



# Characterization of polyphenolic constituents and radical scavenging ability of ripe tomato and red pepper fruits

Seun F. Akomolafe<sup>1,2</sup>, Ganiyu Oboh<sup>2</sup>

<sup>1</sup>Department of Biochemistry, Ekiti State University, Ado Ekiti, Nigeria, <sup>2</sup>Functional Foods and Nutraceuticals Unit, Department of Biochemistry, Federal University of Technology, Akure, Nigeria

## Address for correspondence:

Seun F. Akomolafe,  
Department of Biochemistry,  
Ekiti State University, P.M.B  
5363, Ado Ekiti, Nigeria.  
E-mail: purposefulseun@  
yahoo.co.uk

Received: October 29, 2014

Accepted: March 03, 2015

Published: March 03, 2015

## ABSTRACT

**Objective:** Characterization of polyphenolic contents and the inhibitory effects of aqueous extracts of ripe tomato (*Lycopersicon esculentum*) and red pepper (*Capsicum annuum*) fruits on the sodium nitroprusside (SNP) and iron(II) ( $\text{Fe}^{2+}$ )-induced lipid peroxidation in rat liver were examined in this study. **Materials and Methods:** Various experimental models such as the ABTS • (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) scavenging ability and ferric reducing power were used to characterize the antioxidant activity of the extracts. High-performance liquid chromatography (HPLC) was used to determine the phenolic content of the extracts. Malondialdehyde (MDA) was used as a measure of oxidative stress in the rat's liver tissue. **Results:** The ABTS • scavenging ability and ferric reducing power of the aqueous extract of ripe red pepper were significantly higher than that of ripe tomato. Ripe red pepper and tomato fruits extracts inhibited  $\text{NO} \bullet$  in a concentration dependent manner. Furthermore, the introduction of extracts of ripe red pepper and tomato caused a significant concentration-dependent decrease in the MDA content of the SNP and  $\text{Fe}^{2+}$ -stressed liver homogenates. In addition, HPLC analyses of the extracts revealed the presence of different phenolic compounds. **Conclusion:** With respect to the results of the current study, ripe tomato, and red pepper could be considered to be potential sources of natural antioxidants.

**KEY WORDS:** Antioxidant activity, liver, malondialdehyde, phenolic content, red pepper, tomato

## INTRODUCTION

Reactive oxygen species (ROS) have been implicated in the pathogenesis of many disease states [1]. Oxidative stress results from either a decrease of natural cell antioxidant capacity or an increased amount of ROS in organisms. It is well-established that free radicals are associated with the process that leads to cell degeneration, especially in organs such as liver, kidney, and testes [2]. Although iron is physiologically necessary as a component of many enzymes and proteins, free iron in the cytosol and mitochondria could cause considerable oxidative damage by acting catalytically in the production of ROS, which have the potential to damage cellular lipids, nucleic acids, proteins, and carbohydrate resulting in wide-ranging impairment in cellular function and integrity [3].

Sodium nitroprusside (SNP) is an anti-hypertensive drug, which acts by relaxing smooth vascular muscle; consequently it dilates peripheral arteries and veins. However, it could cause cytotoxicity through the release of cyanide ion and/or nitric oxide (NO). ROS can directly attack and degrade the polyunsaturated fatty acids of the cell membranes and induce

lipid peroxidation. Malondialdehyde (MDA) is the end product of lipid peroxidation; it is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism [4].

However, the most likely and practical way to fight degenerative diseases is to improve body antioxidant status, which could be achieved by higher consumption of fruits and vegetables. Phenolic compounds are an important group of secondary metabolites, which are synthesized by plants because of plant adaptation to biotic and abiotic stress conditions such as infection, water stress and cold stress [5].

Red pepper fruits (*Capsicum annuum* L. [Solanaceae]) are important vegetables used as vegetable foods, as the spice added to food fresh, dried, refined, and ground, and as the principal or incidental ingredient in sauces. The phytochemicals in peppers have been reported to possess many biochemical and pharmacological properties, such as antioxidant, anti-inflammatory, anti-allergic and anticarcinogenic activities [6,7]. Ripe red peppers are naturally rich in ascorbic acid (vitamin C)

and provitamin A [8,9], which neutralize free radicals in the human body and, thus, reduce the risk of diseases, such as arthritis, cardiovascular disease [10], and cancer [11], in addition to delaying the aging process [12]. Carotenoids, which are fat-soluble antioxidants found in peppers, have received considerable interest by researchers due to their antioxidant properties [13] and the necessity for human epithelial cellular differentiation [14]. In addition, several studies have demonstrated the antimicrobial activity of peppers [15,16].

