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Assessment of the Bioactive Composition and in vitro Antioxidant Effect of the Aqueous and Ethanol Extract of Two Pepper Cultivars

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ABSTRACT

Pepper is one of the most important economic fruits with abundant of nutritional qualities. This study was carried out to assess the important bioactive compounds present in the two selected pepper cultivars. Ripe-fruit extracts were used to determine total phenolic content, flavonoid content, ascorbic acid (Vitamin C), beta-carotene and lycopene among the cultivars quantitatively. The results showed that there were significant variations on the nutrients and bioactive compound concentrations. The highest amount of vitamin C was 187.62 ± 0.60 mg/100g recorded from bird eye variety. The highest concentration of Phenol was 52.98 ± 0.00 mgGAE/ 100g obtained from bird eye pepper. High variability was observed among the pepper cultivars; bird eye cultivar with the highest concentrations of total phenol, flavonoid and ascorbic acid, showed to have the highest radical scavenging capacity. Both pepper cultivars can be a cheap source of natural antioxidants, therefore recommended for future development of healthy food products and conveying great benefits for food and pharmaceutical industries, consumers, and producers.

Keywords: Pepper, beta-carotene, phenol, flavonoid, antioxidant, ascorbic acid and cultivar

INTRODUCTION

Pepper is one of the important cultivated vegetable crops in the word which belongs to the Solanaceae family like as tomatoes, potatoes and eggplants [1]. It is believed that pepper originated from tropical American countries such as Peru, Bolivia and Mexico and it rapidly spread to Europe and then to other parts of the world such as African countries [1], [2]. In Africa, they are generally considered together as *Capsicum annuum*. L [3]. It is the most important crop used as spice and food vegetable in the world [4]. In addition, *Capsicum* species have been used as medicines and lachrymatory agents. In Ethiopia, it is a high value crop due to its high pungency which serves as food and source of cash earning for small household farmers or producers in both green and dry form [4]. *Capsicum spp* is a herbaceous plant belonging to the family Solanaceae. This genus comprises variable number of species (25- 200) among which five common cultivated species include *Capsicum annuum* L. (bell, cyenne, thai and paprika pepper), *Capsicum frutescens* L. (bird eye, tabasco pepper), *Capsicum chinense* Jacq. (scotch-bonnet pepper), *Capsicum baccatum* L. (bishop's crown and Malawi piquante pepper), and *Capsicum pubescens* L. (rocoto pepper) [5]. *Capsicum annum* L. (tribe Capsiceae, Solanaceae) is an American genus distributed ranging from the southern United States of America to central Argentina and Brazil. The genus includes chili peppers, bell peppers, ajíes, habaneros, jalapeños, ulupicas and pimientos, well known for their economic importance around the globe [6]. Chilli was first discovered by Christopher Columbus in tropical America, and its use spread rapidly throughout the world because of its pungent flavour. Variously classified as herb, fruit or vegetable, it is now an inseparable part of Asian cuisine. The name "chilli" is derived from the Mexican word, chilli [7]. In Nigeria, pepper occupies the third place of importance among cultivated vegetables. However, Nsukka yellow pepper is an important commercial fruit vegetable. Its cultivation forms a major and sometimes the only agricultural activity of rural women in Enugu state [8].

Anambra state is also known with the cultivation of *Capsicum fructescens* known as bird eye pepper or tabasco pepper. The plant is usually planted from seeds which are extracted from ripe fruits, dried and stored until required for planting. They are usually grown as a rain-fed crop and are always green at maturity but deep yellow and orange varieties has also evolved [9]. It is an important spice crop that is produced and consumed either fresh or processed [10]. The pepper is used as pungent spice for flavouring stew, soup and sauces. It is a rich source of vitamins A and C. It has both nutritive and medicinal values; hence, it is used by the food manufacturing industries for the seasoning of processed food. It is also used by the pharmaceutical industries in the preparation of stimulant and counter irritant balms for external application [11].

Habitually, people eat this botanical spice in raw, dried, and cooked form, and it is also used in making paste, pickle, and sauce. Although, from place to place, the name and type of *Capsicum* berries vary, the most common variety is called "pepper or chili pepper," which itself can vary greatly in color, shape, size, appearance, flavor, and pungency. Basically, the color diversity of *Capsicum* fruit is linked to the presence of pigments like chlorophyll (green), anthocyanins (violet/purple), α-carotene, βcarotene, zeaxanthin, lutein, and β-cryptoxanthin (yellow/ orange) [12]. Pungency of pepper varies in different varieties. Red chillies get their colour from a colouring compound called capsanthin and have a hot, pungent taste due to a chemical called capsaicin [13].

