E-only Article

Journal of Evidence-Based Complementary & Alternative Medicine
2016, Vol. 21(4) NP67-NP76

DOI: 10.1177/2156587216636505
cam.sagepub.com
SAGE

Phenolic Composition and Evaluation of Methanol and Aqueous Extracts of Bitter Gourd (*Momordica charantia* L) Leaves on Angiotensin-I-Converting Enzyme and Some Pro-oxidant-Induced Lipid Peroxidation In Vitro

Sidiqat A. Shodehinde, PhD[1,2], Stephen A. Adefegha, PhD[2], Ganiyu Oboh, PhD[2], Sunday I. Oyeleye, MTech[2], Tosin A. Olasehinde, MTech[2,3], Emmem E. Nwanna, MTech[2], Bukola C. Adedayo, PhD[2], and Aline A. Boligon, PhD[4]

Abstract

This study sought to assess the effects of bitter gourd leaf extracts (methanol and aqueous) on enzyme linked with hypertension (angiotensin-I-converting enzyme and some pro-oxidants (iron sulfate, sodium nitroprusside, and cisplatin]–induced lipid peroxidation in rat kidney homogenates. Chromatographic analysis of the extract was done using high-performance liquid chromatography–diode array detection. The results revealed that methanol extract (IC_{50} = 109.63 μg/mL) had significantly ($P < .05$) higher angiotensin-I converting enzyme inhibitory activity than aqueous extract (IC_{50} = 182.95 μg/mL). Similarly, methanol extract had significantly ($P < .05$) higher inhibitory effect on Fe^{2+}- and cisplatin-induced lipid peroxidation than aqueous extract. However, there was no significant ($P > .05$) difference in the inhibition of sodium nitroprusside–induced lipid peroxidation of both extracts. High-performance liquid chromatography–diode array detection analysis revealed the presence of quercetin, caffeic, gallic, and chlorogenic acids in the leaf. Thus, inhibition of angiotensin-I converting enzyme activity and the antioxidant properties of the extracts could be linked to the presence of phenolic phytochemicals.

Keywords

bitter gourd, angiotensin-I converting enzyme, lipid peroxidation, antihypertension, renoprotection, antioxidant

Received November 10, 2015. Received revised January 27, 2016. Accepted for publication February 5, 2016.

Recent reports have revealed that hypertension is the fourth major cause of premature death in the world and its prevalence is rapidly increasing.[1,2] Hypertension, also known as high blood pressure, is a risk factor for cardiovascular diseases and a long-term complication of type 2 diabetes.[3] Pathophysiological factors such as deficiency of vasodilators like nitric oxide (NO), increased or inappropriate renin secretion with resultant increased production of angiotensin II and oxidative stress have been linked to the development of hypertension.[4]

Oxidative stress is known to play a significant role in the development of hypertension.[5] Overproduction of reactive oxygen species in cells may overwhelm its antioxidant capacity, thereby inducing various physiological responses that are capable of raising the blood pressure.[6] Beg et al[4] reported that oxidative damage to the renal cells may lead to renovascular injury which may subsequently induce hypertension and mediates cardiovascular pathologies. Furthermore, elevated levels of superoxide anions, hydrogen peroxide and reduced synthesis of NO have been

[1] Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria
[2] Federal University of Technology, Akure, Nigeria
[3] Federal Institute of Industrial Research, Oshodi, Nigeria
[4] Federal University of Santa Maria, Santa Maria, Brazil

Corresponding Authors:
Ganiyu Oboh, PhD, Functional Foods and Nutraceutical Unit, Department of Biochemistry, Federal University of Technology, Akure, Ondo State 340001, Nigeria.
Email: goboh2001@yahoo.com
Tosin A. Olasehinde, MTech, Food Technology Department, Nutrition and Toxicology Division, Federal Institute of Industrial Research, 2 FIIRO Road Cappa Oshodi Lagos, Oshodi 21023, Nigeria.
Email: tosinolasehinde26@yahoo.com

discovered in hypertensive patients.[4,7] In this condition, reactive oxygen species may react with NO to form peroxynitrite, which may subsequently cause oxidative damage to cells, impair NO pathway and decrease the bioavailability of NO.[5,6]

Angiotensin II is a potent vasoconstrictor capable of contracting blood vessel and stimulating the production of vasopressin and aldosterone.[8] Angiotensin II is formed from angiotensin I via a reaction catalyzed by angiotensin-I-converting enzyme (ACE) in the renin-angiotensin system. It has also been reported to induce the release of reactive oxygen species by activating the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) and NADP oxidase in the smooth vascular muscles.[6,8] Modern therapeutic approach for the management and treatment of hypertension and hypertension induced-renal damage involves the inhibition of ACE and dietary phenolic compounds and flavonoids in plants have shown promising potentials.[9,10]

Bitter gourd (*Momordica charantia* L) is a common herb usually consumed as vegetable for medicinal purpose in different parts of the world, including Asia, Africa, the Caribbean, Central America, and South America.[11] Bitter gourd contains several bioactive components and has been identified by researchers to play a major role in the treatment of various human ailment including malaria, menstrual disorder, dysentery, leucorrhoea, hemorrhoid, hypoglycemia, jaundice, tumors, wounds, and antiviral diseases such as measles hepatitis, feverish conditions.[11,12] Bitter gourd has been well studied and several mechanisms of action have been proposed especially for its antidiabetic properties.[13] However, there is dearth of information on its antihypertensive and renoprotective potentials coupled with the possible mechanism of action.

Plant phenolics have attracted a lot of attention in the past two decades because of their interesting biological effects.[14] In addition, most of the biological actions of plants and plant foods have been linked to the phenolic composition rather than the ascorbic acid and tocopherol.[15] Thus, this study seeks to characterize the phenolic constituents of bitter gourd extract, assess their inhibitory effects against ACE (enzyme linked to hypertension), and determine their antioxidative and renoprotective properties in vitro.

Materials and Methods

Materials

Plant Material and Authentication. Freshly matured bitter gourd leaves of about 12 to 15 cm long were harvested from the botanical garden of Federal University of Technology, Akure and authenticated at the Biology Department, Federal University of Technology, Akure, Nigeria with voucher number (FUTA/BIO/0125). The leaves were separated from the stem, and washed under running tap water to remove any unwanted materials. The leaves were cut into pieces, air dried at room temperature, ground into powder using a laboratory blender, sieved by passing through 60 mesh sieve and stored in air tight container. Unless stated otherwise, all other chemicals and reagents used were of analytical grades and the water was glass distilled. A JENWAY UV-visible spectrophotometer (Model 6305; Jenway, Barlo World Scientific, Dunmow, United Kingdom) was used to measure the absorbance.