Tomatoes (*Lycopersicon esculentum* Mill. [Solanaceae]), are consumed fresh or as processed products (sauces, juice, ketchup, soup). The consumption of fresh tomatoes and tomato products has been inversely related to the development of some types of cancer [17] and to plasma lipid peroxidation [18]. Tomato contains different classes of substances with antioxidant properties such as carotenoids, ascorbic acid, and phenolics. Lycopene is the major carotenoid present in tomato and has been revealed to show strong antioxidant activity both *in vitro* and *in vivo* [19]. In our previous study, we reported the inhibitory effect of the aqueous extract of ripe tomato and red pepper fruits on  $\text{Fe}^{2+}$ -induced lipid peroxidation in rat testes and kidneys *in vitro* and we discovered that the aqueous extract of tomato had the highest total phenolic content, flavonoid content, vitamin C content, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH $\cdot$ ), and hydroxyl radical ( $\cdot\text{OH}$ ) scavenging ability while the iron(II) ( $\text{Fe}^{2+}$ )-chelating ability of the two extracts were almost the same. Furthermore, the two extracts caused a significant decrease in the MDA contents of the testes and kidney tissues, with aqueous extract of tomato having the highest effect. We therefore concluded that the protective effect of the extracts on  $\text{Fe}^{2+}$ -induced lipid peroxidation in rat testes and kidneys could be attributed to their polyphenolic compounds [20].

Although several works have been reported on the antifungal, antiviral, anti-carcinogenic and antioxidant properties of tomato and red pepper fruits [6,7,19], there is, however, limited information on its potential in the prevention of oxidative stress in the liver. Hence, the objective of this study was to characterize its polyphenolic contents and investigate the inhibitory effect of water extractable phytochemicals from ripe tomato and red pepper fruits on iron(II) sulfate ( $\text{FeSO}_4$ ) and SNP-induced lipid peroxidation in rat liver *in vitro* as continuation of our earlier studies.

## MATERIALS AND METHODS

### Collection and Identification of Plant Samples

Fresh ripe samples of tomato and red pepper fruits were obtained from a local farmland in Akure metropolis, Nigeria. Authentication of the samples was carried out by Mr. Ajayi at the Department of Plant Science, Ekiti State University, Ado Ekiti, Nigeria, where voucher specimens (numbers UHAE 333 and UHAE 334 for tomato and red pepper fruits, respectively) were deposited at the herbarium.

## Experimental Animals

A total of 10 adult male Wistar strain rats were purchased from the Animal Production and Health Department, Federal University of Technology, Akure, and acclimatized for 2 weeks, during which period they were maintained *ad libitum* on a commercial diet and water. The experimental study was approved by the Institutional Animal Ethics Committee of the Ekiti State University, Nigeria. The handling of animals was carried out in accordance with the recommended international standards.

## Chemicals and Reagents

Thiobarbituric acid (TBA), 1,10-phenanthroline, deoxyribose, ascorbic acid, Folin–Ciocalteu's reagent, Catechin, gallic acid, chlorogenic acid, caffeic acid, coumarin, quercetin, quercitrin, rutin, luteolin, and kaempferol were procured from Sigma-Aldrich Inc, St. Louis, MO, USA. Trichloroacetic acid (TCA), MDA, and DPPH were sourced from Sigma-Aldrich Chemie GmbH, Steinheim, Germany. Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), methanol, acetic acid, and hydrochloric acid (HCl) were sourced from BDH Chemicals Ltd, Poole, England. Sodium carbonate, aluminum chloride ( $\text{AlCl}_3$ ), potassium acetate, Tris-HCl buffer, sodium dodecyl sulfate (SDS),  $\text{FeSO}_4$ , potassium ferricyanide and ferric chloride were of analytical grade while the water was glass distilled. A UV-visible spectrophotometer (Jenway Model 6305; Barloworld Scientific Ltd, Dunmow, United Kingdom) was used to measure absorbance. High performance liquid chromatography with photodiode array detection (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC Solution v1.22 SP1 software.

## Aqueous Extract Preparation

The samples were washed under running water to remove any contaminant, air-dried in order to preserve the sample; thereafter the dried samples were ground to powdered form, and kept dry in an airtight container prior to the extraction. The powdered sample (1 g) was weighed into 20 ml of distilled water and was left for 24 h [21]. Then, the mixture was filtered, and the filtrate centrifuged at 400 g for 10 min. The clear supernatant was collected and used for subsequent analysis.

## Reducing Property

The reducing properties of the two extracts were determined by assessing the ability of the extract to reduce a  $\text{FeCl}_3$  solution as described by Pulido *et al* [22]. A 2.5 ml aliquot was mixed with 2.5 ml, 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml, 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, and then 2.5 ml, 10% TCA was added and centrifuged at 650 g for 10 min. 5 ml of the supernatant was mixed with an equal volume of water and 1 ml, 0.1% ferric chloride. The same treatment was performed to a standard

ascorbic acid solution and the absorbance taken at 700 nm. The reducing power was then calculated and expressed as ascorbic acid equivalents (AAE).

### ABTS (2,2-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid]) Radical Scavenging Ability

The ABTS• scavenging ability of both extracts was determined according to the method of Re *et al.* [23]. ABTS• was generated by reacting an ABTS• aqueous solution (7 mmol/l) with potassium persulfate ( $K_2S_2O_8$ ; 2.45 mmol/l final concentration) in the dark for 16 h; 0.2 ml of appropriate dilution of the extract was added to 2 ml ABTS• solution and the absorbance were measured at 734 nm after 15 min. The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated.

