



Radical scavenging potentials: Vitamin-derived antioxidants and total phenolic contents of raw and hydrothermal processed herbs *in vitro*

Okey Alphonsus Ojiako¹, Paul Chidoka Chikezie², Doris I. Ukairo¹, Chiedozi Onyejiaka Ibegbulem¹, Reginald N. Nwaoguikpe¹

ABSTRACT

Aim/Background: The present study evaluated the antioxidant vitamins and total phenolics contents from *Monodora myristica*, *Chromolaena odorata*, *Bucchozia coriacea* and *Sphenostylis stenocarpa* in connection with their comparative radical scavenging potentials between the raw and hydrothermal processed herbs using *in vitro* models.

Methods: Antioxidant vitamins and total phenolics contents as well as radical scavenging capacities and ferric reducing antioxidant power of the herbs were measured using standard spectrophotometric methods. **Results:** The hydrothermal processed herbs exhibited relatively lower antioxidant vitamins and total phenolics contents compared with the raw herbs. Total phenolics contents of the raw and hydrothermal processed herbs varied within relatively narrow range: $0.26 \pm 0.04 - 7.97 \pm 0.20$ mg GAE/g dry sample. The SC_{50} of raw herbal extracts against $O_2^{\bullet-}$ was within the range of $201.61 \pm 4.09 - 305.21 \pm 5.11$ μ g/mL, whereas those of corresponding hydrothermal processed herbs gave $211.02 \pm 4.15 - 531.66 \pm 8.14$ μ g/mL. For the most part, SC_{50} of the raw herbs against $NO^{\bullet-}$ were significantly lower ($p < 0.05$) than those of corresponding hydrothermal processed herbs. Likewise, AP_{50} of the raw herbs were significantly lower ($p < 0.05$) than those of corresponding hydrothermal processed herbs. **Conclusion:** The ambivalent radical scavenging and antioxidant capacities of antioxidant vitamins and phenolics contents of raw and hydrothermal processed herbs against the different oxidizing species were indications that the absolute herbal ascorbic acid, α -tocopherol and total phenolics contents did not exclusively dictate radical scavenging and antioxidant activities *in vitro*.

KEY WORDS: Antioxidants; herbs; hydrothermal; *in vitro*; free radical

¹Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.

²Department of Biochemistry, Imo State University, Owerri, Nigeria.

Address for correspondence:

Paul Chidoka Chikezie,
Department of Biochemistry, Imo State University, Owerri, Nigeria.
p_chikezie@yahoo.com

Received: July 19, 2016

Accepted: October 19, 2016

Published: December 31, 2016

INTRODUCTION

The non-enzymatic antioxidants in biologic systems include ascorbic acid, α -tocopherol, β -carotene, selenium, uric acid, reduced glutathione (GSH), bilirubin and some phytochemicals [1-5]. Ascorbic acid is the major water-soluble and peroxidation chain-breaking antioxidant in biologic systems capable of scavenging reactive oxygen and nitrogen species (RONS), namely, superoxide ($O_2^{\bullet-}$), hydroxyl ($\bullet OH$), singlet oxygen (1O_2), alkoxyl ($RO_2^{\bullet-}$), peroxyxynitrite ($\bullet ONOO$) radicals and pro-radicals, hypochlorous acid (HOCl) [6]. The antioxidant activity of ascorbic acid is hinged on its capacity to readily donate electrons to RONS and reducing equivalents in multiple enzymatic reactions [7]. Furthermore, the delocalized structure of ascorbic acid permits a stable ascorbic acid radical following the donation of electrons to RONS [2]. The complex formed between α -tocopherol and an unpaired electron of RONS, which serves to stabilize the radicals and thereby impedes lipid peroxidation, is responsible for its antioxidant activity [8]. In a similar manner to ascorbic acid in aqueous phase reaction, α -tocopherol neutralizes $O_2^{\bullet-}$, $\bullet OH$, 1O_2 , $RO_2^{\bullet-}$, $\bullet ONOO$, and H_2O_2 in the lipid phase of biologic tissues, which in the process cause the production of water, alcohol and lipid hydroperoxides [1,

7]. Additionally, the α -tocopherol is converted to phenyl radicals that are subsequently neutralized by ascorbic acid [2]. Thus, ascorbic acid and α -tocopherol exhibit synergistic antioxidant activity [9].

In addition to the vitamin-derived antioxidants, phenolics from plants exhibit antioxidant activity [10-12]. Structurally, all phenolics possess one or more aromatic groups with one or more hydroxyl groups [10]. Phenolics are widespread in the plant kingdom and are biosynthesized from the pentose phosphate, phenylpropanoid and shikimate pathways [13-15]. Plant phenolics are broadly classified as derivatives of benzoic acid such as gallic acid, and derivatives of cinnamic acid such as coumaric, caffeic and ferulic acid, which include phenolics acids, flavonoids, tannins and the less common stilbenes and lignans [10, 11, 16].

Vast array of herbal remedies exert therapeutic benefits in oxidative stress induced-pathologic conditions by virtue of their antioxidant vitamins and phenolics contents [11, 16-21]. Some notable Nigerian indigenous herbs used in ethno-medicinal practices include the following. *Monodora myristica*, also known as calabash nutmeg, is a perennial edible plant of the Annonaceae family. The plant is prevalent in Southern part of Nigeria where it is commonly called 'ehuru' or 'ehiri' and 'ariwo' by the Yorubas [22, 23].

Phytochemical studies showed that *M. myristica* is rich in flavonoids, saponins and sterols but minimal anti-nutrients like cyanogenic glycosides, tannins, oxalates and phytates [24, 25]. Pulverized kernel of *M. myristica* is used in Nigerian ethno-medicinal practices for relieve of constipation and control of uterine haemorrhage after parturition [26]. The kernel also exhibit anti-diuretic, anti-septic and anti-haemorrhagic properties [27-29]. *Chromolaena odorata* (Linn) is a perennial herb belonging to the flowering shrub of the sunflower family-Asteraceae [30, 31]. The herb is commonly referred to as Siam weed, 'Elizabeth', 'Independence leaf', whereas in South-Eastern Nigeria, it is called 'Enugu plantation weed' and 'Awolowo' [32]. The proximate composition and phytochemical content of the herb have been reported elsewhere [33]. Some trado-medicinal usefulness of *C. odorata* include urinary retention in Yucatan healing, anti-malarial, anti-bacterial and anti-rheumatism preparations [30], wound healing [31], cure of sore throat, common cold, leach and poisonous bites, skin infection and dendo-alveolitis [32]. *Bucchozia coriacea* is a perennial plant of the family-Capparaceae, commonly called 'Wonderful Kola' due to its popular use in trado-medicine [34]. There several local names of *B. coriacea* among ethnic groups in Nigeria and Africa: 'uwuro' (Yoruba), 'esson bossi' (Central Africa), 'uke' (Igbo), 'ovu' (Bini), and 'aponmu' (Akure) [35]. The proximate composition and phytochemical content of *B. coriacea* have been reported elsewhere [35, 36]. Ethno-medicinal uses of *B. coriacea* include: anti-malarial, anti-helminthics, anti-bacterial, anti-diabetes, anti-hypertensive agent as well as treatment of fibroid, hypoglycemia, hypercholesterolemia, respiratory disorders, dysmenorrhea, ulcers and chest pain [34; 36-38]. *Sphenostylis stenocarpa* or African yam bean is a leguminous herbaceous plant cultivated throughout tropical Africa [39]. The low acceptability of the African yam bean has been associated with its relatively high anti-nutritional contents and the hard seed testa, which requires several hours of boiling before suitable for consumption. The nutraceutical usefulness of the African yam bean has been reported elsewhere [40, 41].