Plate 1: Photo of dried red bell pepper (*Capsicum annuum*)

Plate 2: Photo of dried bird eye pepper (*Capsicum frutescens*)

MATERIALS AND METHODS

Study area

This study was carried out at Nnamdi Azikiwe University, Awka, Anambra State. Anambra State is located in the south-eastern part of Nigeria and lies between latitudes 6° 13' and 16' N and longitude 7° 4' and 7° 41' E AND Altitude 160.8m respectively [14]. The research is based on the assessment of the bioactive composition and invitro antioxidant effect of the aqueous and extract of two pepper cultivars.

Materials and reagent used

The materials used were red bell pepper (Capsicum annuum) from Enuugu State and bird eye pepper (Capsicum frutescens) from Anambra State. Other materials and reagents used are; Test tube, pipette, pipette rack, beaker, water bath, spectrophotometer, oven, morter and pestle, Ethanol, phosplate buffer (0.2 mole), KFCN (potassium percyanate), TCA, distilled water, 0.5ml ferric chloride, folin C, 10% $NaCO_3$, 7ml of water, acetone.

Preparation of sample

Extraction:

Two grams (10g) of the ground sample was homogenized with 20ml of 70% ethanol. This was centrifuged at 1500rpm for 10mins using Axiom 90-3 bench centrifuge. The supernatant was used for the analysis.

Assay of Bioactive Compounds

Total Phenol

The total phenol content of the pepper extract was determined using the method of [15]. The stock solution was diluted to 1mg/ml. The diluted extract solution (1 ml) was mixed with Folin and Ciocalteu's phenol reagent (1 ml). After 3 minutes, saturated sodium carbonate solution (1 ml) was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 minutes, after which the absorbance was read at 725 nm using spectrophotometer. Gallic acid was used to prepare the standard curve and the results were expressed as mg of gallic acid equivalents (GAEs) per g of extract.

Total Flavonoids

The flavonoid content was determined by using the slightly modified colorimetry method described previously by [15]. An aliquot, 0.5 ml of appropriately diluted pepper sample solution (250μg/ml), was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5 % $NaNO_2$ solution. After 6 minutes, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 minutes 2 ml of 4% NaOH solution was added to the mixture. Water was added to bring the final volume to 5ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 minutes. The absorbance of the mixture was read at 510 nm versus water blank with reference standard prepared with catechin concentrations. The analyses were performed in duplicate. The results were expressed as mg Catechin equivalents per 100g of sample (mg CE/100 g).

Beta Carotene and Lycopene Contents

These were determined by the method of [15]. The concentrated extract (100 mg) was vigorously shaken with 6 ml acetone-hexane mixture in the ratio of (4:6) for one minute and filtered using Whatman No.4 filter paper. The absorbance of the filtrate was read at 453, 505 and 663 nm respectively.The contents of lycopene and β-carotene were calculated according to the following equations:

Lycopene (mg/100ml) = $-0.0458A_{663}+0.372A_{505}+0.0806A_{453}$

β-carotene (mg/100ml) = $0.216A_{663}+-0.304A_{505}+0.452A_{453}$

Ascorbic Acid

Ascorbic acid content of the sample was determined according to the method of [16]. An aliquot (20mg equivalent) of the extract was extracted with 10ml of 1% metaphosphoric acid. It was allowed to stand for 45 min at a temperature of 28°C (Laboratory temperature) after which it was filtered through Whatman No.4 filter paper. An aliquote (1ml) of the filterate was mixed with 9ml of 50μM 2,6-dichlorophenolindophenol sodium salt hydrate. The absorbance was measured at 515nm using a UV-Vis spectrophotometer after 30min. Ascorbic acid content was calculated from the calibration curve of authentic L-ascorbic acid and the result expressed as mg ascorbic acid equivalent per gram (mgAAE/100g) of the sample.

DPPH scavenging activity Assay

The stable 2,2-diphenyl-1-picryl hydrazyl radical (DPPH) was used for the determination of free radical scavenging activity of the ethanolic extract of the sample. This was assayed using the method of [15]. An aliquote (0.3ml) of different concentrations of the pepper extract (0-100mg/ml) were mixed with 2.7ml of methanolic solution of DPPH (100 μM) in test tubes. The mixture was shake and kept in dark for 60mins. The absorbance was taken at a wavelength of 517nM using Axiom 752 spectrophotometer. Butylated hydroxyanisole (BHA) was used as standard antioxidant. The percentage scavenging activity was calculated using the formular:

$\%RSA= [(A_{DPPH}-A_s)/A_{DPPH}]\times 100$

Where A_s is the absorbance of the test solution with the sample and A_{DPPH} is the absorbance of DPPH solution. The EC50 (concentration of sample at 50% RSA) was calculated from the graph of %RSA against the sample concentration.