### NO Scavenging Assay

The NO scavenging activity of the extracts was evaluated by the method of Igbino *et al.* [24]. 25 millimole SNP (1 ml) prepared in 0.5 mM phosphate buffer saline (pH 7.4) was added to 0.5 ml of plant extract or standard (100-400 µg/ml) and vortexed. The mixture was incubated for 2 h at 37°C and, thereafter, 1 ml of the mixture was taken and mixed with 1 ml of Griess reagent (equal volumes of 1% sulfanilic acid prepared in 2% orthophosphoric acid and 0.01% (w/v) naphthalenediamine dichloride) and incubated at room temperature for 30 min. The absorbance was read at 546 nm, and the percentage NO inhibition by the extracts was calculated using the following equation:

$$\text{NO scavenging activity (\%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{control}})} \times 100$$

-Abs<sub>control</sub>: absorbance of NO radicals.

-Abs<sub>sample</sub>: absorbance of NO radical plus sample or standard.

### Preparation of Tissue Homogenate

The rats were decapitated under mild diethyl ether anesthesia, and the liver tissue was rapidly dissected and placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1:10 w/v) with about 10-up-and-down strokes at approximately 1200 g in a Teflon-glass homogenizer. The homogenate was centrifuged for 10 min at 3000 g to yield a pellet that was discarded, and a low-speed supernatant (S1) containing mainly water, proteins and lipids (cholesterol, galactolipid, individual phospholipids, gangliosides) was kept for the lipid peroxidation assay [25].

### Lipid Peroxidation and TBA Reactions

The lipid peroxidation assay was carried out using the method of Ohkawa *et al.* [26]. Briefly, 100 ml S1 fraction was mixed with a reaction mixture containing 30 ml of 0.1 M Tris-HCl buffer (pH 7.4), extracts of red pepper and tomato (0-100 ml) and 30 ml of 250 mM freshly prepared FeSO<sub>4</sub>. The volume was

made up to 300 ml with water before incubation at 37°C for 1 h. The color reaction was developed by adding 300 ml, 8.1% SDS to the reaction mixture containing S1; this was subsequently followed by the addition of 500 ml of acetic acid/HCl (pH 3.4) and 500 ml, 0.8% TBA. This mixture was incubated at 100°C for 1 h. The absorbance of TBA reactive species produced was measured at 532 nm. MDA produced was expressed as % control, The IC<sub>50</sub> (extract concentration required to inhibit 50% of MDA produced) values were calculated using nonlinear regression analysis.

### Quantification of Compounds by HPLC-DAD

Reverse phase chromatographic analyses were carried out under gradient conditions using C<sub>18</sub> column (4.6 mm × 150 mm) packed with 5 µm diameter particles; the mobile phase was water containing 2% formic acid (A) and acetonitrile (B), and the composition gradient was: 17% of B until 10 min and changed to obtain 20, 30, 50, 60, 70, 20, and 10% B at 20, 30, 40, 50, 60, 70, and 80 min, respectively, following the method described by Kamdem *et al.* [27] with slight modifications. Aqueous extracts were analyzed at concentrations of 20 mg/ml. The presence of ten antioxidants compounds was investigated, namely, gallic acid, chlorogenic acid, caffeic acid, catechin, coumarin, quercetin, quercitrin, rutin, luteolin, and kaempferol. Identification of these compounds was performed by comparing their retention time and UV absorption spectrum with those of the commercial standards. The flow rate was 0.7 ml/min, injection volume 40 µl and the wavelength were 270 nm for gallic acid, 276 nm for coumarin, 280 nm for catechin, 327 nm for caffeic and chlorogenic acids, and 365 nm for quercetin, quercitrin, rutin, kaempferol, and luteolin. The samples and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.025-0.25 mg/ml for catechin, coumarin, quercetin, quercitrin, rutin, kaempferol, and luteolin; and 0.03-0.35 mg/ml for caffeic, chlorogenic, and gallic acids.

The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200-600 nm). [Calibration curves for catechin:  $Y = 13649x + 1257.8$  ( $r = 0.9995$ ); coumarin:  $Y = 11974x + 1309.5$  ( $r = 0.9999$ ); gallic acid:  $Y = 12890x + 1253.7$  ( $r = 0.9998$ ); caffeic acid:  $Y = 13078x + 1186.3$  ( $r = 0.9991$ ); chlorogenic acid:  $Y = 11952x + 1187.5$  ( $r = 0.9996$ ); rutin:  $Y = 12657x + 1238.9$  ( $r = 0.9999$ ); quercetin:  $Y = 13591x + 1318.7$  ( $r = 0.9998$ ); quercitrin:  $Y = 11783x + 1263.8$  ( $r = 0.9994$ ); kaempferol:  $Y = 11974x + 1271.0$  ( $r = 0.9999$ ); luteolin  $Y = 13528x + 1367.2$  ( $r = 0.9997$ )].