Sample Preparation

Aqueous Extract Preparation. The aqueous extract of the leaves was prepared according to the modified method of Molehin and Adefegha.[16] Five grams of the bitter gourd leaves were soaked in 100 mL of distilled water for about 16 hours; the mixture was spanned at 1914 × *g*, filtered, and the filtrate was kept in a freezer for 24 to 36 hours. The dried extract was recovered using a freeze drier. The extract was later reconstituted in water for subsequent analysis.

Methanol Extract Preparation. The methanol extract of the leaves were prepared in line with the modified method of Molehin and Adefegha[16] for ethanol extraction. Five grams of the powdered bitter gourd leaves were soaked in 100 mL of methanol for about 16 hours in an air-tight container; the mixture was centrifuged, filtered, and the filtrate was evaporated under pressure at 40°C using a rotary evaporator. The dried extract obtained was reconstituted with distilled water for subsequent analysis. All the extracts were stored in a sealed vial at +4°C for further analysis.

Enzyme Inhibition Assay

Angiotensin-I Converting Enzyme Inhibition Assay. The ACE inhibitory activity by the extracts was measured according to the method of Cushman and Cheung.[17] Different concentrations (50-200 µg/mL) of the extract and 50 µL of rabbit lungs ACE (EC 3.4.15.1) solution (4 mU/mL) were preincubated at 37°C for 15 minutes. Thereafter, enzymatic reaction was initiated by adding 150 µL of 8.33 mM of ACE substrate (hippuryl-L-histidyl-L-leucine) in 125 mM Tris-HCl buffer (pH 8.3) to the reaction mixture and incubated at 37°C for 30 minutes. The reaction was terminated by adding 250 µL of 1 M HCl. The hippuric acid (Bz-Gly) produced by the reaction was extracted with 1.5 mL ethyl acetate. The mixture was then centrifuged to separate the ethyl acetate layer, after which the 1 mL of the ethyl acetate layer was transferred to a clean test tube and evaporated to dryness. The residue was redissolved in distilled water and its absorbance was measured at 228 nm. The average value of triplicate reading were taken and used to calculate the ACE inhibition and expressed as percentage inhibition using the following formula:

$$\%\ \text{Inhibition} = [(\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}})/\text{Abs}_{\text{ref}}] \times 100.$$

where Abs_{ref} is the absorbance without the essential oil and Abs_{sam} is the absorbance of the extracts.

Handling of Experimental Animals. Five adult male Wistar strain albino rats weighing 180 to 210 g were purchased from the animal breeding colony of Animal Production and Health, Federal University of Technology, Akure. Their handling was in accordance with the Guide for Care and Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institutes of Health, United States.[18] The rats were kept for 14 days to acclimatize and were maintained at room temperature under laboratory conditions with access to standard animal feed and water ad libitum.

Lipid Peroxidation Assay. The rats were decapitated under mild diethyl ether anesthesia and dissected. The kidneys were rapidly isolated, placed on ice pack, weighed, and subsequently homogenized in cold saline (1/10 w/v) with about 10-up-and-down strokes at approximately 172 × *g* in a Teflon glass homogenizer. The homogenate was

centrifuged for 10 minutes at 3000 × *g* to yield a pellet that was discarded, and a low-speed supernatant that was kept for lipid peroxidation assay. One hundred microliters of the supernatant was mixed with a reaction mixture containing 30 µL of 0.1 M Tris-HCl buffer (pH 7.4), extracts (0-100 µL) and 30 µL of the 250 µM of iron(II) sulfate solution (similar procedure was carried out for 7 mM sodum nitroprusside [SNP] and 1 mM cisplatin). The volume was made up to 300 µL with distilled water before incubation at 37 C for 2 hours. The color reaction was developed by adding 300 µL of 8.1% sodium dodecyl sulfate to the reaction mixture containing the homogenate, followed by the addition of 600 µL of acetic acid/HCl (pH 3.4) and 600 µL of 0.8% thiobarbituric acid. This mixture was incubated at 100°C for 1 hour. The absorbance of thiobarbituric acid reactive species produced were measured at 532 nm. Malondialdehyde produced was expressed as % control.[19,20]

Antioxidant Assays

Determination of Total Antioxidant Capacity. The total antioxidant capacity of the extracts was determined based on 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonate radical (ABTS*) scavenging ability of the extracts according to the method described by Re et al.[21] The ABTS* was generated by reacting an (7 mmol/L) ABTS aqueous solution with $K_2S_2O_8$ (2.45 mmol/L, final concentration) in the dark for 16 hours and adjusting the absorbance at 734 nm to 0.700 with ethanol. Appropriate dilution of the extracts was added to 2.0 mL ABTS* solution and the absorbance were measured at 734 nm after 15 minutes. The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated.

Nitric Oxide Scavenging Ability. The scavenging effect of the extract on nitric oxide radical (NO*) was measured according to the method of Mercocci et al.[22] One hundred to 400 µL of the extracts were added in the test tubes to 1 mL of SNP solution (25 mM) and tubes were incubated at 37°C for 2 hours. An aliquot (0.5 mL) of the incubated mixture was removed and diluted with 0.3 mL Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% naphthlethylenediamine dihydrochloride). The absorbance of the chromophore formed was immediately measured at 570 nm against the blank (distilled water). The NO* scavenging ability was subsequently calculated and reported as % NO* radical scavenging ability.

Hydroxyl Radical Scavenging Ability. The method of Halliwell and Gutteridge[23] was used to determine the ability of the extracts to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose. The extract 0 to 100 µL was added to a reaction mixture containing 120 µL of 20 mg deoxyribose, 400 µL of 0.1 M phosphate buffer, 40 µL of 500 µM of $FeSO_4$, and the volume was made up to 800 µL with distilled water. The mixture was incubated at 37°C for 30 minutes and the reaction was then stopped by the addition of 0.5 mL of 28% trichloroacetic acid. This was followed by addition of 0.4 mL of 0.8% thiobarbituric acid solution. The tubes were subsequently incubated in boiling water for 20 minutes. The absorbance was measured at 532 nm in a spectrophotometer.