Depending on the therapeutic protocol adopted in ethno-medicinal practices, medicinal plants may be administered raw and fresh or subjected to hydrothermal treatment, which entails boiling of the herbs, prior usage in order to achieve desired therapeutic benefits. However, constraints in the use of raw herbal products may not be unconnected with toxicity concerns. For instance, raw and fresh pulses and legumes contain high levels of anti-nutritional factors, which may provoke acute toxicity and thereby negate their medicinal value [16]. Hydrothermal treatment, in combination with other processing schemes, serves to eliminate or attenuate toxic components from herbs and foods [42-45]. However, the molecular stability and efficacy of bioactive principles in herbal remedies following hydrothermal processing are still being controversially discussed as a result of conflicting research outcomes [46-50].

Accordingly, the present study evaluated the antioxidant vitamins and total phenolics contents from Nigerian indigenous herbs in connection with their comparative radical scavenging and antioxidant capacities between the raw and hydrothermal processed herbs using *in vitro* models.

MATERIALS AND METHODS

Collection and preparation of samples

High-grade raw seeds of *M. myristica*, *B. coriacea* and *S. stenocarpa* were purchased from Relief and Obazu-Mbieri Markets located in Owerri Capital Territory, Imo State, Nigeria. Fresh leaves of *C. odorata* were harvested from a private garden in Amakihia, Owerri-North Local Government Area, Imo State, Nigeria. The samples were transported to the laboratory, identified and authenticated by Dr. E.S. Willie at the Herbarium of the Department of Agronomy, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. All samples were collected between the months of February and March, 2015. Voucher specimens were deposited at the Herbarium for reference purposes.

The various samples were washed separately in continues flow of distilled water for 15 min and allowed to dry at laboratory ambient temperature ($24 \pm 5^\circ\text{C}$). The samples were divided into two portions on equal weight basis and designated as follows:

Group R: Raw samples

Group H: Hydrothermal processed samples

Appropriate separate quantities of Group R samples were pulverized using Thomas-Wiley milling machine (ASTM D-3182, India). The ground samples were transferred into corresponding vacuum desiccators and allowed to dry at $T = 24 \pm 5^\circ\text{C}$ until a constant weight was achieved. Appropriate separate quantities of Group H samples were boiled in distilled water in corresponding conical flasks (sample/water ratio = 1:4 w/v). According to local traditional medicine practice, hydrothermal processing of the seed samples was in the following durations: *M. myristica* = 10 min, *B. coriacea* = 1 h and *S. stenocarpa* = 1.5 h, whereas leaves of *C. odorata* were subjected to hydrothermal processing for 5 min. Next, the Group H samples were dried separately in an oven (Gallenkamp Oven 300 plus series, England) at 50°C until a constant weight was achieved. Finally, Group H samples were ground using the Thomas-Wiley milling machine (ASTM D-3182; India), after which the samples were stored in air-tight plastic bottles with screw caps pending extraction.

Extraction of samples

Extraction of Group R and Group H samples was according to the methods previously described [51]. Portion of 10 g each of the ground and dried Group R and Group H samples were subjected to repeated Soxhlet extraction cycles for 2

h using 96% CH₃OH (BDH, U.K) as solvent to obtain final volume of 250 mL of corresponding extracts. The volumes of the extracts were concentrated and recovered in a rotary evaporator (Büch Rotavapor R-200) for 12 h at 50°C under reduced pressure. The extracts were dried in vacuum desiccators for 24 h, wrapped in aluminum foil and stored in air-tight plastic bottles with screw caps at $\leq 4^\circ\text{C}$. The yields were calculated to be as follows:

Extract R1; Raw seeds of *M. myristica* = 8.94% (w/w).

Extract R2; Raw leaves of *C. odorata* = 6.22% (w/w).

Extract R3; Raw seeds of *B. coriacea* = 8.07% (w/w).

Extract R4; Raw seeds of *S. stenocarpa* = 14.02% (w/w).

Extract H1; Hydrothermal processed seeds of *M. myristica* = 6.41% (w/w).

Extract H2; Hydrothermal processed leaves of *C. odorata* = 4.39% (w/w).

Extract H3; Hydrothermal processed seeds of *B. coriacea* = 7.12% (w/w).

Extract H4; Hydrothermal processed seeds of *S. stenocarpa* = 13.51% (w/w).

The separate extracts were reconstituted in phosphate buffered saline (PBS) solution, osmotically equivalent to 100 g/L PBS (90.0 g NaCl, 17.0 Na₂HPO₄·2H₂O and 2.43 g NaH₂PO₄·2H₂O). Appropriate serial concentrations of the herbal extracts were prepared and measured for ascorbic and tocopherol contents as well as radical scavenging capacity index (RSCI).

Ascorbic acid content

Ascorbic acid contents of the herbal extracts were measured by iodine titrimetric methods as described [52]. A 0.2 g of each herbal extract (Extract R1–R4; Extract H1–H4) was dispersed in 100 mL distilled water and allowed to stand, with continuous shaking, at $T = 24 \pm 5^\circ\text{C}$ for 3 h. Next, 10 mL of 1.0% starch solution was added to the herbal extract suspension, mixed thoroughly and filtered with Whatman No. 1 filter paper. The filtrate (20 mL) was titrated against iodine solution (2.0 g KI, 0.11 g KIO₃ and 12 mL of 3.0 M H₂SO₄ in 1.0 L of distilled water). The end point was indicated by the appearance of pale blue-black colour. The procedure was repeated using standard ascorbic acid solution. Ascorbic acid content of the herbal extract was calculated thus:

$$\text{AAC} = V_{\text{st}} \times \frac{C_{\text{std-c}}}{V_{\text{std-v}}} \quad \text{Eq 1}$$

Where AAC = Ascorbic acid concentration of herbal extract (mg/100 g sample).

V_{st} = Titer volume of herbal extract.

$C_{\text{std-c}}$ = Standard concentration of ascorbic acid.

$V_{\text{std-v}}$ = Titer volume of standard of ascorbic acid.

Tocopherol content

The α -tocopherol contents of the herbal extracts were measured according to the methods of Kirk and Sawyer, [53]. A 10 g of each herbal extract (Extract R1–R4; Extract H1–H4) was introduced into a test tube containing 10 mL of ethanolic 1.0 M H₂SO₄ (1:1 v/v) and boiled gently under reflux for 30 min. The content was transferred into a separating funnel and treated thrice with 30 mL of diethyl ether, recovering the ether layer each time. The ether extract was transferred into a desiccator and allowed to stand for 30 min, after which it was evaporated to dryness at $T = 24 \pm 5^\circ\text{C}$. Equal weights of the extract and α -tocopherol were dissolved in separate 10 mL absolute C₂H₅OH. Aliquots of 1.0 mL each of the extract and standard α -tocopherol solutions were transferred into corresponding test tubes containing 5.0 mL absolute C₂H₅OH and 1.0 mL of concentrated HNO₃ and allowed to stand for 5 min. The absorbance, at maximum absorptivity (λ_{max}) = 410 nm, of the test sample was compared with that of the standard α -tocopherol sample using a spectrophotometer (Digital Blood Analyzer; SPECTRONIC 20; Labtech, LabX, Bay Street, Midland, ON, Canada).

