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Original Article

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

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ABSTRACT

Aim/Background: The present study evaluated the antioxidant vitamins and total phenolics contents from *Monodora myristica*, *Chromolaena odorata*, *Buccholzia 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 ± 0.04 – 7.97 ± 0.20 mg GAE/g dry sample. The SCl_{50} of raw herbal extracts against $O_2^{\bullet-}$ was within the range of 201.61 ± 4.09 – 305.21 ± 5.11 μg/mL, whereas those of corresponding hydrothermal processed herbs gave 211.02 ± 4.15 – 531.66 ± 8.14 μg/mL. For the most part, SCl_{50} of the raw herbs against NO^- 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

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 ($RO_2^{\bullet-}$), peroxynitrite (^-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 $O_2^{\bullet-}$, $^{\bullet-}OH$, $^1O_2$, $RO_2^{\bullet-}$, ^-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-Asteraeae [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]. *Buccholzia 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 ± 5°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-Willey milling machine (ASTM D-3182, India). The ground samples were transferred into corresponding vacuum desiccators and allowed to dry at T = 24 ± 5°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-Willey 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% CH_3OH (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 ≤ 4 °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 $Na_2HPO_4.2H_2O$ and 2.43 g $NaH_2PO_4.2H_2O$). 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 $T = 24 \pm 5$°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 KIO_3 and 12 mL of 3.0 M H_2SO_4 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:

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

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

V_{st} = Titer volume of herbal extract.

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

V_{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 H_2SO_4 (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 $T = 24 \pm 5$°C. Equal weights of the extract and α-tocopherol were dissolved in separate 10 mL absolute C_2H_5OH. 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 C_2H_5OH and 1.0 mL of concentrated HNO_3 and allowed to stand for 5 min. The absorbance, at maximum absorptivity (ƛmax) = 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% Na_2CO_3 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 ƛmax = 750 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:

$$Y = 0.0221X + 0.001 \qquad Eq\ 2$$

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 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 µg/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 λmax = 530 nm. The herbal extract superoxide radical scavenging capacity index (SORSCI) was calculated thus:

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

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 (µg/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 µg/mL) of the herbal extracts were incubated at 25°C for 30 min. A 0.85 mL FOX Reagent (100 µM xylenol 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-xylenol orange complex of the mixtures were measured against a blank at λmax = 560 nm.

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

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

The HPSCI% was expressed as SCI_{50}, which is defined as the concentration (µg/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 $^{\bullet-}OH$ 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 µg/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 λmax = 532 nm against a blank.

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

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

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

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 µg/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 λmax = 540 nm using quercetin as blank [21]. The nitric oxide scavenging capacity index (NOSCI) of the herbal extract was calculated thus:

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

The NOSCI% was expressed as SCI_{50}, which is defined as the concentration (µg/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 µg/mL) of each herbal extract (Extract R1–R4; Extract H1–H4) and 1.0% $K_3Fe(CN)_6$ 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 λmax = 700 nm against a blank.

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

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

The FRAP% was expressed as AP_{50}, which is defined as the concentration (µg/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 $O_2^{\bullet-}$ was within the range of 201.61 ± 4.09 – 305.21 ± 5.11 μg/mL, whereas those of corresponding Extracts H1–H4 gave 211.02 ± 4.15 – 531.66 ± 8.14 μg/mL. Figure 1 showed that SCI_{50} of Extract R1 against $O_2^{\bullet-}$ was not significantly different ($p < 0.05$) from that of corresponding Extract H1. Conversely, SCI_{50} of Extracts R2–R4 against $O_2^{\bullet-}$ was comparatively lower than those of corresponding Extracts H2–H4; $p < 0.05$. Overall, SCI_{50} of Extract H4 against $O_2^{\bullet-}$ gave the highest value, whereas that of Extract R2 represented the lowest SCI_{50}; $p < 0.05$.