Fe^{2+} Chelation Assay. The Fe^{2+}-chelating ability of the extracts was determined using the method of Minotti and Aust[24] with slight modification by Puntel et al.[25] Freshly prepared 500 µM $FeSO_4$ (150 µL) was added to a reaction mixture containing 168 µL 0.1 M Tris-HCl (pH 7.4), 218 µL saline and the extract (0-25 µL). The reaction mixture was incubated for 5 minutes, before the addition of 13 µL 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe(II) chelating ability was subsequently calculated.

Determination of Total Phenol Content. The total phenol content of the extracts was determined according to the method of Singleton et al.[26] Briefly, the extract was oxidized with 2.5 mL 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 mL of 7.5% Na_2CO_3. The mixture was incubated for 40 minutes at 45°C and the absorbance was measured at 765 nm in the spectrophotometer. Gallic acid was used as standard phenol and the total phenol content was subsequently calculated as gallic acid equivalent

Determination of Total Flavonoid Content. The total flavonoid content of the extracts was determined according to the modified method of Meda et al.[27] Briefly, 0.5 mL of the extract was mixed with 0.5 mL absolute methanol, 50 µL of 10% $AlCl_3$, 50 µL of 1 M potassium acetate and 1.4 mL H_2O. The mixture was incubated at room temperature for 30 minutes. Thereafter, the absorbance of the mixture was subsequently measured at 415 nm. Quercetin was used as standard flavonoid and the total flavonoid content was subsequently calculated as quercetin equivalent.

High-Performance Liquid Chromatography Coupled Diode Array Detector Analyses. Chromatographic analyses were carried out under gradient conditions using C_{18} 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: 13% of B until 10 minutes and changed to obtain 20%, 30%, 50%, 60%, 70%, 20%, and 10% B at 20, 30, 40, 50, 60, 70, and 80 minutes, respectively, following the method described by Adedayo et al[28] with slight modifications. *Momordica charantia* leaf extract and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use, bitter gourd leaf extracts were analyzed at a concentration of 20 mg/mL. The flow rate was 0.7 mL/min, injection volume 40 µL and the wavelength were 254 nm for gallic acid, 280 nm for catechin and epicatechin, 325 nm for chlorogenic and ellagic acids, and 365 nm for quercetin, quercitrin, isoquercitrin, rutin, and kaempferol. Stock solutions of standard references were prepared in the high-performance liquid chromatography mobile phase at a concentration range of 0.030 to 0.250 mg/mL for kaempferol, quercetin, quercitrin, isoquercitrin, rutin, catechin, and epicatechin; and 0.050 to 0.450 mg/mL for ellagic, gallic, and chlorogenic acids. Chromatography peaks were confirmed by comparing its retention time with those of reference standards and diode array detection spectra (200-500 nm).

Data Analysis

The results of triplicate readings were pooled and expressed as mean ± standard deviation. One-way analysis of variance was used to analyze the mean and the post hoc treatment was performed using Duncan multiple test. Significance was accepted at $P \leq .05$. IC_{50} (extract concentration causing 50% enzyme/antioxidant activity) was determined using nonlinear regression analysis.

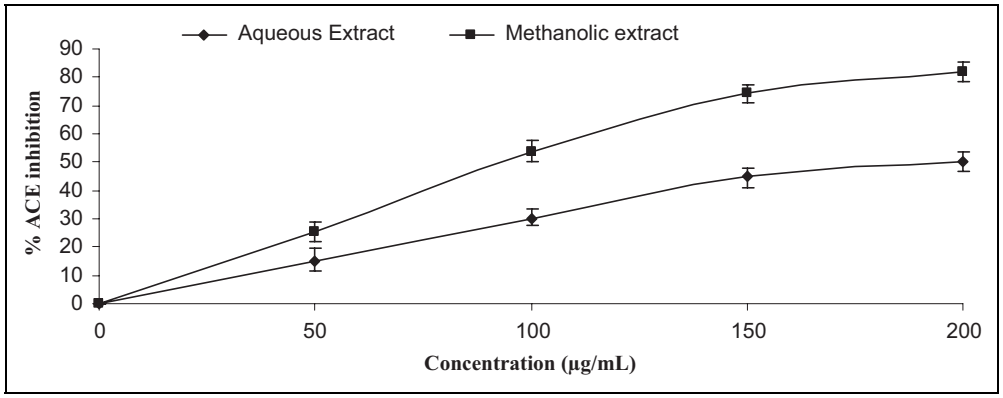

Figure 1. Angiotensin-I-converting enzyme (ACE) inhibitory ability of aqueous and methanolic extracts from *Momordica charantia* leaves.

Table 1. Extract Concentration Causing 50% Enzyme/Antioxidant Concentration (IC_{50}) of Inhibition of Angiotensin-I-Converting Enzyme Activity, Inhibition of Fe^{2+}-, Cisplatin-, and Sodium Nitroprusside–Induced Lipid Peroxidation in Rat Kidney Homogenates and Radicals (DPPH, NO*, OH*) Scavenging and Fe^{2+}-Chelating Abilities of Aqueous and Methanolic Extracts of *Momordica charantia* (µg/mL).[†]

Parameter	Aqueous Extract	Methanolic Extract
Angiotensin-I-converting enzyme	182.95 ± 6.45[a]	109.63 ± 4.11[b]
Lipid peroxidation		
Fe^{2+} induced	23.25 ± 1.45[a]	19.71 ± 1.02[b]
Cisplatin induced	26.08 ± 1.22[a]	21.06 ± 0.95[b]
Sodium nitroprusside induced	24.35 ± 2.50[a]	21.50 ± 1.81[a]
Radicals scavenging ability		
OH*	24.53 ± 1.83[a]	20.02 ± 1.01[b]
NO*	61.53 ± 2.32[a]	48.43 ± 1.98[b]
Fe^{2+}-chelating ability	29.05 ± 1.75[a]	23.92 ± 1.54[b]

[†]Values represent means of triplicate. Values with the same superscript along the same row are not significantly ($P > .05$) different.

Results

Enzyme Inhibition

Interaction of aqueous and methanol extracts of bitter gourd with ACE is presented in Figure 1. The extracts inhibited ACE activity in a dose-dependent pattern (50-200 µg/mL). However, considering the IC_{50} (Table 1) values, methanol extracts (109.63 µg/mL) had significantly ($P < .05$) higher inhibitory activity than aqueous extract (182.95 µg/mL).