Total phenolics content

The total phenolics contents of the extracts were determined using the Folin-Ciocalteu method as previously described [54]. A 0.1 mL of 20 mg/mL (w/v) of each extract (Extract R1–R4; Extract H1–H4) was added to corresponding 1.0 mL, 7% Na₂CO₃ solutions and mixed thoroughly. Next, 0.1 mL of Folin-Ciocalteu reagent was introduced into the mixtures. The final mixture volume was made up to 2.5 mL using distilled water and was allowed to stand for 90 min during intermittent shaking. The absorbance of the mixture was measured at $\lambda_{\text{max}} = 750 \text{ nm}$. Total phenolics contents of the samples were obtained by comparing the absorbance with that of standard gallic acid calibration curve, and expressed as milligram of gallic acid equivalent per gram (mg GAE/g) of dry weight of the extracts. The equation of the calibration curve is presented thus:

$$Y = 0.0221X + 0.001 \quad \text{Eq 2}$$

Y: Absorbance of medium

X: Concentration of gallic acid (mg/mL)

$$R^2 = 0.948$$

Standard concentration range of gallic acid = 4.3 – 7.02 mg/mL

Measurement of radical scavenging capacities of extracts

RSCIs of the herbal extracts were measured according to the methods previously described [55] with minor modifications [21].

Superoxide oxide

Each herbal extract (Extract R1–R4; Extract H1–H4)

scavenging capacity index (SCI) against $O_2^{\bullet-}$ was measured according to the methods of Nishimiki *et al.*, [56] with minor modifications. Briefly, 0.5 mL of different concentrations (100 – 800 $\mu\text{g/mL}$) of the herbal extracts were introduced into test tubes containing 6.0 μL of 0.1 mM EDTA, 0.5 mL of 0.1 mM NaCN, 0.5 mL of riboflavin and 0.5 mL of 150 mM nitroblue tetrazolium (NBT). The assay mixtures were made up to 3.0 mL mark by the addition of 0.1 M phosphate buffer (pH = 7.4). The test tubes containing the test and control samples were illuminated with incandescent lamp for 15 min. Absorbance of the samples were measured before and after illumination at λ_{max} = 530 nm. The herbal extract superoxide radical scavenging capacity index (SORSCI) was calculated thus:

$$\text{SORSCI\%} = \left(1 - \frac{\text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Blank}}}\right) \times 100 \quad \text{Eq 3}$$

Where absorbance of the samples = absorbance after illumination minus absorbance before illumination of the media.

The SORSCI% was expressed as SCI_{50} , which is defined as the concentration ($\mu\text{g/mL}$) of the herbal extract required to scavenge 50% of $O_2^{\bullet-}$.

Hydrogen peroxide

Each herbal extract (Extract R1–R4; Extract H1–H4) SCI against H_2O_2 was according to the methods of Banerjee *et al.*, [57] but with minor modifications. Separate volumes of 50 μL , 1.0 mM H_2O_2 and 100 μL of various concentrations (100 – 800 $\mu\text{g/mL}$) of the herbal extracts were incubated at 25°C for 30 min. A 0.85 mL FOX Reagent (100 μM xylene orange, 250 μM ammonium ferrous sulphate and 25 mM H_2SO_4) was added to the reaction mixtures and allowed to stand at 25°C for 30 min. The absorbance of ferric-xylene orange complex of the mixtures were measured against a blank at λ_{max} = 560 nm.

The hydrogen peroxide scavenging capacity index (HPSCI) of the herbal extract was calculated thus:

$$\text{HPSCI\%} = \left(1 - \frac{\text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Blank}}}\right) \times 100 \quad \text{Eq 4}$$

The HPSCI% was expressed as SCI_{50} , which is defined as the concentration ($\mu\text{g/mL}$) of the herbal extract required to scavenge 50% of H_2O_2 .

Hydroxyl radicals

Each herbal extract (Extract R1–R4; Extract H1–H4) SCI against $\bullet\text{OH}$ was carried out as previously described [58] but with minor modification. Briefly, the reaction mixtures containing 100 μL of 28 mM 2-deoxyribose, 500 μL of various concentrations (100 – 800 $\mu\text{g/mL}$) of the herbal extracts in phosphate buffer (pH = 7.4), 200 μL of 200 μM FeCl_3 in 1.04 mM aqueous EDTA (1:1, v/v), 100 μL of 1.0 mM H_2O_2 , and 100 μL of 1.0 mM ascorbic acid was incubated at 37°C for 1 h. The reaction was terminated by the addition of 1.0 mL of 28% trichloroacetic acid (TCA).

A 1.0 mL of 10% thiobarbituric acid (TBA) was added and the mixture was again incubated on a water bath at 80°C for 20 min. After cooling to 25°C, the absorbance the mixtures were measured at λ_{max} = 532 nm against a blank.

The hydroxyl radical scavenging capacity index (HRSCI) of the herbal extract was calculated thus:

$$\text{HRSCI\%} = \left(1 - \frac{\text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Blank}}}\right) \times 100 \quad \text{Eq 5}$$

The HRSCI% was expressed as SCI_{50} , which is defined as the concentration ($\mu\text{g/mL}$) of the herbal extract required to scavenge 50% of $\bullet\text{OH}$.

Nitric oxide

Each herbal extract (Extract R1–R4; Extract H1–H4) SCI against NO^- was according to previous methods [59] but with minor modifications. Reaction mixtures containing of 2.0 mL of 10 mM NaN_3 in phosphate buffered saline (pH = 7.4) and 1.0 mL of various concentrations (100 – 800 $\mu\text{g/mL}$) of the herbal extracts were incubated at 25°C for 150 min. Next, 1.0 mL of 0.33% sulfanilic acid in 20% glacial CH_3COOH was added to 0.3 mL of the incubated solution and was allowed to stand for 5 min. A 0.5 mL, 1.0% N-(1 naphthyl) ethylenediamine dihydrochloride] was added to the mixture and incubated at 25°C for 30 min. The absorbance was measured at λ_{max} = 540 nm using quercetin as blank [21]. The nitric oxide scavenging capacity index (NOSCI) of the herbal extract was calculated thus:

$$\text{NOSCI\%} = \left(1 - \frac{\text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Blank}}}\right) \times 100 \quad \text{Eq 6}$$

The NOSCI% was expressed as SCI_{50} , which is defined as the concentration ($\mu\text{g/mL}$) of the herbal extract required to scavenge 50% of NO^- .

Ferric reducing antioxidant power

The ferric (Fe^{3+}) reducing antioxidant power was measured as previously reported [60] but with minor modifications. Equal volumes (0.5 mL) of various concentrations (100 – 800 $\mu\text{g/mL}$) of each herbal extract (Extract R1–R4; Extract H1–H4) and 1.0% $\text{K}_3\text{Fe}(\text{CN})_6$ with 0.2 M phosphate buffer (pH = 6.6) were mixed and incubated at 50°C in a water bath for 20 min. TCA (0.5 mL) was added to the mixture and centrifuged at 3000 rpm for 10 min. Finally, 0.5 mL of the supernatant was mixed with equal volume of distilled water and 0.1 mL of 0.1% FeCl_3 solution. The reaction mixture was left to stand at 25°C for 10 min and the absorbance measured at λ_{max} = 700 nm against a blank.

The ferric reducing antioxidant power (FRAP) of the herbal extract was calculated thus:

$$\text{FRAP\%} = \left(1 - \frac{\text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Blank}}}\right) \times 100 \quad \text{Eq 7}$$

The FRAP% was expressed as AP_{50} , which is defined as the concentration ($\mu\text{g/mL}$) of the herbal extract required to reduce 50% of FeCl_3 .