Reducing Power Capacity Assay

The reducing power was determined according to the method of [15]. This method is based on the principle of increase in the absorbance of the reaction mixture. The pepper extract (2.5ml) of various concentration (0-100mg/ml) was mixed with 2.5ml of 0.2M sodium phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20mins. 2.5ml 0f 10% Trichloroacetic acid was added and the mixture centrifuged at 1000rpm for 8min. the upper layer (5ml) was mixed with 5ml of deionised water followed by the addition of 1ml of 0.1% ferric chloride. The absorbance was measured at 700nM using Axiom 752 spectrophotometer. The graph of absorbance at 700nM against the extract concentrations was plotted. Butylated Hydroxyanisole (BHA) was used as a standard antioxidant.

Statistical Analysis

All analysis was conducted in duplicates. The results obtained therein were used for the statistical analysis using the statistical package for social sciences (SPSS), version 26 (SPSS Inc., Chicago, IL, USA). Descriptive statistics and Independent sample T-test was used to analyze result.

RESULTS

Bioactive Compound Composition of the two Pepper Type

For all the bioactive compounds measured, the bird eye chili pepper had a much higher total phenol, flavonoid, lycopene and ascorbic acid than the red eye chili pepper, except for the beta carotene (Table 1). There was significant difference in the total phenol and beta carotene ($p<0.05$), while there was no significant difference in the flavonoid, lycopene and ascorbic acid ($p>0.05$) content among pepper varieties (Table 1).

Table 1: Bioactive Compound Composition of the two Pepper Type

Bioactive Compounds	Red Bell Pepper	Bird Eye Chili	P-value	Remark
Total Phenol (mgGAE/100g)	37.33±0.116	52.98±0.000	.003	Significant
Flavonoid (mgCE/100g)	12.27±1.928	47.27±9.000	.102	Not Significant
Lyopene (mg/100g)	0.41±0.001	0.42±0.000	.058	Not Significant
Beta carotene (mg/100g)	0.56±0.001	0.46±0.001	.000	Significant
Ascorbic acid (mg/100g)	150.03±17.169	187.62±0.601	.199	Not Significant

Results are in mean ± standard deviation of duplicate determination.

GAE: Gallic acid equivalent
CE: Catechin equivalent

Total Phenol Composition

The result shows that the Bird chili pepper had higher phenol (52.98±0.00mgGAE/100G) content compared to Red Bell pepper (37.33±0.116mgGAE/100g) (Table 2).

Table 2: Total Phenol Composition of the two Pepper Type

Pepper Type	Total Phenol (mgGAE/100g)
Red Bell Pepper	37.33±0.116[b]
Bird Chili Pepper	52.98±0.000[a]

Results are in mean ± standard deviation of duplicate determination.
Columns with different superscripts are significantly ($p<0.05$) different.
GAE: Gallic acid equivalent

Total Flavonoid Composition of the two Pepper Type

The result shows that Bird Eye Chili exhibits higher antioxidant capacity due to its significantly ($p<0.05$) higher flavonoid (47.27±9.000 mg CE/100g) content compared to Red Bell Pepper (12.27±1.928 mg CE/100g) (Table 3).

Table 3: Total Flavonoid Composition of the two Pepper Type

Pepper Type	Flavonoid (mgCE/100g)
Red Bell Pepper	12.27±1.928[b]
Bird Chili Pepper	47.27±9.000[a]

Results are in mean±standard deviation of duplicate determination.
Columns with different superscripts are significantly ($p<0.05$) different.
CE: Catechin equivalent

Lycopene Composition of the two Pepper Type

The lycopene (0.41± 0.001 mg/100g) content of Red Bell Pepper was slightly lower compared to Bird Chili Pepper (0.42±0.000 mg/100g). And this difference was not significant ($p<0.05$) (Table 4).

Table 4: Lycopene Composition of the two Pepper Type

Pepper Type	Lyopene (mg/100g)
Red Bell Pepper	0.41±0.001[a]
Bird Chili Pepper	0.42±0.000[a]

Results are in mean ± standard deviation of duplicate determination.
Columns with different superscripts are significantly ($p<0.05$) different.

Beta Carotene Composition of the two Pepper Type

From the result, Red Bell Pepper contains slightly more beta-carotene (0.56±0.000 mg/100g) than Bird Eye Chili (0.46±0.001mg/100g) (Table 5).

Table 5: Beta Carotene Composition of the two Pepper Type

Pepper Type	Beta carotene (mg/100g)
Red Bell Pepper	0.56±0.00[a]
Bird Chili Pepper	0.46±0.001[b]

Results are in mean ± standard deviation of duplicate determination.
Columns with different superscripts are significantly ($p<0.05$) different.