Inhibition of Pro-oxidant-Induced Lipid Peroxidation

The ability of the aqueous and methanol extracts of bitter gourd leaves to inhibit Fe^{2+}-, SNP-, and cisplatin-induced lipid peroxidation in rat kidney (in vitro) are presented in Figures 2, 3, and 4, respectively. The result revealed that the extracts inhibited Fe^{2+}-induced malondialdehyde production in a dose-dependent manner (12.50-31.25 µg/mL). However, judging by the IC_{50} values in Table 1, the methanol extract (IC_{50} = 19.71 µg/mL) had higher inhibitory effect than the aqueous extract (IC_{50} = 23.25 µg/mL).

Furthermore, the extracts inhibited SNP-induced malondialdehyde production in rat kidney tissue homogenates in a dose-dependent manner (12.50-31.25 µg/mL). The IC_{50} values in Table 1 shows that there was no significant ($P > .05$) difference in the inhibitory effects of the methanol (21.50 µg/mL) and aqueous (24.35 µg/mL) extracts. Also, the ability of the extracts to inhibit cisplatin-induced malondialdehyde production was also evaluated and presented in Figure 4. The methanol and aqueous extracts also inhibited cisplatin-induced malondialdehyde production in rat's kidney homogenates in a dose-dependent manner (12.50-31.25 µg/mL). However, methanol extract (IC_{50} = 21.06 µg/mL) had higher inhibitory effect than the aqueous extract (IC_{50} = 26.08 µg/mL).

Radical Scavenging and Chelating Ability

The radical scavenging and chelating abilities of the extracts were assessed through their abilities to scavenge free radicals and chelate metallic ion. As presented in Figures 5 and 6, the extracts scavenged NO and OH radicals in a dose-dependent pattern. The ability of the extracts to scavenge these radicals may prevent the initiation or cause rapid termination of lipid peroxidation. The methanol extract (IC_{50} = 48.43 and 20.02 µg/mL) had significantly ($P < .05$) higher NO and OH radical scavenging abilities than aqueous extract (IC_{50} = 61.53 and 24.53 µg/mL) respectively. In addition, the extracts were able to chelate Fe^{2+} in a dose dependent manner as shown in Figure 7. The result follows similar trend as that of the radicals scavenging abilities where methanol extract (IC_{50} = 23.92 µg/mL) had significantly ($P < .05$) higher Fe^{2+}-chelating ability than the aqueous extract (IC_{50} = 29.05 µg/mL).

Total Antioxidant Capacity of Bitter Gourd Leaf Extracts

ABTS* scavenging ability of the extracts reported as trolox equivalent antioxidant capacity (TEAC) is presented in Table 2. The result revealed that the methanol extract (11.75 mmol TEAC/100 g) had significantly ($P < .05$) higher antioxidant capacity than the aqueous extract (8.34 mmol TEAC/100 g).

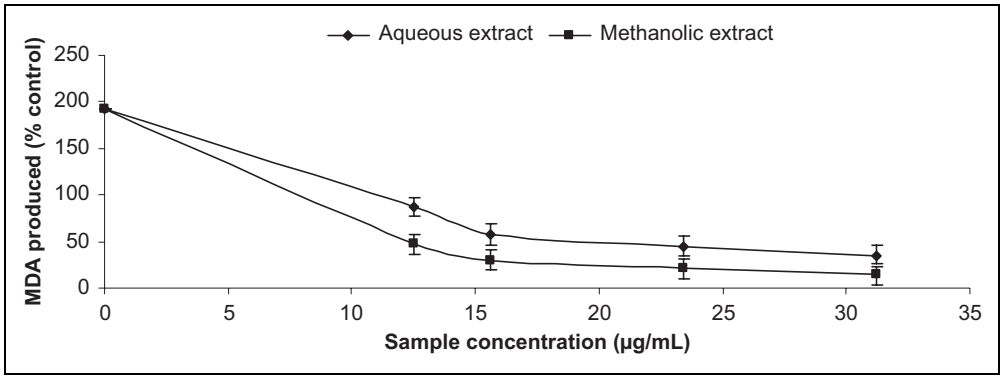

Figure 2. Inhibition of Fe^{2+}-induced lipid peroxidation in rat kidney tissue homogenates by aqueous and methanolic extracts from *Momordica charantia* leaves.

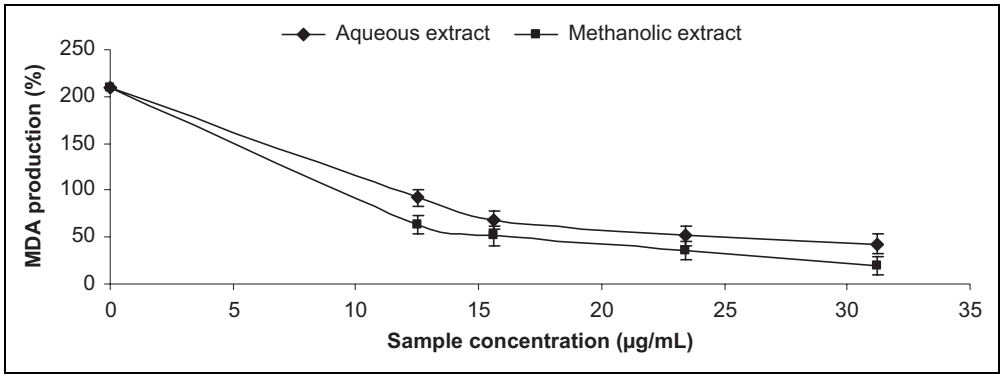

Figure 3. Inhibition of sodium nitroprusside (SNP)–induced lipid peroxidation in rat kidney tissue homogenates by aqueous and methanolic extracts from *Momordica charantia* leaves.