All chromatography operations were carried out at ambient temperature and in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves, as defined by Kamdem *et al.* [27]. LOD and LOQ were calculated as 3.3 and 10 σ/S,

respectively, where  $\sigma$  is the standard deviation of the response and  $S$  is the slope of the calibration curve.

## Data Analysis

The result of three replicate experiments was pooled and expressed as mean  $\pm$  standard deviation [28]. A one-way Analysis of Variance, Tukey test and the least significant difference (LSD) were carried out. Significance was accepted at  $P < 0.05$ .

## RESULTS

The ABTS $\bullet$  scavenging ability of the two fruits reported as TEAC is presented in Table 1. The result revealed that the ABTS $\bullet$  scavenging ability of the aqueous extract of ripe red pepper (3.7 mmol/100 g) was significantly higher than that of ripe tomato (1.4 mmol/100 g). Both extracts were able to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  and the results are presented in Table 2 as AAE.

The aqueous extract of red pepper and tomato fruits inhibited  $\text{NO}\bullet$  in a concentration-dependent manner [Figure 1]. Incubation of the rat liver in the presence of  $\text{Fe}^{2+}$  caused a significant increase ( $P < 0.05$ ) in the MDA content of the liver (156%). However, the introduction of water-extractable phytochemicals (156-625  $\mu\text{g/ml}$ ) from red pepper and tomato caused a significant concentration-dependent decrease

( $P < 0.05$ ) in the MDA content of the  $\text{Fe}^{2+}$ -stressed liver homogenates [Figure 2]. Likewise, incubation of rat's liver tissue homogenates in the presence of SNP also caused a significant increase in the rat liver MDA content as shown in Figure 3; however, both extracts inhibited MDA production content in both tissues in a dose-dependent manner (156-625  $\mu\text{g/ml}$ ).

**Table 1:**  $\text{IC}_{50}$  values of inhibition of  $\text{Fe}^{2+}$  - and SNP-induced lipid peroxidation in rat's liver by aqueous extract of ripe red pepper (*C. annuum*) and tomato (*L. esculentum*) fruits ( $\mu\text{g/ml}$ )

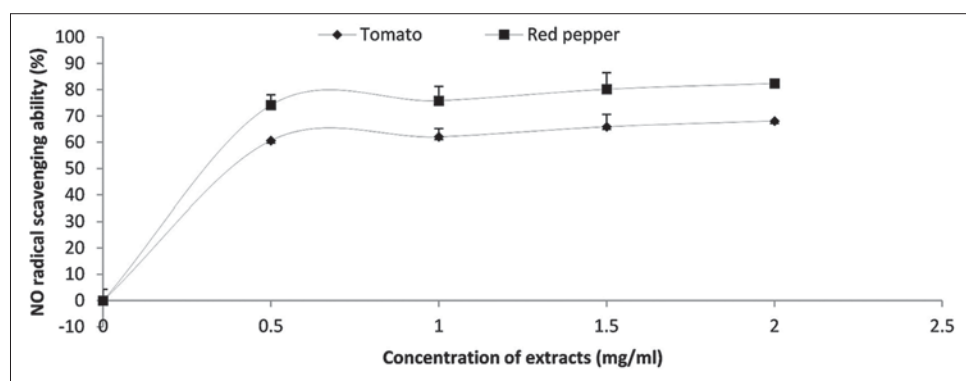
	Red pepper	Tomato
$\text{Fe}^{2+}$ -induced lipid peroxidation	$432.92 \pm 1.65$	$516.34 \pm 9.14$
SNP-induced lipid peroxidation	$431.48 \pm 1.05$	$591.55 \pm 1.67$

Values represent mean  $\pm$  standard deviation; number of samples=5 per group, SNP: Sodium nitroprusside, *L. esculentum*: *Lycopersicon esculentum*, *C. annuum*: *Capsicum annuum*

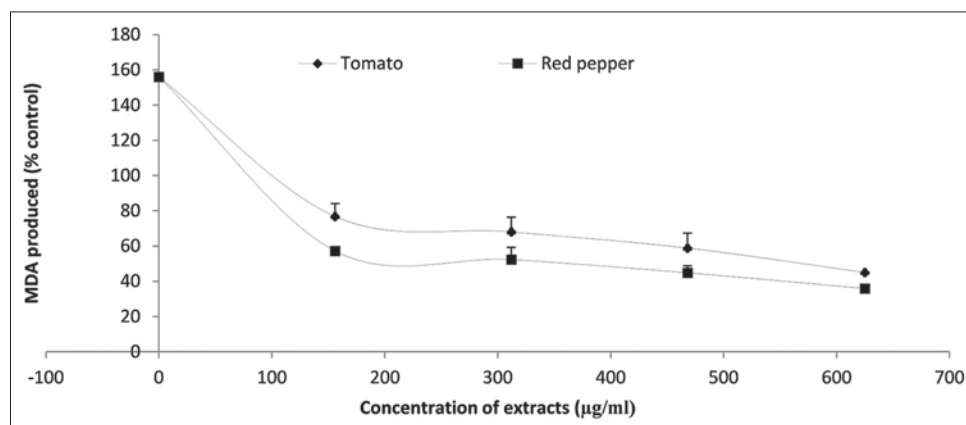
**Table 2:** Antioxidant capacity and ferric reducing antioxidant property of aqueous extract of ripe red pepper (*C. annuum*) and tomato (*L. esculentum*)