Statistical analysis

The data collected were analyzed by the analysis of variance procedure while treatment means were separated by the least significance difference (LSD) incorporated in the statistical analysis system (SAS) package of 9.1 version, (2006). Correlation coefficients were determined using Excel Software (Microsoft, 2010 version).

RESULTS

Table 1 showed that the concentrations of ascorbic acid in the herbal extracts were such that: Extract R2 > Extract R4 > Extract R1 > Extract R3; $p < 0.05$. The relative reductions in ascorbic acid concentrations in the herbs following hydrothermal processing was in the increasing order: Extract R3→H3 = 4.35%, Extract R1→H1 = 11.89%, Extract R2→H2 = 61.99% and Extract R4→H4 = 79.35%.

Extract R4 gave the highest concentration of α -tocopherol, which was not significantly different ($p > 0.05$) from that of the corresponding Extract H4. Likewise, the concentrations of α -tocopherol in Extract R1 and Extract R3 were not significantly different ($p > 0.05$) from those of their corresponding Extract H1 and Extract H3. Contrary, the concentration of α -tocopherol in Extract H2 was significantly lower ($p < 0.05$) than that of the corresponding Extract R2 and represented 26.04 folds decrease in α -tocopherol concentration.

The total phenolics content of Extract H1 was significantly lower ($p < 0.05$) than that of corresponding Extract R1, whereas other herbs exhibited no significant difference ($p > 0.05$) between their raw and hydrothermal processed extracts.

The SCI_{50} of Extracts R1–R4 against $O_2^{\cdot-}$ was within the range of 201.61 ± 4.09 – $305.21 \pm 5.11 \mu\text{g/mL}$, whereas those of corresponding Extracts H1–H4 gave 211.02 ± 4.15 – $531.66 \pm 8.14 \mu\text{g/mL}$. Figure 1 showed that SCI_{50} of Extract R1 against $O_2^{\cdot-}$ was not significantly different ($p < 0.05$) from that of corresponding Extract H1. Conversely,

SCI_{50} of Extracts R2–R4 against $O_2^{\cdot-}$ was comparatively lower than those of corresponding Extracts H2–H4; $p < 0.05$. Overall, SCI_{50} of Extract H4 against $O_2^{\cdot-}$ gave the highest value, whereas that of Extract R2 represented the lowest SCI_{50} ; $p < 0.05$.

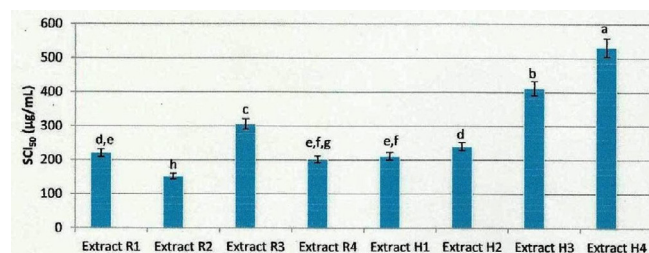


Figure 1. Superoxide radicals scavenging capacities of raw and hydrothermal processed herbal extracts. Means denoted by the same letter are not significantly different at $p > 0.05$ according to LSD.

Figure 2 showed that SCI_{50} of Extracts R1–R4 against H_2O_2 ranged between 119.23 ± 2.99 – $162.49 \pm 3.31 \mu\text{g/mL}$. Extract H1–H4 gave a relatively wider range compared with that of Extract R1–R4, which was within the range of 134.61 ± 3.13 – $401.12 \pm 3.32 \mu\text{g/mL}$. The SCI_{50} of Extract R1, Extract R3 and Extract R4 against H_2O_2 were significantly lower ($p < 0.05$) than those of corresponding Extract H1, Extract H3 and Extract H4. Extract R4 gave the lowest SCI_{50} against H_2O_2 .

The SCI_{50} of Extract R1 and Extract R3 against $\cdot\text{OH}$ were not significantly different ($p > 0.05$) from those of corresponding Extract H1 and Extract H3 (Figure 3). However, SCI_{50} of Extract R2 and Extract R4 and the corresponding Extract H2 and Extract H4 showed significant difference ($p < 0.05$) such that Extract H2 and Extract H4 gave higher SCI_{50} than those of Extract R2 and Extract R4.

The SCI_{50} of Extracts R1–R3 against $\text{NO}^{\cdot-}$ were significantly lower ($p < 0.05$) than those of corresponding Extracts H1–H3, whereas SCI_{50} of Extract R4 was not significantly different ($p > 0.05$) from that of corresponding Extract

Table 1. Ascorbic acid, α -tocopherol and total phenolics contents of raw and hydrothermal processed herbal extracts

Extract	Concentration (mg/100 g dry sample)		
	Ascorbic acid	α -Tocopherol	*Total phenolics
R1	$193.50 \pm 2.10^{\text{c,d}}$	$3.85 \pm 0.27^{\text{d,e,f}}$	$4.04 \pm 0.01^{\text{b}}$
R2	$581.25 \pm 4.36^{\text{a}}$	$43.49 \pm 0.32^{\text{a}}$	$7.97 \pm 0.20^{\text{a}}$
R3	$89.13 \pm 3.28^{\text{f}}$	$4.36 \pm 0.18^{\text{d}}$	$3.02 \pm 0.03^{\text{b,c,d}}$
R4	$356.50 \pm 6.56^{\text{b}}$	$10.62 \pm 0.15^{\text{b}}$	$1.00 \pm 0.03^{\text{c,d,e,f}}$
H1	$170.50 \pm 8.29^{\text{d,e}}$	$1.67 \pm 0.18^{\text{e,f,g,h}}$	$3.12 \pm 0.19^{\text{b,c}}$
H2	$220.88 \pm 3.17^{\text{c}}$	$3.86 \pm 0.09^{\text{d,e}}$	$1.97 \pm 0.09^{\text{b,c,d,e}}$
H3	$85.25 \pm 5.67^{\text{f,g}}$	$3.54 \pm 0.45^{\text{d,e,f,g}}$	$0.59 \pm 0.03^{\text{c,d,e,f,g}}$
H4	$73.63 \pm 6.33^{\text{f,g,h}}$	$9.85 \pm 0.31^{\text{b,c}}$	$0.26 \pm 0.04^{\text{c,d,e,f,g,h}}$

The mean ($\bar{X}$) $\pm$ S.D of three ($n = 3$) determinations; *mg GAE/g dry sample. Means in the column with the same letter are not significantly different at $p > 0.05$ according to LSD.

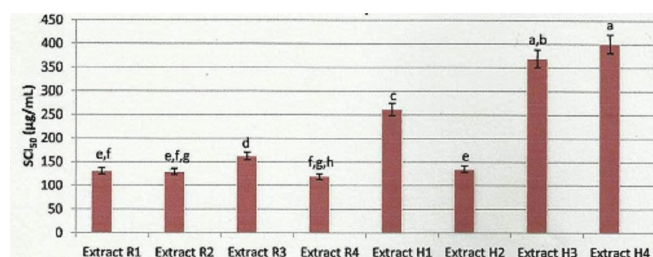


Figure 2. Hydrogen peroxide scavenging capacities of raw and hydrothermal processed herbal extracts. Means denoted by the same letter are not significantly different at $p > 0.05$ according to LSD.