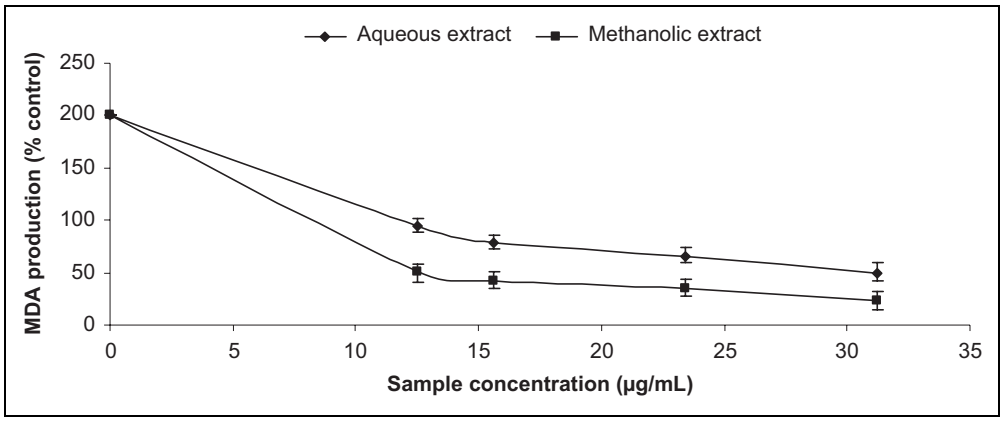

Figure 4. Inhibition of cisplatin-induced lipid peroxidation in rat kidney tissue homogenates by aqueous and methanolic extracts from *Momordica charantia* leaves.

The Total Phenol and Total Flavonoid Content

The total phenol and total flavonoid content of aqueous and methanol extracts are presented in Table 2. The phenol and flavonoid contents of the methanol extract (52.43 mg/100 g and 38.75 mg/100 g) were significantly higher ($P < .05$) than that of aqueous extract (46.95 mg/100 g and 29.46 mg/100 g).

Identification of Phenolic Acids and Flavonoids in Bitter Gourd Leaves

The high-performance liquid chromatography–diode array detector analysis as presented in Table 3 and Figure 8 revealed the presence of some phenolic acids such as gallic acid (97.35 mg/g), catechin (28.19 mg/g), chlorogenic acid

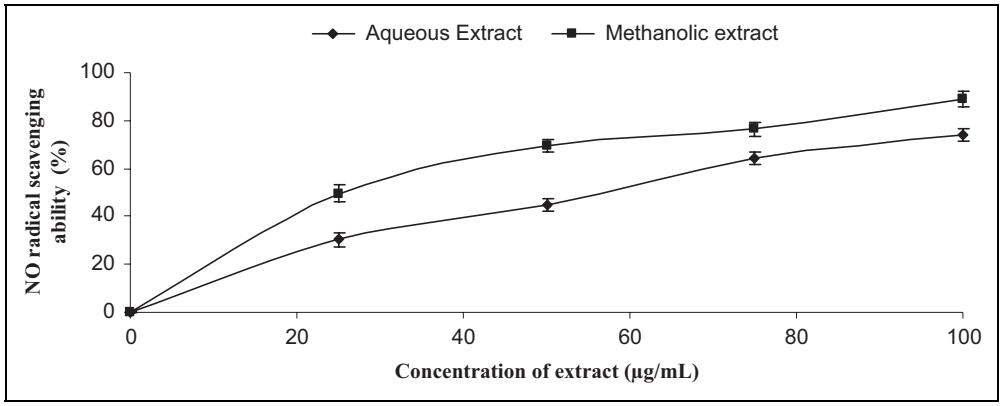

Figure 5. Nitric oxide radical (NO*) scavenging ability of aqueous and methanolic extracts from *Momordica charantia* leaves.

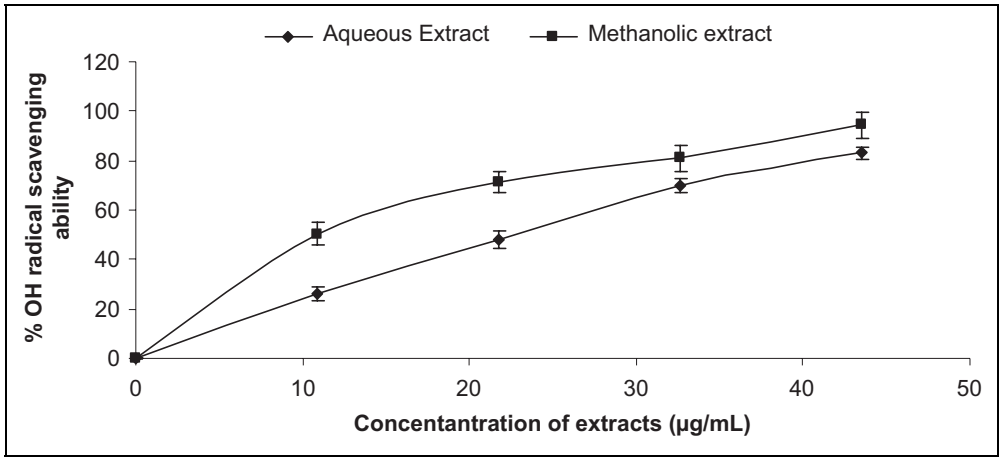

Figure 6. Hydroxyl radical (OH*) scavenging ability of aqueous and methanolic extracts from *Momordica charantia* leaves.

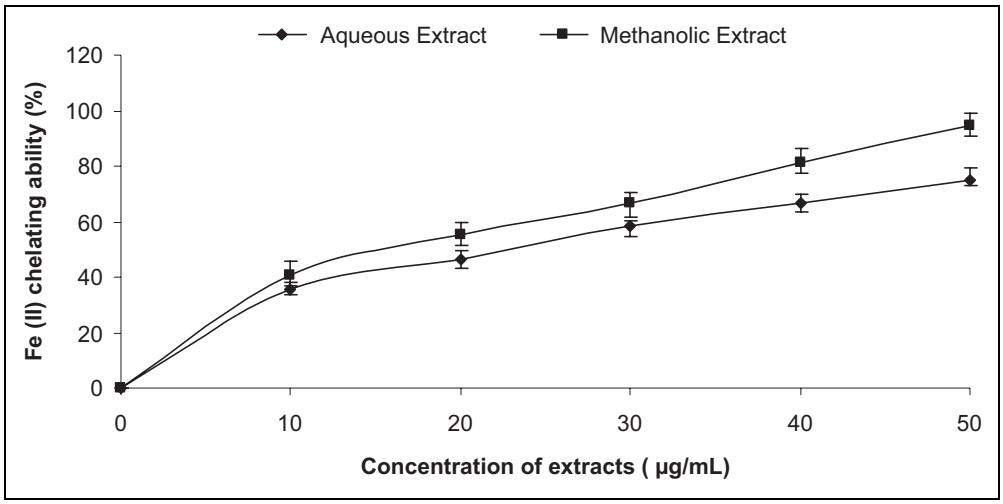

Figure 7. Fe^{2+}-chelating ability of aqueous and methanolic extracts from *Momordica charantia* leaves.