	Red pepper	Tomato
Antioxidant capacity (mmol TEAC/100 g)	$3.70 \pm 0.06$	$1.40 \pm 0.01$
Ferric reducing antioxidant property (mmol AAE/g)	$11.98 \pm 1.73$	$4.71 \pm 1.79$

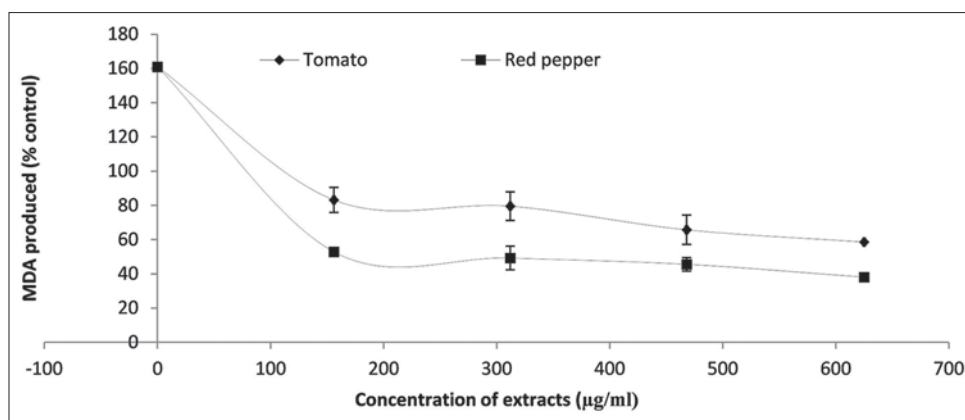
Values represent mean  $\pm$  standard deviation; number of samples=5 per group, *L. esculentum*: *Lycopersicon esculentum*, *C. annuum*: *Capsicum annuum*



**Figure 1:** Nitric oxide radical scavenging ability of aqueous extract of ripe red pepper (*Capsicum annuum*) and tomato (*Lycopersicon esculentum*)



**Figure 2:** Inhibition of iron(II)-induced lipid peroxidation in rat's liver by aqueous extract of ripe red pepper (*Capsicum annuum*) and tomato (*Lycopersicon esculentum*)



**Figure 3:** Inhibition of sodium nitroprusside-induced lipid peroxidation in rat's liver by aqueous extract of ripe red pepper (*Capsicum annuum*) and tomato (*Lycopersicon esculentum*)

**Table 3:** Constituents of aqueous extract of ripe red pepper (*C. annuum*) and tomato (*L. esculentum*) fruits

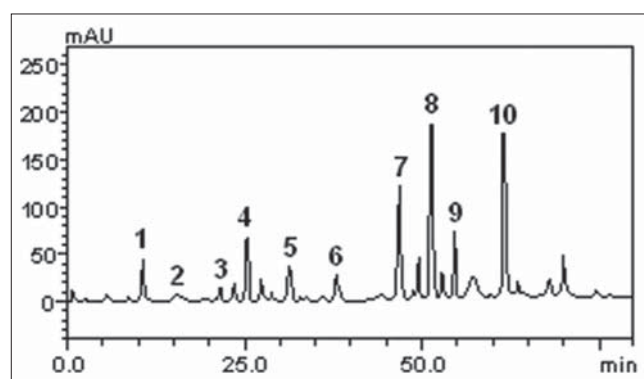
Compounds	Red pepper (mg/g)	Tomato (mg/g)
Gallic acid	4.58±0.01 <sup>a</sup>	4.42±0.01 <sup>a</sup>
Catechin	1.4±0.02 <sup>b</sup>	2.07±0.01 <sup>b</sup>
Chlorogenic acid	1.74±0.01 <sup>b</sup>	15.52±0.02 <sup>c</sup>
Caffeic acid	5.16±0.02 <sup>c</sup>	4.63±0.03 <sup>a</sup>
Coumarin	2.67±0.01 <sup>d</sup>	4.92±0.03 <sup>a</sup>
Rutin	2.13±0.03 <sup>d</sup>	1.87±0.03 <sup>d</sup>
Quercitrin	6.09±0.02 <sup>e</sup>	26.94±0.01 <sup>e</sup>
Quercetin	38.79±0.01 <sup>f</sup>	17.10±0.02 <sup>f</sup>
Kaempferol	6.05±0.01 <sup>e</sup>	2.37±0.01 <sup>b</sup>
Luteolin	13.04±0.03 <sup>g</sup>	65.66±0.01 <sup>g</sup>

Results are expressed as mean±standard deviations of three determinations. Values followed by different superscript letters on the same column differ by Tukey test at  $P < 0.05$ . *L. esculentum*: *Lycopersicon esculentum*, *C. annuum*: *Capsicum annuum*

