPPH
TP	-(0.821)[1)]	0.926	-(0.909)	-(0.925)
TF	-(0.925)	0.849	-(0.830)	-(0.782)
VC	-(0.829)	0.960	-(0.774)	-(0.746)

[1)]All correlation coefficients are significant at p<0.01 (two-tailed).
[2)]TP, Total phenol; TF, Total flavonoid, VC, Vitamin C; ACE, Angiotensin-I-converting enzyme; FRAP, Ferric reducing antioxidant property; OH, Hydroxyl radical; DPPH, 1, 1-diphenyl-2-picrylhydrazyl

Vitamin C and phenolic compounds have protective potentials against several diseases (33) and have been considered as strong antioxidants capable of scavenging free radicals and chelate metal ions, activating antioxidant enzymes, reducing α-tocopherol radicals, and inhibiting oxidases. They also act as good nephroprotective agents because of their abilities to donate electron or hydrogen atoms (8,16,17). Reports have shown that the correlations between the antioxidant capacities of plant extracts and their phenolic constituents are statistically significant (2,34,35). Therefore, in this study, the observed highest flavonoid content and varieties of phenolic compounds found in the VA extract could be responsible for being the most potent for inhibition of ACE activity and antioxidant properties.

In this study, all the tested vegetable extracts were able to inhibit ACE activity and prevent CP-induced lipid peroxidation in a rat kidney

Table 4. Phenolic acid and flavonoid composition of leafy vegetables (mg/g dry weight sample)

Compounds	VA[3)]	TO	TT	AH
Gallic acid	0.49±0.02[1)a2)]	1.49±0.03[c]	1.65±0.02[d]	1.29±0.01[b]
Catechin	ND[4)]	ND	0.73±0.03	ND
Chlorogenic acid	ND	1.45±0.01[a]	ND	2.73±0.02[b]
Caffeic acid	5.17±0.01[d]	3.27±0.01[b]	3.04±0.01[a]	3.65±0.01[c]
Ellagic acid	1.03±0.01[a]	3.18±0.02[c]	2.58±0.01[b]	10.48±0.03[d]
p-Coumaric acid	9.38±0.02	ND	ND	ND
Orientin	4.27±0.01[b]	ND	3.91±0.03[a]	ND
Vitexin	ND	ND	ND	3.81±0.01
Rutin	1.56±0.03[c]	4.65±0.04[d]	0.74±0.01[a]	1.32±0.01[b]
Quercitrin	ND	10.37±0.01	ND	ND
Quercetin	4.31±0.01[b]	ND	2.53±0.01[a]	ND
Luteolin	6.45±0.02[b]	2.06±0.03[a]	ND	ND
Apigenin	0.52±0.01[a]	ND	2.59±0.02[c]	1.28±0.04[b]

[1)]Results are expressed as mean±standard deviations of three determinations.
[2)]Mean values with different superscript letters across each row are significantly different (p<0.05)
[3)]VA, *V. amygdalina*; TO, *T. occidentalis*; TT, *T. triangulare*; AH, *A. hybridus*
[4)]ND, not detected

tissue homogenate in a concentration-dependent manner. These bioactivities could be attributed to their phenolic composition and vitamin C contents, as revealed by their Pearson correlation coefficients. Therefore, the antioxidant properties and ACE inhibitory effects of the vegetables could suggest some possible scientific justification and mechanism of action for their efficacy in the prevention and management of hypertension and oxidative-stress-induced kidney dysfunction. Among the vegetables studied, VA appeared to be the most potent in possible physiological functions, proven by this *in vitro* study.

Acknowledgment The author(s) received no financial support from any public or private organization towards this work, authorship, and/or publication of this article, and all activities were solely conducted and funded by the authors. Oboh G. and Akinyemi A. J. design the experiment and interpretation of data. Akinyemi A. J., Adeleye B., Oyeleye S. I., Ogunsuyi O. B., Ademosun A. O., and Ademiluyi A. O. involved in bench work, data collection, and manuscript preparation. Boligon A. A. carried out phenolic characterization.

Disclosure The authors declare no conflict of interest.

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