Table 2. The Total Phenol Content Reported as Gallic Acid Equivalent and Total Flavonoid Content Reported as Quercetin Equivalent and Total Antioxidant Capacity.*

Sample	Total Phenol (mg GAE/100 g)	Total Flavonoid (mg QE/100 g)	Total Antioxidant Capacity (mmol TEAC/100 g)
Aqueous extract	46.95 ± 1.21[a]	29.46 ± 0.91[a]	8.34 ± 0.42[a]
Methanolic extract	52.43 ± 1.50[b]	38.75 ± 1.42[b]	11.75 ± 0.83[b]

Abbreviations: GAE, gallic acid equivalent; QE, quercetin equivalent; TEAC, trolox equivalent antioxidant capacity.
*Values represent means of triplicate readings. Values with the same superscript on the same column are not significantly ($P > .05$) different.

Table 3. Phenolics and Flavonoids Composition of *Momordica charantia* Leaf.[a]

Parameter	mg/g	%
Gallic acid	97.35 ± 0.02	9.73
Catechin	28.19 ± 0.01	2.81
Chlorogenic acid	82.51 ± 0.01	8.25
Caffeic acid	116.27 ± 0.01	11.62
Ellagic acid	27.90 ± 0.01	2.79
Epicatechin	11.48 ± 0.03	1.14
Rutin	26.54 ± 0.02	2.65
Quercitrin	50.39 ± 0.01	5.03
Isoquercitrin	44.91 ± 0.02	4.49
Quercetin	80.62 ± 0.01	8.06
Kaempferol	25.78 ± 0.03	2.57

[a]Values represent means ± standard deviations of triplicate readings.

(82.51 mg/g), caffeic acid (116.27 mg/g), ellagic acid (27.90 mg/g), epicatechin (11.48 mg/g); and flavonoids such as quercetin (80.62 mg/g), quercitrin (50.39 mg/g), isoquercitrin (44.91 mg/g), kaempferol (25.78 mg/g), and rutin (26.54 mg/g).

Discussion

Angiotensin-I-Converting Enzyme Inhibitory Ability

The inhibition of ACE is a common therapeutic target for the treatment and management of hypertension.[8,29] Inhibition of ACE activity by the extracts from bitter gourd indicates their antihypertensive effects. This could also represent the possible mechanism and the scientific/biochemical basis for the folkloric use of bitter gourd leaf in the management of hypertension and related diseases. The ACE inhibitory ability of these extracts could be attributed to the presence of important phenolics in the leaves as ACE inhibition by the plant extract has been linked to their phenolic content.[30,31] Moreover, Guerrero et al[32] also reported that ACE inhibitory activity may be due to structure-activity relationship of some flavonoids such as rutin, quercetin, kaempferol, catechin, and epicatechin, which were identified in bitter gourd leaves. The observed inhibitory effects of the extracts could be due to the association of the functional groups of the flavonoids including the catechol group in the B-ring and the double bond between C-2 and C-3 at the C-ring present in the extracts.[32]

Inhibition of Pro-oxidant-Induced Lipid Peroxidation

The link between oxidative stress and hypertension has been established via the action of angiotensin II by inducing the formation of reactive oxygen species.[8] Angiotensin II and aldosterone are capable of interacting with pulse pressure and increased systolic blood pressure thereby activating NADPH oxidase, which releases reactive oxygen species.[32] The reactive oxygen species produced are capable of initiating chain reactions and attacking renal cells thereby causing oxidative damage to the kidney.[33]

Iron (Fe^{2+}) participates in Fenton reaction by breaking down hydrogen peroxide to form hydroxyl radicals (OH*), which in turn, abstract an electron from polyunsaturated fatty acids and initiate a chain reaction.[6,32] This study indicates that the extracts could prevent lipid peroxidation originating from iron influx in the cell. SNP releases NO radicals which can be metabolized into peroxynitrite ($ONOO^{-\dot{c}}$). It can subsequently induce lipid peroxidation leading to oxidative damage in cells.[34,35] Furthermore, cisplatin is an effective antitumor agent used in the treatment of various cancers.[36] However, it can cause acute renal failure via induction of lipid peroxidation.[36] Therefore elevated levels of cisplatin found in renal epithelial cells can induce reactive oxygen species formation by decreasing antioxidant enzymes and decomposing hydrogen peroxide to form OH*.[37,38]

The observed inhibition of malondialdehyde production induced by Fe^{2+}, SNP, and cisplatin in kidney homogenates may indicate that bitter gourd leaf extracts possess strong antioxidant agents that are capable of protecting the kidneys from oxidative damage and renal dysfunction.[39]

The higher radical scavenging abilities observed in the methanol extract could be attributed to the quantity and quality of the phenolic compounds/constituents that have dissolved in the extracting solvent. This finding is in agreement with the result obtained by Rezaeizadeh et al.[40] Scavengers of reactive oxygen species may thus prevent and manage complications of hypertension and renal damage in both human and animal cells. The observed chelating ability of the extracts could be attributed to their phenolic constituents. Gryszczyńska et al.[41] reported that phenolic acids such as gallic, chlorogenic, ellagic, and caffeic acid are capable of forming complexes with Fe^{2+}. This prevents the oxidation of Fe^{2+} to Fe^{3+}, which could be initiated by hydrogen peroxide. The Fe^{2+}-chelating ability of the extracts may hinder the production of OH radicals and initiation of lipid peroxidation, hence preventing cell/tissue oxidative damage.