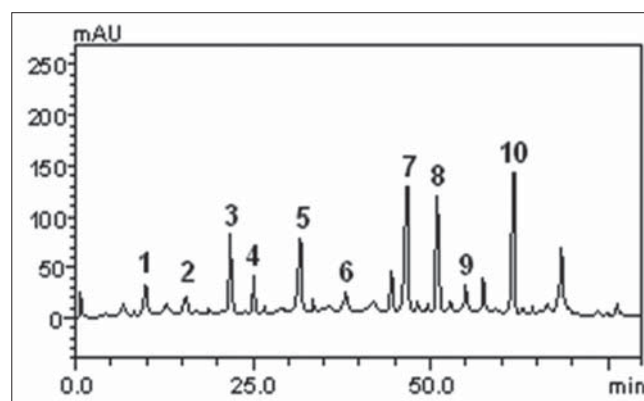
Characterization of the extract with HPLC revealed that the major constituent of the aqueous extract of ripe red pepper and tomato is coumarin, P-coumaric acid, O-coumaric acid, vanillic acid, gallic acid, caffeic acid, ferulic acid, syringic acid, sinapinic acid, apigenin, naringenin, kaempferol, luteolin, epicatechin, epigallocatechin, quercetin, chlorogenic acid, capsaicin, and dihydrocapsaicin [Table 3, Figures 4 and 5].

## DISCUSSION

ABTS•, a blue-green chromophore is mostly reactive toward phenolics, thiols, and other antioxidants [29]. The ABTS• free radical scavenging ability model has the advantage of being more versatile due to the minimal spectral interference as the absorption maximum used is 760 nm, a wavelength not normally encountered with natural products [23]. The ABTS• scavenging ability of the two fruits reported as TEAC [Table 1] in the present study, revealed that the activity of the aqueous extract of ripe red pepper was significantly higher than that of ripe tomato. This result was lower than what was reported for ripe and unripe pepper fruits [30]. The ABTS• scavenging ability of the two fruits could be due to the hydrogen donating ability of the phenolics present in the aqueous extract of ripe red pepper and tomato to the lone pair of ABTS radical.



**Figure 4:** Representative HPLC profile of red pepper aqueous extract: gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), coumarin (peak 5), rutin (peak 6), quercitrin (peak 7), quercetin (peak 8), kaempferol (peak 9), and luteolin (peak 10)



**Figure 5:** Representative HPLC profile of tomato aqueous extract: gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), coumarin (peak 5), rutin (peak 6), quercitrin (peak 7), quercetin (peak 8), kaempferol (peak 9), and luteolin (peak 10)

The prevention of the chain initiation step by scavenging various reactive species like free radicals is considered to be an important antioxidant mode of action. Reducing power is a novel antioxidant defense mechanism; electron transfer and hydrogen atom transfer are the two mechanisms that are available to affect this property [31]. In the present study, both



extracts were able to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  [Table 2]; aqueous extract of red pepper had a higher reducing power than that of a tomato. This trend agrees with the trend by ABTS• scavenging abilities earlier discussed. Nevertheless, the reducing capacity of the two extracts may be an indication of their potential antioxidant activities due to the presence of reductants [32].

The NO radical is generated from SNP at physiological pH. It has the ability of changing the structural and functional behavior of many cellular components because it is a highly reactive compound [33]. The aqueous extract of red pepper and tomato fruits inhibited NO• in a concentration-dependent manner [Figure 1]; the percentage inhibition of aqueous extract of red pepper was higher than that of a tomato. This inhibitory effect of the two extracts against NO• can be attributed to their ability to compete with reactive oxygen and its derivatives [34].

One of the major mechanisms of cell injury in aerobic organisms subjected to oxidative stress is lipid peroxidation of biological membranes [5]. Incubation of the rat liver in the presence of  $\text{Fe}^{2+}$  caused a significant increase in the MDA content of the liver. These findings agree with our earlier reports on the interaction of  $\text{Fe}^{2+}$  with the testes [35], in which  $\text{Fe}^{2+}$  was shown to be a very potent initiator of lipid peroxidation (a pro-oxidant) in the testes. The increased lipid peroxidation in the presence of  $\text{Fe}^{2+}$  could be attributed to the fact that  $\text{Fe}^{2+}$  can catalyze one-electron transfer reactions that generate ROS, such as the reactive •OH, which is formed from  $\text{H}_2\text{O}_2$  through the Fenton reaction. Iron also decomposes lipid peroxides, thus generating peroxy and alkoxy radicals, which favors the propagation of lipid (per) oxidation [36].

On the other hand, the introduction of water-extractable phytochemicals from red pepper and tomato caused a significant concentration-dependent decrease in the MDA content of the  $\text{Fe}^{2+}$ -stressed liver homogenates with the least MDA production occurring at the introduction of the highest concentration (625  $\mu\text{g/ml}$ ) of the two fruits extracts. The mode of inhibition of  $\text{Fe}^{2+}$ -induced lipid peroxidation could be because of the possibility of the water-extractable phytochemicals to form complexes with  $\text{Fe}^{2+}$ , thereby preventing them from catalyzing the initiation of lipid peroxidation, or the phytochemicals could have mopped up the free radicals produced by the  $\text{Fe}^{2+}$ -catalyzed reaction [5].