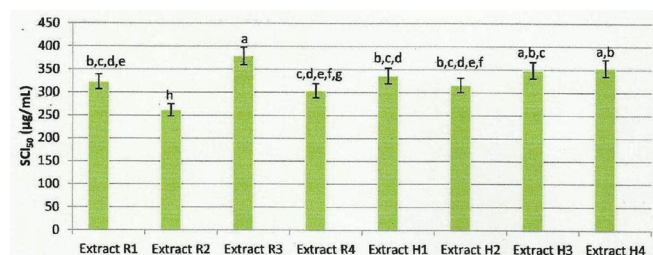


Figure 3. Hydroxyl radicals scavenging capacities of raw and hydrothermal processed herbal extracts- Means denoted by the same letter are not significantly different at $p > 0.05$ according to LSD.

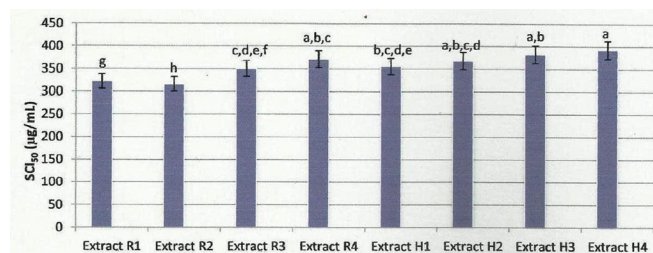


Figure 4. Nitric oxide radicals scavenging capacities of raw and hydrothermal processed herbal extracts. Means denoted by the same letter are not significantly different at $p > 0.05$ according to LSD.

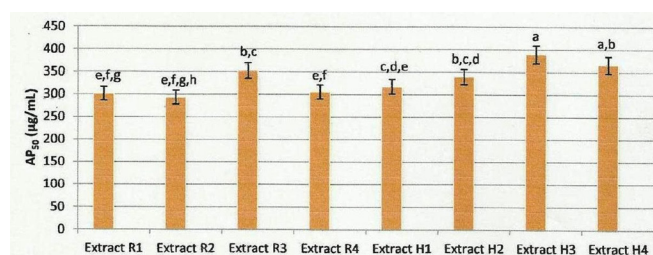


Figure 5. Ferric reducing antioxidant powers of raw and hydrothermal processed herbal extracts. Means denoted by the same letter are not significantly different at $p > 0.05$ according to LSD.

H4. Generally, SCI₅₀ of Extracts R1–R4 and corresponding Extracts H1–H4 against NO[•] was within the range of $316.42 \pm 3.13 - 393.14 \pm 3.22 \mu\text{g/mL}$ (Figure 4).

Figure 5 showed that AP₅₀ of Extracts R1–R4 were significantly lower ($p < 0.05$) than those of corresponding Extracts H1–H4. Extract R2 gave the lowest AP₅₀, whereas that of Extract H3 was significantly higher ($p < 0.05$) than other experimental extracts.

The ascorbic acid contents of raw and hydrothermal processed extracts and their corresponding SCI₅₀ against

O₂^{•-}, H₂O₂, [•]OH and NO[•] as well as AP₅₀ against ferric ion gave negative correlation coefficients. Specifically, the ascorbic acid content of raw herbal extract and SCI₅₀ against NO[•] showed a weak negative correlation coefficient, whereas that of SCI₅₀ against H₂O₂ gave a fair negative correlation coefficient (Table 2). The ascorbic acid contents of hydrothermal processed herbal extracts and their corresponding SCI₅₀ against O₂^{•-}, H₂O₂, [•]OH and NO[•] gave strong negative correlation coefficients. Additionally, the correlation coefficients between ascorbic acid contents and SCI₅₀ against O₂^{•-} and [•]OH as well as AP₅₀ against ferric ion of raw and hydrothermal processed herbal extracts were comparable.

The α-tocopherol contents of raw herbal extracts and their corresponding SCI₅₀ against O₂^{•-}, H₂O₂, [•]OH and NO[•] gave negative correlation coefficients, whereas those of corresponding hydrothermal processed herbal extracts exhibited positive correlation coefficients (Table 3). Furthermore, α-tocopherol contents of raw herbal extracts and their corresponding SCI₅₀ against H₂O₂ and NO[•] gave poor negative correlation coefficients except SCI₅₀ against O₂^{•-} and [•]OH. Conversely, α-tocopherol contents of hydrothermal processed herbal extracts and their corresponding SCI₅₀ against O₂^{•-} and NO[•] gave strong positive correlation coefficients except corresponding SCI₅₀ against H₂O₂ and [•]OH. The α-tocopherol contents of raw herbal extracts and corresponding AP₅₀ against ferric ion gave a weak positive correlation coefficient, whereas that of the raw herbal extract gave a weak negative correlation coefficient.

Table 2. Correlation between ascorbic acid contents and antioxidant capacity indices of raw and hydrothermal processed herbal extracts

Parameters (SCI ₅₀ /AP ₅₀)	Correlation coefficient (r)	
	Raw herbal extracts	Hydrothermal processed herbal extracts
O ₂ ^{•-}	-0.92714	-0.89865
H ₂ O ₂	-0.64174	-0.98787
[•] OH	-0.95789	-0.97706
NO [•]	-0.31234	-0.80278
FRAP	-0.75709	-0.76458

Raw herbal extracts: R1–R4; Hydrothermal processed herbal extracts: H1–H4.

Table 3. Correlation between α-tocopherol contents and antioxidant capacity indices of raw and hydrothermal processed herbs

Parameters (SCI ₅₀ /AP ₅₀)	Correlation coefficient (r)	
	Raw herbal extracts	Hydrothermal processed herbal extracts
O ₂ ^{•-}	-0.76256	0.857854
H ₂ O ₂	-0.31071	0.542208
[•] OH	-0.81517	0.516216
NO [•]	-0.49860	0.852013
FRAP	-0.54161	0.432524

Raw herbal extracts: R1–R4; Hydrothermal processed herbal extracts: H1–H4.

Table 4. Correlation between total phenolics contents and antioxidant capacity indices of raw and hydrothermal processed herbs

Parameters (SCI ₅₀ /AP ₅₀)	Correlation coefficient (r)	
	Raw herbal extracts	Hydrothermal processed herbal extracts
O ₂ ^{•-}	-0.52938	-0.93651
H ₂ O ₂	-0.00741	-0.68936
•OH	-0.56375	-0.64588
NO [•]	-0.88417	-0.98601
FRAP	-0.40959	-0.91467

Raw herbal extracts: R1–R4; Hydrothermal processed herbal extracts: H1–H4.

The total phenolics contents of raw and hydrothermal processed herbal extracts and their corresponding SCI₅₀ against O₂^{•-}, H₂O₂, •OH and NO[•] as well as AP₅₀ against ferric ion gave negative correlation coefficients. Markedly, the total phenolics contents of raw herbal extracts and SCI₅₀ against H₂O₂ gave a very poor negative correlation coefficient (Table 4). Furthermore, correlation coefficients between total phenolics contents and SCI₅₀ against NO[•] of the raw herbal extracts exhibited a strong negative index. Additionally, the correlation coefficients between total phenolics contents and SCI₅₀ against O₂^{•-} as well as AP₅₀ against ferric ion of hydrothermal processed herbal extracts were comparable.

DISCUSSION

A measure of the relationship between antioxidant contents and antioxidant potentials of herbs is a relevant tool for preliminary investigation into their capacities to ameliorate oxidative stress-induced pathologic conditions. The outcome of the present study showed disproportionate reductions in ascorbic acid, α-tocopherol and total phenolics contents of the raw herbs following hydrothermal processing. Previous reports had noted that hydrothermal processing of herbs caused changes in their biochemical compositions and physical characteristics [48, 61-63], which may alter their nutraceutical properties [3, 46, 64, 65]. For instance, Song *et al.*, [66] noted that hydrothermal processing of *Saururus chinensis* gave differential quercetin contents in the extracts and corresponding varying radical scavenging potentials *in vitro*.