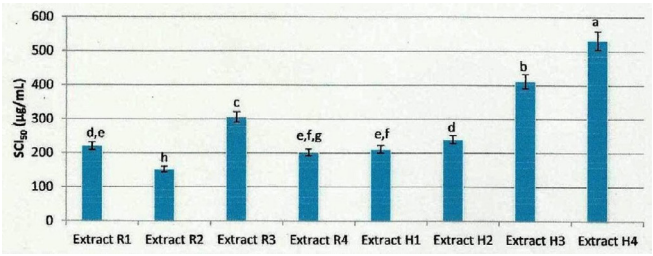

Figure 1. Superoxide radicals scavenging capacities of" raw and hydrothermal processed herbal extracts. Means denoted by the same letter are not significantly different at ***p*** > 0.05 according to LSD.

Figure 2 showed that SCI_{50} of Extracts R1–R4 against H_2O_2 ranged between 119.23 ± 2.99 – 162.49 ± 3.31 μg/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 ± 3.32 μg/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 $^{\bullet-}OH$ 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 NO^- 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

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

The mean (X) ± 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.

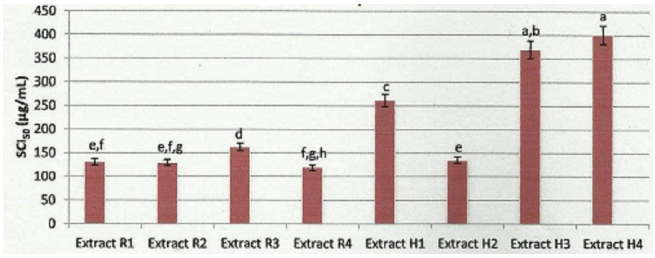

Figure 2. Hydrogen peroxide scavenging capacities of raw and hydrothermal processed herbal extracts. Means denoted by the same letter are not significantly different at $p > 0.05$ according to LSD.

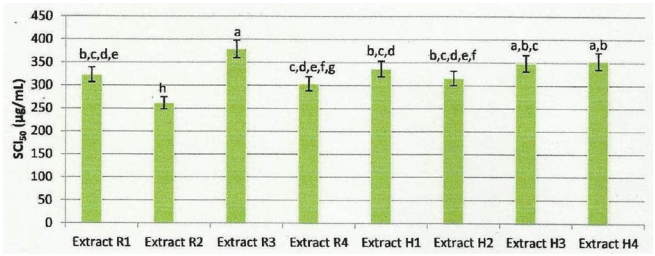

Figure 3. Hydroxyl radicals scavenging capacities of raw and hydrothermal processed herbal extracts- Means denoted by the same letter are not significantly different at ***p>*** 0.05 according to LSD.

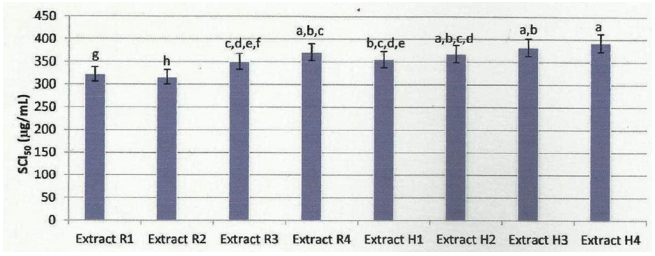

Figure 4. Nitric oxide radicals scavenging capacities of raw and hydrothermal processed herbal extracts. Means denoted by the same letter are not significantly different at $p > 0.05$ according to LSD.

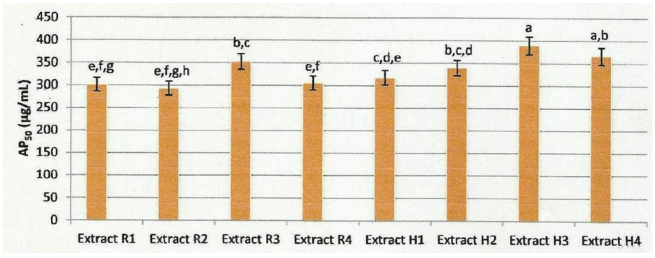

Figure 5. Ferric reducing antioxidant powers of raw and hydrothermal processed herbal extracts. Means denoted by the same letter are not significantly different at $p > 0.05$ according to LSD.