Likewise, incubation of rat's liver tissue homogenates in the presence of SNP also caused a significant increase in the rat liver MDA [Figure 3]; both extracts inhibited MDA production content in both tissues in a dose-dependent manner. Nevertheless, judging by the  $\text{IC}_{50}$  values [Table 1], aqueous extract of red pepper had a significantly higher inhibitory effect on  $\text{Fe}^{2+}$ -induced lipid peroxidation in the liver homogenate than tomato. SNP, a component of antihypertensive drugs, causes cytotoxicity through the release of cyanide and NO [37]. The protective properties of the red pepper and tomato against SNP-induced lipid peroxidation in the liver could be because of the ability of the antioxidant phytochemicals present in the aqueous extract to scavenge the nitrous and Fe radicals produced from the decomposition of the SNP.

The antioxidant properties of plant foods have been linked to the presence of an array of important phenolic and non-phenolic phytochemicals including phenolic acids, flavonoids, and alkaloids [38]. However, characterization of the extract with HPLC revealed that the major constituents of the aqueous extract of ripe red pepper and tomato are gallic acid, catechin, chlorogenic acid, caffeic acid, coumarin, rutin, quercitrin, quercetin, kaempferol, and luteolin of which the level of quercetin, luteolin, quercitrin, and kaempferol were very high in ripe red pepper while luteolin, quercitrin, quercetin and chlorogenic acid were very high in ripe tomato [Table 3, Figures 4 and 5].

Phenolic compounds are strong antioxidants capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce  $\alpha$ -tocopherol radicals and inhibit oxidases [39]. They can protect the human body from free radicals, whose formation is associated with the normal metabolism of aerobic cells.

In conclusion, the results obtained in the present study showed that the two fruits tested possessed good reducing power and NO and ABTS radical scavenging abilities. Therefore, the protection of liver tissue from the SNP and  $\text{Fe}^{2+}$ -induced lipid peroxidation by the aqueous extract of ripe red pepper and tomato could be attributed to their phenolic compound and the mechanism through which they possibly do this, could be by their radical scavenging abilities and reducing power.

## REFERENCES

1. Iwasaki A, Gagnon C. Formation of reactive oxygen species in spermatozoa of infertile patients. *Fertil Steril* 1992;57:409-16.
2. Shulman RG, Rothman DL, Behar KL, Hyder F. Energetic basis of brain activity: Implications for neuroimaging. *Trends Neurosci* 2004;27:489-95.
3. Britton RS, Leicester KL, Bacon BR. Iron toxicity and chelation therapy. *Int J Hematol* 2002;76:219-28.
4. Murray RK, Granner DK, Mayes PA, Rodwell VW. *Harper's Biochemistry*. 25<sup>th</sup> ed. New York: The McGraw-Hill Companies; 2000. p. 927.
5. Oboh G, Rocha JB. Antioxidant in Foods: A New Challenge for Food Processors: Leading Edge Antioxidants Research. New York, USA: Nova Science Publishers Inc.; 2007. p. 35-64.
6. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radic Res* 1995;22:375-83.
7. Lee JJ, Crosby KM, Pike LM, Yoo KS, Lescobar DI. Impact of genetic and environmental variation of development of flavonoids and carotenoids in pepper (*Capsicum* spp.). *Sci Hortic* 2005;106:341-52.
8. Niizu P, Rodriguez-Amaya DB. New data on the carotenoid composition of raw salad vegetables. *J Food Comp Anal* 2005;18:739-49.
9. Kidmose U, Yang RY, Thilsted SH, Christensen LP, Brandt K. Content of carotenoids in commonly eaten vegetables and stability and extractability during frying. *J Food Comp Anal* 2006;19:562-71.
10. Kritchevsky D. Antioxidant vitamins in the prevention of cardiovascular disease. *Nutr Today* 1992;27:30-3.
11. Nishino H, Murakoshi M, Tokuda H, Satomi Y. Cancer prevention by carotenoids. *Arch Biochem Biophys* 2009 15;483:165-8.
12. Packer L. Possible factors influencing aging. United Nations and World Health Organization First Joint Conference on Healthy Aging, New York, NY, 29 April-1 May, 1996.
13. Rao AV, Rao LG. Carotenoids and human health. *Pharmacol Res* 2007;55:207-16.
14. Byers T, Perry G. Dietary carotenes, vitamin C, and vitamin E as protective antioxidants in human cancers. *Annu Rev Nutr*