An overview of the results of the present *in vitro* antioxidant evaluation models showed that raw and hydrothermal processed herbs gave comparatively lower ascorbic acid, α-tocopherol and total phenolics contents with varying capacities to scavenge oxygen active species and differential FRAP indicators. Additionally, the present findings appeared to suggest that, to a large extent, the raw herbs exhibited greater capacities to scavenge O₂^{•-}, H₂O₂ and NO[•] than the hydrothermal processed herbs; whereas both the raw and hydrothermal processed herbs exhibited equivalent FRAP. The present findings corroborated the report of Nithiyantham *et al.*, [63], in which they noted

that raw seed extracts of *Cicer arietinum* L. and *Pisum sativum* L. were the most potent antioxidant suppliers and free radical scavengers compared to the corresponding processed samples. On a general note, studies had shown that radical scavenging capacities of plant specimen were associated with certain intrinsic factors such as maturation, genera and cultivars differences [67-69] in conjunction with extrinsic factors such as agronomic practices and environmental conditions [10, 70, 71].

It is presumed that bioactive principles from hydrothermal processed herbs exhibited reduced antioxidant activity [72, 73] as a result of changes in their chemical properties elicited by oxidation, hydrolysis, denaturation [61] and perhaps Millard reaction. On the contrary, previous reports had shown that hydrothermal processed pods, tomatoes and sweet corn exhibited increased radical scavenging properties [50, 74] and boiled broccoli and Brussels sprout contained raised levels of carotenoids and polyphenols respectively [46, 48]. According to Dewanto *et al.* [74], hydrothermal processed fruits and vegetables may retain their antioxidant activity, despite the loss of antioxidant vitamin like ascorbic acid, due to synergistic antioxidant activity of other antioxidant phytochemicals against RONS. Additionally, Sommano *et al.*, [49] reported that hydrothermal processing of Australian bush foods stabilized their bioactive principles and specifically caused increase in the levels of lycopene and β-carotene in the finished products, though the ascorbic acid content was minimized.

Based on general principles, the radical scavenging capacities of the herbal extracts are inversely proportional to the experimentally derived SCI₅₀ or AP₅₀. Accordingly, the correlation coefficients between the antioxidant vitamins/total phenolics contents and experimentally derived SCI₅₀ or AP₅₀ of the raw and hydrothermal processed herbal extracts defined their proportionate capacities to scavenge active oxygen species or reduce ferric ion in connection with the absolute concentrations of the antioxidant bioactive principles *in vitro*. For instance, a strong negative correlation coefficient between specific antioxidant contents and the experimentally derived SCI₅₀ or AP₅₀ was an indication that the antioxidant, to a large extent, dictated the capacity of the herbs to scavenge active oxygen species or reduce ferric ion as exemplified by ascorbic acid contents of raw herbal extracts and their SCI₅₀ against O₂^{•-} and •OH. Likewise, the capacity of hydrothermal processed herbal extracts to scavenge O₂^{•-} were largely dictated by their total phenolics contents as indicated by a strong negative correlation coefficient.

The present study showed that hydrothermal processing of the herbs impacted negatively on the capacities of α-tocopherol contents to scavenge active oxygen species as well as altered FRAP indicator as typified by the positive correlation coefficients between α-tocopherol contents of the hydrothermal processed herbs and their corresponding SCI₅₀ against O₂^{•-}, H₂O₂, •OH and NO[•] or AP₅₀ against ferric ion. In a related study, which concurred with the present findings, O₂^{•-} scavenging activity was found to be

significantly higher in raw and dry heated seed extracts of two varieties of cowpea (*Vigna unguiculata*) than the hydrothermal processed seed samples of the respective varieties [75].

Previous reports had noted that radical scavenging potentials and antioxidant activities against different active oxygen species depended on series of synergic and redox interactions among the divergent antioxidant molecules [46, 76-78] as well as the presence of interfering elements and antioxidant antagonist in biologic systems [4, 46, 79]. The present study showed that hydrothermal processing of the raw herbs lowered their antioxidant vitamins and total phenolics contents with concomitant alterations in radical scavenging and antioxidant activities of the herbal extracts, which by implications, resulted to re-adjustments in synergistic interactions of antioxidants as well as antioxidant antagonist as previously described [46, 74]. Furthermore, the outcomes of correlation evaluations/studies revealed that hydrothermal processing of the herbs, to a large extent, increased the propensity of ascorbic acid contents of the herbs to scavenge H_2O_2 and NO^- in spite of losses in their ascorbic acid contents. These findings appeared to suggest a dynamic interplay between ascorbic acid and other reactive oxygen species scavenging components, including phytochemicals such as ferulic acid [74] present in the herbal extracts, which was analogous with the synergy between (poly)phenolics and other antioxidant bioactive molecules as previously proposed [46, 78, 80].

CONCLUSION

Generally, scavenging and antioxidant activities were observed to be comparatively higher in the raw herbs than hydrothermal processed herbs. Hydrothermal processing of the herbs caused alterations in the radical scavenging and antioxidant activities of ascorbic acid contents. Conversely, hydrothermal processing of the herbs obliterated the radical scavenging and antioxidant activities of α -tocopherol contents, whereas the capacities of total phenolics contents of the herbs, following hydrothermal processing, to exert radical scavenging and antioxidant activities were significantly improved.

Precisely, the ambivalent radical scavenging and antioxidant capacities of antioxidant vitamins and phenolics contents of raw and hydrothermal processed herbs against the different oxidizing species were indications that the absolute herbal ascorbic acid, α -tocopherol and total phenolics contents did not exclusively dictate radical scavenging and antioxidant activities *in vitro*.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