H4. Generally, SCI_{50} of Extracts R1–R4 and corresponding Extracts H1–H4 against NO^- was within the range of 316.42 ± 3.13 – 393.14 ± 3.22 μg/mL (Figure 4).

Figure 5 showed that AP_{50} of Extracts R1–R4 were significantly lower ($p < 0.05$) than those of corresponding Extracts H1–H4. Extract R2 gave the lowest AP_{50}, 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 SCI_{50} against $O_2^{\bullet-}$, H_2O_2, $^{\bullet-}OH$ and NO^- as well as AP_{50} against ferric ion gave negative correlation coefficients. Specifically, the ascorbic acid content of raw herbal extract and SCI_{50} against NO^- showed a weak negative correlation coefficient, whereas that of SCI_{50} against H_2O_2 gave a fair negative correlation coefficient (Table 2). The ascorbic acid contents of hydrothermal processed herbal extracts and their corresponding SCI_{50} against $O_2^{\bullet-}$, H_2O_2, $^{\bullet-}OH$ and NO^- gave strong negative correlation coefficients. Additionally, the correlation coefficients between ascorbic acid contents and SCI_{50} against $O_2^{\bullet-}$ and $^{\bullet-}OH$ as well as AP_{50} against ferric ion of raw and hydrothermal proceeded herbal extracts were comparable.

The α-tocopherol contents of raw herbal extracts and their corresponding SCI_{50} against $O_2^{\bullet-}$, H_2O_2, $^{\bullet-}OH$ 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 SCI_{50} against H_2O_2, and NO^- gave poor negative correlation coefficients except SCI_{50} against $O_2^{\bullet-}$ and $^{\bullet-}OH$. Conversely, α-tocopherol contents of hydrothermal processed herbal extracts and their corresponding SCI_{50} against $O_2^{\bullet-}$ and NO^- gave strong positive correlation coefficients except corresponding SCI_{50} against H_2O_2 and $^{\bullet-}OH$. The α-tocopherol contents of raw herbal extracts and corresponding AP_{50} 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

	Correlation coefficient (*r*)	
Parameters (SCI_{50}/AP_{50})	Raw herbal extracts	Hydrothermal processed herbal extracts
$O_2^{\bullet-}$	-0.92714	-0.89865
H_2O_2	-0.64174	-0.98787
$^{\bullet-}OH$	-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

	Correlation coefficient (*r*)	
Parameters (SCI_{50}/AP_{50})	Raw herbal extracts	Hydrothermal processed herbal extracts
$O_2^{\bullet-}$	-0.76256	0.857854
H_2O_2	-0.31071	0.542208
$^{\bullet-}OH$	-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
$O_2^{\bullet-}$	-0.52938	-0.93651
H_2O_2	-0.00741	-0.68936
$^{\bullet-}OH$	-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 SCI_{50} against $O_2^{\bullet-}$, H_2O_2, $^{\bullet-}OH$ and NO^- as well as AP_{50} against ferric ion gave negative correlation coefficients. Markedly, the total phenolics contents of raw herbal extracts and SCI_{50} against H_2O_2 gave a very poor negative correlation coefficient (Table 4). Furthermore, correlation coefficients between total phenolics contents and SCI_{50} against NO^- of the raw herbal extracts exhibited a strong negative index. Additionally, the correlation coefficients between total phenolics contents and SCI_{50} against $O_2^{\bullet-}$ as well as AP_{50} against ferric ion of hydrothermal proceeded 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 $O_2^{\bullet-}$, H_2O_2 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 Nithiyanantham *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 SCI_{50} or AP_{50}. Accordingly, the correlation coefficients between the antioxidant vitamins/total phenolics contents and experimentally derived SCI_{50} or AP_{50} 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 SCI_{50} or AP_{50} 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 SCI_{50} against $O_2^{\bullet-}$ and $^{\bullet-}OH$. Likewise, the capacity of hydrothermal processed herbal extracts to scavenge $O_2^{\bullet-}$ 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 SCI_{50} against $O_2^{\bullet-}$, H_2O_2, $^{\bullet-}OH$ and NO^- or AP_{50} against ferric ion. In a related study, which concurred with the present findings, $O_2^{\bullet-}$ 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.

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