Total Antioxidant Capacity

The total antioxidant capacity was assessed based on ABTS radical (ABTS*). The ABTS* radical–based model of free

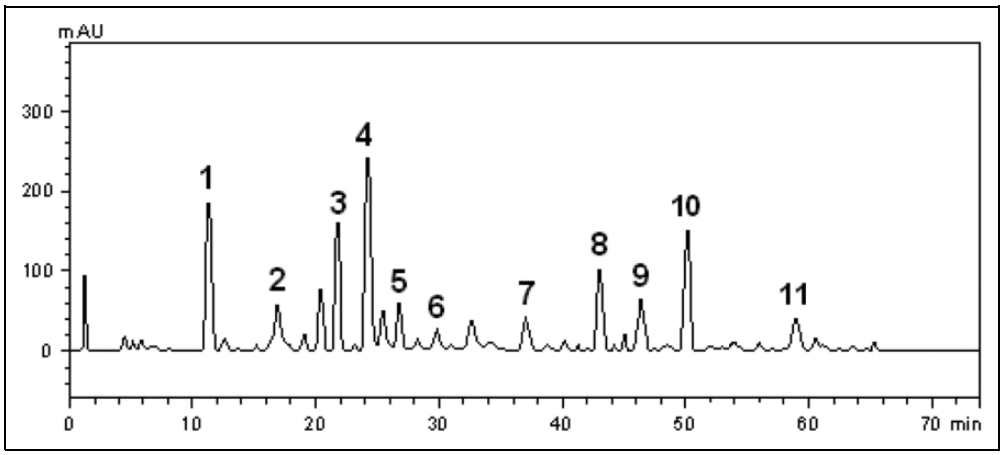

Figure 8. Representative high-performance liquid chromatography profile of *Momordica charantia* extract. Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), epicatechin (peak 6), rutin (peak 7), quercitrin (peak 8), isoquercitrin (peak 9), quercetin (peak 10), and kaempferol (peak 11).

radical scavenging ability has preference over other models because ABTS* can be assessed on both the non-polar and polar samples, hence the method can be assessed on wide range of polarity. The high antioxidant capacity observed in the methanol extract may be attributed to its high phenolic and flavonoid content.

Phenol and Flavonoid Contents

Our findings correlates with earlier reports on some plant foods where phenolic content of methanol extract was significantly ($P < .05$) higher than that of aqueous extract.[42] The same trend was also observed in the flavonoid distribution in which methanol extract had higher flavonoid content than the aqueous extract. The differences in the total phenolic and flavonoid content of the extracts could be attributed to the solubility and polarity of the solvents used for the extraction. This is in agreement with the findings of Sadeghi et al,[43] who reported higher phenolic content in methanol extracts from Iranian plants compared with the aqueous extracts. The result obtained in this study also agrees with previous work on total phenol and flavonoids content of some vegetables.[44]

Phenolic compounds have strong antioxidant properties and are capable of reducing cellular oxidative stress.[37] Studies have revealed that majority of the antioxidant capacity of plants are associated with their phenolic constituents.[45,46] The antioxidant activity of these bioactive compounds may be linked to the ability of the phenolic constituents of the extracts to donate hydroxyl groups to mop up and/or chelate free radicals thereby terminating free radical chain reaction.[40,47]

Conclusion

Bitter gourd leaf extracts inhibited ACE, protected the kidney from Fe^{2+}, SNP, and cisplatin-induced lipid peroxidation, chelated Fe^{2+} and scavenged ABTS, NO, and OH radicals in vitro. These biological effects could be attributed to the phenolic contents of bitter gourd extracts. This study suggests that part of the possible mechanisms by which bitter gourd exerts its antihypertensive and renoprotective properties could be through the inhibition of ACE activity, prevention of lipid peroxidation in the kidney and radicals scavenging abilities. Further studies are ongoing in our laboratory to assess the antihypertensive and renoprotective properties of the leaf in vivo.

Acknowledgments

The authors are grateful to the research staff of Functional Foods and Nutraceutical Unit of Biochemistry Department, Federal University of Technology Akure for their assistance and support in conducting this study.

Author Contributions

SAS, EEN, SIO, and BCA were involved in the collection of materials, experimental work, data collection and analysis. SAA, TAO, and SIO participated in the design of the research, supervised the experiment and drafted the article. GO participated in the design of the experiment, proof read the manuscript and provided mentorship support. All authors proof read and approved the final version of the manuscript.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Ethical Approval

The handling and use of animals for this study was approved by the institution's ethical committee for the use of animals in laboratory experiments with reference no FUTA/SOS/1413 and was in accordance with the NIH guide for the use and handling of experimental animals.