1. Ha HL, Shin HJ, Feitelson MA, Yu DY. Oxidative stress and antioxidants in hepatic pathogenesis. *World J Gastroenterol*. 2010; 16:6035-43.
2. Lu JM, Lin PH, Yao Q, Chen C. Chemical and molecular mechanisms of antioxidants, Experimental approaches and model systems. *J Cell Mol Med*. 2010; 14:840-60.
3. Pisoschi AM, Negulescu GP. Methods for total antioxidant activity determination: A Review. *Biochem Anal Biochem*. 2011; 1:12 pages.
4. Saeed N, Khan M, Shabbir M. Antioxidant activity, total phenolics and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Compl Altern Med*. 2012; 12:221.
5. Khan RA, Khan MR, Sahreen S. Protective effect of *Sonchus asper* extracts against experimentally induced lung injuries in rats: A novel study. *Exp Toxicol Pathol*. 2012; 64:725-31.
6. Adikwu E, Deo O. Hepatoprotective effect of vitamin C (Ascorbic Acid). *Pharmacol Pharm*. 2013; 4:84-92.
7. Singal AK, Jampana SC, Weinman SA. Antioxidants as therapeutic agents for liver disease. *Liver Int*. 2011; 31:1432-48.
8. Kohen R, Nyska A. Oxidation of biological systems: Oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol Pathol*. 2002; 30:620-50.
9. Li S, Chen G, Zhang C, Wu M, Wu S, Liu Q. Research progress of natural antioxidants in foods for the treatment of diseases. *Food Sci Hum Wellness* 2014; 3:110-6.
10. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Mole*. 2010; 15:7313-52.
11. Bahadoran Z, Mirmiran P, Azizi F. Dietary polyphenols as potential nutraceuticals in management of diabetes: A review. *J Diabetes Metab Disord*. 2013; 12:9 pages.
12. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. *The Sci World J*. 2013:16 pages.
13. Randhir R, Lin Y-T, Shetty K. Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. *Asia Pac J Clin Nutr*. 2004; 13:295-307.
14. Ramawat KG, Dass S, Mathur M. The chemical diversity of bioactive molecules and therapeutic potential of medicinal plants. In: *Herbal drugs: Ethnomedicine to modern medicine*. Ramawat KG. (Ed.). Springer-Verlag Berlin Heidelberg. 2009; pp. 7-31.
15. Tsao R. Chemistry and biochemistry of dietary polyphenols. *Nutr*. 2010; 2:1231-46.
16. Chikezie PC, Ibegbulem CO, Mbagwu FN. Bioactive principles from medicinal plants. *Res J Phytochem*. 2015; 9:88-115.
17. Aqil F, Ahmad I, Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turk J Biol*. 2006; 30:177-83.
18. Yoshimi N, Keita S, Fumihiko Y, Masahiro K, Toshihiko O. Extensive screening for herbal extracts with potent antioxidant properties. *J Clin Biochem Nutr*. 2011; 48:78-84.
19. Jyothi AN, Sasikiran BN, Balagopalan C. Optimization of glutamic acid production from cassava starch factory residues using *Brevibacterium divaricatum*. *Process Biochem*. 2005; 40:3576-9.
20. Belonwu DC, Ibegbulem CO, Nwokocha MN, Chikezie PC. Some phytochemicals and hydrophilic vitamins of *Anacardium occidentale*. *Res J Phytochem*. 2014; 8:78-91.
21. Ojiako AO, Chikezie PC, Ogbuji CA. Radical scavenging potentials of single and combinatorial herbal formulations *in vitro*. *J Tradit Compl Med*. 2016; 6:153-9.
22. Adegoke E, Akinsanya A. Studies of Nigerian medicinal plants. *J West Afr Sci Assoc*. 1970; 132:15-65.
23. Ojiako OA, Igwe CU, Agha NC, Ogbuji CA, Onwuliri VA. Protein and amino acid compositions of *Sphenostylis stenocarpa*, *Sesamum indicum*, *Monodora myristica* and *Azela africana* seeds from Nigeria. *Pak J Nutr*. 2010; 94:368-72.
24. Middleton E, Kandaswami C, Theohardes TC. The effects of plant flavonoids on mammalian cells, implication for inflammation, heart disease and cancer. *Pharmacol Rev*. 2000; 52:673-751.
25. Ekeanyanwu CR, Ugu IG, Nwachuku UP. Biochemical characteristics of the African nutmeg, *Monodora myristica*. *J Agric Food Chem* 2010; 5:303-8.
26. Akinwunmi KF, Oyedapo OO. Evaluation of antioxidant potentials of *Monodora myristica* (Gaertn) dunel seeds. *Afr J Food Sc*. 2013; 7:317-24.
27. Okafor JC. Development of forest tree crops for food supply in Nigeria. *Forest Ecology and Management* 1987; 1:235-247.
28. Udeala OK. Preliminary evaluation of dike fat a new tablet lubricant. *J Pharm Pharmacol*. 2000; 32:6-9.