- 1992;12:139-59.
15. Cichewicz RH, Thorpe PA. The antimicrobial properties of chile peppers (*Capsicum species*) and their uses in Mayan medicine. *J Ethnopharmacol* 1996;52:61-70.
16. Wahba NM, Ahmed AS, Ebraheim ZZ. Antimicrobial effects of pepper, parsley, and dill and their roles in the microbiological quality enhancement of traditional Egyptian Kareish cheese. *Foodborne Pathog Dis* 2010;7:411-8.
17. Giovannucci E. Tomatoes, tomato-based products, lycopene, and cancer: Review of the epidemiologic literature. *J Natl Cancer Inst* 1999;91:317-31.
18. Parfitt VJ, Rubba P, Bolton C, Marotta G, Hartog M, Mancini M. A comparison of antioxidant status and free radical peroxidation of plasma lipoproteins in healthy young persons from Naples and Bristol. *Eur Heart J* 1994;15:871-6.
19. Agarwal S, Rao AV. Tomato lycopene and its role in human health and chronic diseases. *CMAJ* 2000;163:739-44.
20. Akomolafe SF, Ajayi OB. Aqueous extracts of ripe red pepper and tomato fruits inhibit Fe<sup>2+</sup>-induced oxidative stress in rat testes and kidney. *Oxid Antioxid Med Sci* 2014;3:217-24.
21. Oboh G, Puntel RL, Rocha JB. Hot pepper (*Capsicum annuum*, *tepin* and *Capsicum chinese*, *Habanero*) prevents Fe<sup>2+</sup> – Induced lipid peroxidation in brain – *In vitro*. *Food Chem* 2007;102:178-85.
22. Pulido R, Bravo L, Saura-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J Agric Food Chem* 2000;48:3396-402.
23. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorisation assay. *Free Radic Biol Med* 1999;26:1231-7.
24. Igbinosa OO, Igbinosa IH, Chigor VN, Uzunuigbo OE, Oyedemi SO, Odjajare EE, *et al*. Polyphenolic contents and antioxidant potential of stem bark extracts from *Jatropha curcas* (Linn). *Int J Mol Sci* 2011;12:2958-71.
25. Bellé NA, Dalmolin GD, Fonini G, Rubin MA, Rocha JB. Polyamines reduces lipid peroxidation induced by different pro-oxidant agents. *Brain Res* 2004;1008:245-51.
26. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351-8.
27. Kamdem JP, Olalekan EO, Hassan, W, Kade J, Yetunde O, Boligon AA, *et al*. *Trichilia catigua* (Catuaba) bark extract exerts neuroprotection against oxidative stress induced by different neurotoxic agents in rat hippocampal slices. *Ind Crops Prod* 2013;50:625-32.
28. Zar JH. Biostatistical Analysis. USA: Prentice-Hall; 1984.
29. Walker RB, Everette JD. Comparative reaction rates of various antioxidants with ABTS radical cation. *J Agric Food Chem* 2009 25;57:1156-61.
30. Adedayo BC, Oboh G, Akindahunsi AA. Changes in the total phenol content and antioxidant properties of pepper fruit (*Dennettia tripetala*) with ripening. *Afr J Food Sci* 2010;4:403-9.
31. Dastmalchi K, Dorman HJ, Kosar M, Hiltunen R. Chemical composition and *in vitro* antioxidant evaluation of a water soluble Moldavian balm (*Dracocephalum moldavica* L.) extract. *Lebensm Wiss Technol* 2007;40:239-48.
32. Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract of Harnng Jyur 449 (*Chrysanthemum morifolium* Ramat). *Lebensm Wiss Technol* 1999;32:269-77.
33. Ashokkumar D, Thamilselvan V, Senthilkumar GP, Mazumder UK, Gupta M. Antioxidant and free radical scavenging effects of *Lippia nodiflora*. *Pharm Biol* 2008;46:762-71.
34. Marcocci L, Packer L, Droy-Lefaix MT, Sekaki A, Gardès-Albert M. Antioxidant action of Ginkgo biloba extract EGB 761. *Methods Enzymol* 1994;234:462-75.
35. Akomolafe SF, Oboh G, Akindahunsi AA, Akinyemi AJ, Adeyanju . O. Inhibitory effect of aqueous extract of *Moringa oleifera* and *Newbouldia laevis* Leaves on ferrous sulphate and sodium nitroprusside induced oxidative stress in rat's testes *in vitro*. *Open J Med Chem* 2012;2:119-28.
36. Zago MP, Verstraeten SV, Oteiza PI. Zinc in the prevention of Fe<sup>2+</sup> -initiated lipid and protein oxidation. *Biol Res* 2000;33:143-50.
37. Oboh G, Rocha JB. Antioxidant and neuroprotective properties of sour tea (*Hibiscus sabdariffa*, calyx) and green tea (*Camellia sinensis*) on some pro-oxidants induced lipid peroxidation in brain – *in vitro*. *Food Biophys* 2008;3:382-9.
38. Cheplick S, Kwon Y, Bhowmik P, Shetty K. Clonal variation in raspberry fruit phenolics and relevance for diabetes and hypertension management. *J Food Biochem* 2007;31:656-79.
39. Amic D, Beslo D, Trinajstić N. Structure-radical scavenging activity relationship of flavonoids. *Croat Chem Acta* 2003;76:55-61.

© AKAY; licensee AKAY. 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, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.

Source of Support: Nil, Conflict of Interest: None declared.