References

1. Pradeepa R, Mohan V. Hypertension and pre-hypertension in developing countries. *Indian J Med Res*. 2008;128:688-690.
2. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data, *Lancet*. 2005;365:217-223.
3. Fowler MJ. Microvascular and macrovascular complications of diabetes. *Clin Diabetes*. 2008;26:77-82.
4. Beg M, Gupta MA, Khanna VN. Oxidative stress in essential hypertension and role of antioxidants. *J Indian Acad Clin Med*. 2010;11:287-293.
5. Ceriello A. Possible role of oxidative stress in the pathogenesis of hypertension. *Diabetes Care*. 2008;31(suppl 2):S181-S184.
6. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007;39:44-84.
7. Dessy C, Ferron O. Pathophysiological roles of nitric oxide: in the heart and the coronary vasculature. *Curr Med Chem Anti Inflamm Anti Allergy Agents*. 2004;3:207-216.
8. Hirata Y, Satonaka H. Hypertension and oxidative stress. *JMAJ*. 2001;44:540-545.
9. Erdos EG, Skidgel RA. The angiotensin-I-converting enzyme, *Lab Investig*. 1987;56:345-348.
10. Kwon YI, Jang HD, Shetty K. Evaluation of *Rhodiola crenulata* and *Rhodiola rosea* for management of type II diabetes and hypertension, *Asia Pac J Clin Nutr*. 2006;15:425-432.
11. Nagarani G, Abirami A, Siddhuraju P. A comparative study on antioxidant potentials, inhibitory activities against key enzymes related to metabolic syndrome, and anti-inflammatory activity of leaf extract from different Momordica species, *Food Sci Hum Wellness*. 2014;3:36-46.
12. Taylor L. *Technical Data Report for Bitter Melon (Momordica charantia)*. 2nd ed. Austin, TX: Sage Press; 2002.
13. Efird JT, Choi YM, Davies SW, Mehra S, Anderson EJ, Katunga LA. Potential for improved glycemic control with dietary *Momordica charantia* in patients with insulin resistance and pre-diabetes. *Int J Environ Res Public Health*. 2014;11:2328-2345.
14. Cartea ME, Francisco M, Soengas P, Velasco P. Phenolic compounds in *Brassica* vegetables. *Molecules*. 2011;16:251-280.
15. Wang H, Cao G, Prior RL. Total antioxidant capacity of fruits. *J Agric Food Chem*. 1996;44:701-705.
16. Molehin OR, Adefegha SA. Comparative study of the aqueous and ethanolic extract of *Momordica foetida* on the phenolic content and antioxidant properties. *Int Food Res J*. 2014;21:401-405.
17. Cushman W, Cheung HS. Spectrophotometric assay and properties of the angiotensin-I-converting enzyme of rabbit lung. *Biochem Pharmacol*. 1971;20:1637-1648.
18. Public Health Service. Public Health Service Policy on Humane Care and Use of Laboratory Animals (PL 99-158). *Health Research Extension Act, 1985*. Washington, DC: US Department of Health and Human Services; 1996.
19. Bellé NA, Dalmolin GD, Fonini G, Rubim M, Rocha J. Polyamine reduces lipid peroxidation induced by different prooxidant agents. *Brain Res*. 2004;1008:245-251.
20. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95: 351-358.
21. Re R, Pellegrini N, Proteggente Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Rad Biol Med*. 1999;26: 1231-1237.
22. Mercocci L, Marguire JJ, Droy-Lefaiz MT, Parker L. The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGB 761. *Biochem Biophys Res Commun*. 1994;201:748-755.
23. Halliwell B, Gutterdge JM. Formation of a thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. *FEBS Lett*. 1981; 128:347-352.
24. Minotti G, Aust SD. An investigation into the mechanism of citrate-Fe^{2+}-dependent lipid peroxidation. *Free Rad Biol Med*. 1987;3:379-387.
25. Puntel RL, Nogueira CW, Rocha JB. Krebs cycle intermediates modulate thiobarbituric reactive species (TBARS) production in rat brain in vitro. *Neurochem Res*. 2005;30: 225-235.
26. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau's reagent. *Method Enzymol*. 1999;299: 152-178.
27. Meda A, Lamien CE, Romito Millogo J, Nacoulma O. Determination of the total phenolic, flavonoid and proline contents in Burkina Faso honey, as well as their radical scavenging activity. *Food Chem*. 2005;91:571-577.
28. Adedayo BC, Oboh G, Oyeleye SI, Ejakpovi II, Boligon AA, Athayde ML. Blanching alters the phenolic constituents and in vitro antioxidant and anticholinesterases properties of fireweed (*Crassocephalum crepidioides*). *J Taibah Univ Med Sci*. 2015; 10:419-426.
29. Brunner HR, Laragh JH, Baer L, et al. Essential hypertension: renin and aldosterone, heart attack and stroke. *N Engl J Med*. 1972;286:441-449.
30. Kwon YI, Apostolidis E, Kim YC, Shetty K. Health benefits of traditional corn, beans and pumpkin: in vitro studies for hyperglycemia and hypertension management. *J Med Food*. 2007;10: 266-275.
31. Kwon YI, Apostolidis E, Shetty K. In vitro studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. *Bioresour Technol*. 2008;99:2981-2988.
32. Guerrero L, Castillo J, Quiñones M, et al. Inhibition of angiotensin-converting enzyme activity by flavonoids: structure-activity relationship studies. *PLoS One*. 2012;7(11):e49493.
33. Dabla PK. Renal function in diabetic nephropathy. *World J Diabetes*. 2010;15:148-156.
34. Oboh G, Agunloye OM, Akinyemi AJ, Ademiluyi AO, Adefegha SA. Comparative study on the inhibitory effect of caffeic and chlorogenic acids on key enzymes linked to Alzheimer's disease and some pro-oxidant induced oxidative stress in rats' brain in vitro. *Neurochem Res*. 2012;38:413-419.

35. Zou MH, Shi CM, Cohen RA. Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. *J Clin Invest*. 2002;109:817-826.
36. Kawai Y, Gemba M. Cisplatin-induced renal injury in LLC-PK_1 cells. *Alternat Anim Test Exp J*. 2007;14:453-456.
37. Santos NA, Bezerra CS, Martins NM, Curti C, Bianchi ML, Santos AC. Hydroxyl radical scavenger ameliorates cisplatin-induced nephrotoxicity by preventing oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. *Cancer Chemother Pharmacol*. 2008;61: 145-155.
38. Noori S, Mahboob T. Antioxidant effect of carnosine pretreatment on cisplatin- induced renal oxidative stress in rats. *Indian J Clin Biochem*. 2010;25:86-91.
39. Oboh G, Rocha JBT. Antioxidant in foods: a new challenge for food processors. In: Panglossi HV, ed. *Leading Edge Antioxidants Research*. New York, NY: Nova Science; 2007:35-64.
40. Rezaeizadeh A, Zuki AB, Abdollahi M, et al. Determination of antioxidant activity in methanol and chloroformic extracts of *Momordica charantia*. *Afr J Biotechnol*. 2011;10:4932-4940.
41. Gryszczyńska B, Wielkoszyński MIT, Małecka M, Budzyn-Napierala M, Kasprzak M, Gryszczyńska A. Phenolic acids improve the antioxidant activity of cerulo-plasmin isolated from plasma of healthy volunteers and atherosclerotic patients. *J Elementol*. 2015;20:83-93.
42. Salih NM, Bayar AH, Hindi MJ. Antioxidant activity of dietary plants: peppermint. *Pak J Nutr*. 2013;12:571-574.
43. Sadeghi Z, Valizadeh J, Azyzian Shermeh O, Akaberi M. Antioxidant activity and total phenolic content of *Boerhavia elegans* (choisy) grown in Baluchestan, *Iran.* Avicenna J Phytomed. 2015; 5:1-9.
44. Adefegha SA, Oboh G. Enhancement of total phenolics and antioxidant properties of some tropical green leafy vegetables by steam cooking. *J Food Process Preserv*. 2011;35:615-622.
45. Oboh G, Puntel RL, Rocha JBT. Hot pepper (*Capsicum annuum*, Tepin and *Capsicum chinense*, Habanero) prevents Fe^{2+}-induced lipid peroxidation in brain in vitro. *Food Chem*. 2007; 102:178-185.
46. Materska M, Perucka I. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruits (*Capsicum annuum* L.). *J Agric Food Chem*. 2005;53:1730-1758.
47. Sathishsekar D, Subramanian S. Antioxidant properties of *Momordica charantia* (bitter gourd) seeds on Streptozotocin induced diabetic rats. *Asia Pac J Clin Nutr*. 2005;14:153-158.