29. Iwu MM. Evaluation of the anti-hepatotoxic activity of the bioflavonoids of *Garcinia kola* seeds. *J Ethnopharmacol.* 2002; 21:14–9.
30. Chakraborty AK, Ranbhade S, Patil UK. *Chromolaena odorata* (L): An overview. *J Pharm Res.* 2011; 4:573.
31. Pandith H, Zhang X, Liggert J, Min K, Gritsanapant W, Baek SJ. Hemostatic and wound healing properties of *Chromolaena odorata* leaf extract. *ISRN Dermatol.* 2013; 2013:8 pages.
32. Alisi CS, Nwogu LA, Ibegbulem CO, Ujowundu CU. Antimicrobial action of methanol extract of *Chromolaena odorata* Linn is logistic and exerted by inhibition of dehydrogenase enzymes. *J Res Biol.* 2011; 3:209–16.
33. Anyasor GN, Aina DA, Olushola M, Aniyikaye AF. Phytochemical constituent, proximate analysis, antioxidant, antibacterial and wound healing properties of leaf extracts of *Chromolaena Odorata*. *Ann Biol Res.* 2011; 2:41.
34. Nwaehujor CO, Ode OJ, Nwinyi FC, Udeh NE. Effect of methanol extract of *Buchholzia coriacea* fruits on streptozotocin-induced diabetic rats. *J Pharmacol Toxicol.* 2012; 7:181–91.
35. Ibrahim TA, Fagboun ED. Phytochemical and nutritive quality of dried seed of *Buchholzia coriacea*. *Greener J Phys Sci.* 2013; 2:185–91.
36. Mbata TI, Ikenebomeh MJ, Ezeibe S. Evaluation of mineral content and functional properties of fermented maize (generic and specific) flour blended with bambara groundnut. *Afr J Food Sci* 2009; 3:107–12.
37. Amaechi NC. Nutritive and anti-nutritive evaluation of wonder kola (*Buchholzia coriacea*) seeds. *Pak J Nutr.* 2009; 8:1120–2.
38. Adisa RA, Choudhary MI, Olorunsogo OO. Hypoglycemic activity of *Buchholzia coriacea* (Capparaceae) seeds in streptozotocin-induced diabetic rats and mice. *Exp Toxicol Pathol.* 2010; 63:619–25.
39. Porter D, Doyle JJ. Origins of the African yam bean (*Sphenostylis stenocarpa* Leguminosae): Evidence from morphology, isoenzymes and chloroplast DNA. *Soc Econ Bot.* 1992; 46:276–92.
40. Ajibola C, Fashakin JB, Fagbemi TN, Aluko RE. Effect of peptide size on antioxidant properties of African yam bean seed (*Sphenostylis stenocarpa*) protein hydrolysate fractions. *Int J Mol Sci.* 2011; 12:6685–702.
41. Okonkwo CC, Njoku OU, Ikevude CT, Odo CE. Hepatoprotective effect of methanol extract of *Sphenostylis stenocarpa* (Hoschst ex. A. Rich. Harms) against carbon tetrachloride-induced liver toxicity in Wistar rats. *J Pharm Res* 2013; 6:293–298.
42. Fagbemi TN, Oshodi AA, Ipinmoroti KO. Processing effects on some antinutritional factors and *in vitro* multienzyme protein digestibility (ivpd) of three tropical seeds, breadnut (*Artocarpus altilis*), cashewnut (*Anacardium occidentale*) and fluted pumpkin (*Telfairia occidentalis*). *Pak J Nutr.* 2005; 4:250–6.
43. Hefnawy TH. Effect of processing methods on nutritional composition and anti-nutritional factors in lentils (*Lens culinaris*). *Ann Agric Sci.* 2011; 56:57–61.
44. Chu C, Ho K, Hu A, Chiu C, Wu H, Ye S, Ho K, Chen L. Toxicity attenuation of Atractyloside in traditional Chinese medicinal herbs after hydrothermal processing. *Bot Stud* 2012; 53:459–65.
45. Chen F, Xiong H, Wang J, Ding X, Shu G, Mei Z. Antidiabetic effect of total flavonoids from *Sanguis draxonis* in Type 2 diabetic rats. *J Ethnopharmacol.* 2013; 149:729–36.
46. Gawlik-Dziki U. Changes in the antioxidant activities of vegetables as a consequence of interactions between active compounds. *J Funct Food.* 2012; 4:872–82.
47. Hwang I, Lee J, Huh JY, Park J, Lee HB, Ho YS, Ha H. Catalase deficiency accelerates diabetic renal injury through peroxisomal dysfunction. *Diabetes.* 2012; 61:728–38.
48. Pellegrini N, Chiavato E, Gardama C, Mazzeo T, Continto D, Gallo M, Riso P, Fogliano V, Porrini M. Effect of different cooking methods in color, phytochemical concentration and antioxidant capacity of raw and frozen *Brassica* vegetables. *J Agric Food Chem.* 2010; 58:4310–21.
49. Sommano S, Caffin N, McDonald J, Cocksedge E. The impact of thermal processing on bioactive compounds in Australian native food products (bush tomato and Kakadu plum). *Food Res Int.* 2013; 50:557–61.
50. Thi ND, Hwang E-S. Effects of drying methods on contents of bioactive compounds and antioxidant activities of black chokeberries (*Aronia melanocarpa*). *Food Sci Technol.* 2016; 25:55–61.
51. Ojiako AO, Chikezie PC, Ogbuji CA. Histopathological studies of renal and hepatic tissues of hyperglycemic rats administered with traditional herbal formulations. *Int J Green Pharm.* 2015; 9:184–91.
52. Yebio A, Gebrelibanos M, Karim A, Gebremedhin G, Sintayehu B, Periasamy G. Comparison of vitamin C content in fresh and packed juices of orange and mango. *Int J Pharmacog.* 2015; 2:88–92.
53. Kirk RS, Sawyer R. Vitamins in Pearson. Composition and analysis of foods. 9th Ed. Longman Group Limited, UK. 1991; pp. 330–6.
54. Suanarunsawat T, Ayutthaya WDN, Thirawarapan S, Pongshompoo S. Anti-oxidative, anti-hyperglycemic and lipid-lowering effects of aqueous extracts of *Ocimum sanctum* L. leaves in diabetic rats. *Food Nutr Sci.* 2014; 5:801–11.
55. Kumar V, Hussain PR, Chatterjee S, Variyar PS. Evaluation of *in vitro* antioxidant activity and characterization of phenolics compounds of bottle gourd towards the green synthesis of gold nanoparticles and its bio-efficacy. *Int J Food Nutr Saf.* 2015; 6:125–49.
56. Nishimiki M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem Biophys Res Commun.* 1972; 46:849–53.
57. Banerjee D, Madhusoodanan UK, Sharanabasappa M, Ghosh S, Jacob J. Measurement of plasma hydroperoxide concentration by FOX-3 assay in conjunction with triphenylphosphine. *Clin Chim Acta.* 2003; 337:147–52.
58. Elizabeth K, Rao MNA. Oxygen radical scavenging activity of curcumin. *Int J Pharm.* 1990; 58:237–40.
59. Isfahlan AJ, Mahmoodzadeh J, Hassanzadeh A, Heidari R, Jamei R. Antioxidant and antiradical activities of phenolics extracts from Iranian almond (*Prunus amygdalus* L.) hull and shells. *Turk J Biol.* 2010; 34:165–73.
60. Oyaizu M. Studies on products of browning reaction: Antioxidative activity of products of browning reaction prepared from glucosamine. *Japan J Nutr.* 1986; 44:307–15.
61. Zhang D, Hamazu Y. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. *Food Chem.* 2004; 88:503–9.
62. Adefegha SA, Oboh GO. Cooking enhances the antioxidant properties of some tropical leafy vegetables. *Afr J Biotechnol.* 2011; 10:632–9.
63. Nithiyannantham S, Selvakumar S, Siddhuraju P. Total phenolics content and antioxidant activity of two different solvent extracts from raw and processed legumes, *Cicer arietinum* L. and *Pisum sativum* L. *J Food Composition Anal.* 2012; 27:52–60.
64. Klein BP, Kurlich AC. Processing effects on dietary antioxidants from plant foods. *Hortsci.* 2000; 35:580–4.
65. Kaur P, Bains K, Kaur H. Effect of hydrothermal treatment on free radical scavenging potential of selected green vegetables. *Indian J Nat Prod Resour.* 2012; 3:563–9.
66. Song AS, Lim SW, Kim SJ, Lee SC. Effect of hydrothermal treatment on the antioxidant activity of *Sambaekecho* (*Saururus chinensis*) leaves. *Food Sci Biotechnol.* 2013; 22:825–9.
67. Prior RL, Cao G, Martin A, Sofic E, McEwen J, O'Brien C, Lischner N, Ehlenfeldt M, Kalt W, Krewer G, Mainland MC. Antioxidant capacity as influenced by total phenolics and anthocyanin content, maturity, and variety of *Vaccinium* species. *J Agric Food Chem.* 1998; 46:2686–93.
68. Ehlenfeldt MK, Prior RL. Oxygen radical absorbance capacity (ORAC) and phenolics and anthocyanin concentrations in fruit and leaf tissues of high bush blueberry. *J Agric Food Chem.* 2001; 49:2222–7.
69. Wang SY, Jiao H. Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry. *J Sci Food Agric.* 2000; 48:5672–6.
70. Tomás-Barberán FA, Espín JC. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *J Sci Food Agric.* 2001; 81:853–76.
71. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agro-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem.* 2006; 99:191–203.
72. Podsedek A. Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. *Lebensmittel-Wissenschaft Technol.* 2007; 40:1–11.
73. Azizah AH, Wee KC, Azizah O, Azizah M. Effect of boiling and stir frying on total phenolics, carotenoids and radical scavenging activity of pumpkin (*Cucurbita moschato*). *Int Food Res J.* 2009; 16:45–51.

Ojiako et al: Radical scavenging potentials

74. Dewanto V, Wu X, Liu RH. Processed sweet corn has higher antioxidant activity. J Agric Food Chem. 2002; 50:4959–64.
75. Siddhuraju P, Becker K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. Food Chem. 2007; 101:10–9.
76. Ramadan MF, Kroh LW, Moersel JT. Radical scavenging activity of black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.) and niger (*Guizotia abyssinica* Cass.) crude seed oils and oil fractions. J Agric Food Chem. 2003; 51:6961–9.
77. Ramadan MF, Moersel JT. Impact of enzymatic treatment on chemical composition, physicochemical properties and radical scavenging activity of golden berry (*Physalis peruviana* L.) juice. J Sci Food Agric. 2007; 87:452–60.
78. Kim IS, Yang M, Goo TH, Jo C, Ahn DU, Park JH, Lee OH, Kang SN. Radical scavenging-linked antioxidant activities of commonly used herbs and spices in Korea. Int J Food Sci Nutr. 2011; 2011:1-7.
79. Jing L, Ma H, Fan P, Gao R, Jia Z. Antioxidant potential, total phenolics and total flavonoid contents of *Rhododendron anthopogonoides* and its protective effect on hypoxia-induced injury in PC12 cells. BMC Compl Altern Med. 2015; 15:287.
80. Muchuweti M, Nyamukonda L, Chagonda L, Ndhala A, Mupure C, Benhura M. Total phenolics content and antioxidant activity in selected medicinal plants of Zimbabwe. Int J Food Sci Technol. 2006; 41:33–8.

© TEMKODER. This is an open access article licensed under the terms of the **Creative Commons Attribution Non-Commercial License** (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, noncommercial use, distribution and reproduction in any medium, provided that the work is properly cited.

Conflict of Interest: None declared