Ascorbic Acid Composition of the two Pepper Type

Additionally, Table 6 revealed Bird Eye Chili has higher vitamin C content (187.62±0.601 mg/100g) than Red Bell Pepper (150.03±17.169 mg/100g).

Table 6: Ascorbic Acid Composition of the two Pepper Type

Pepper Type	Ascorbic acid (mg/100g)
Red Bell Pepper	150.03±17.169[b]
Bird Chili Pepper	187.62±0.601[a]

Results are in mean ± standard deviation of duplicate determination.
Columns with different superscripts are significantly ($p<0.05$) different.

Antioxidant Properties of Different Pepper Cultivars

Figure 1 reveals a graph showing the free radical scavenging activities of the extract of Red bell pepper and Bird eye chili compared with that of a standard antioxidant, ascorbic acid. From this graph of the %RSA against the sample concentration it can be seen that the extract of bird eye chili pepper competed favorably with the standard antioxidant. The least activity was observed in the red bell pepper. The reducing power capacity of the extract of red bell pepper and bird eye chili compared with that of a standard antioxidant, ascorbic acid (Fig 2). It was seen that the extract of bird eye chili pepper also competed favourably with the standard antioxidant. The least activity was observed in the red bell pepper.

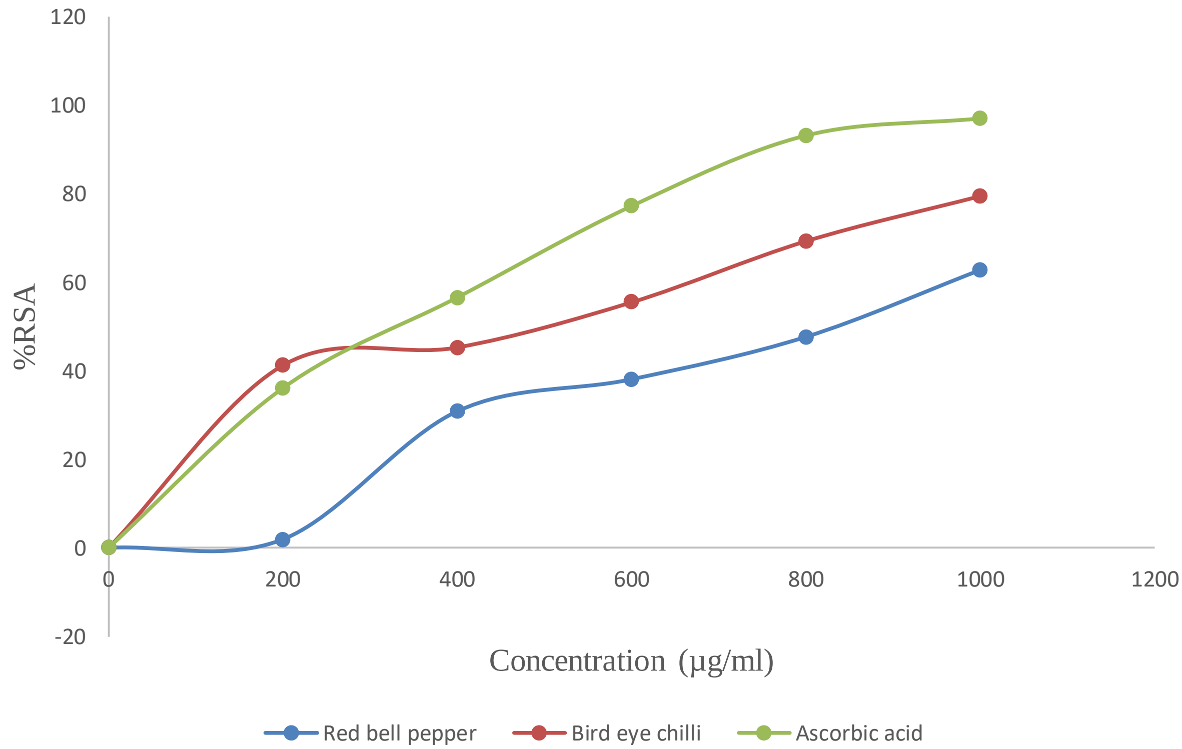

Figure 1: A graph showing the free radical scavenging activities of the extract of Red bell pepper and Bird eye chili compared with that of a standard antioxidant, ascorbic acid.

Antioxidant Properties of Different Pepper Cultivars

The reducing power capacity of the extract of red bell pepper and bird eye chili compared with that of a standard antioxidant ascorbic acid (figure 2). It was seen that the extract of bird eye chili pepper also competed favourably with the standard antioxidant. The least activity was observed in the red bell pepper.

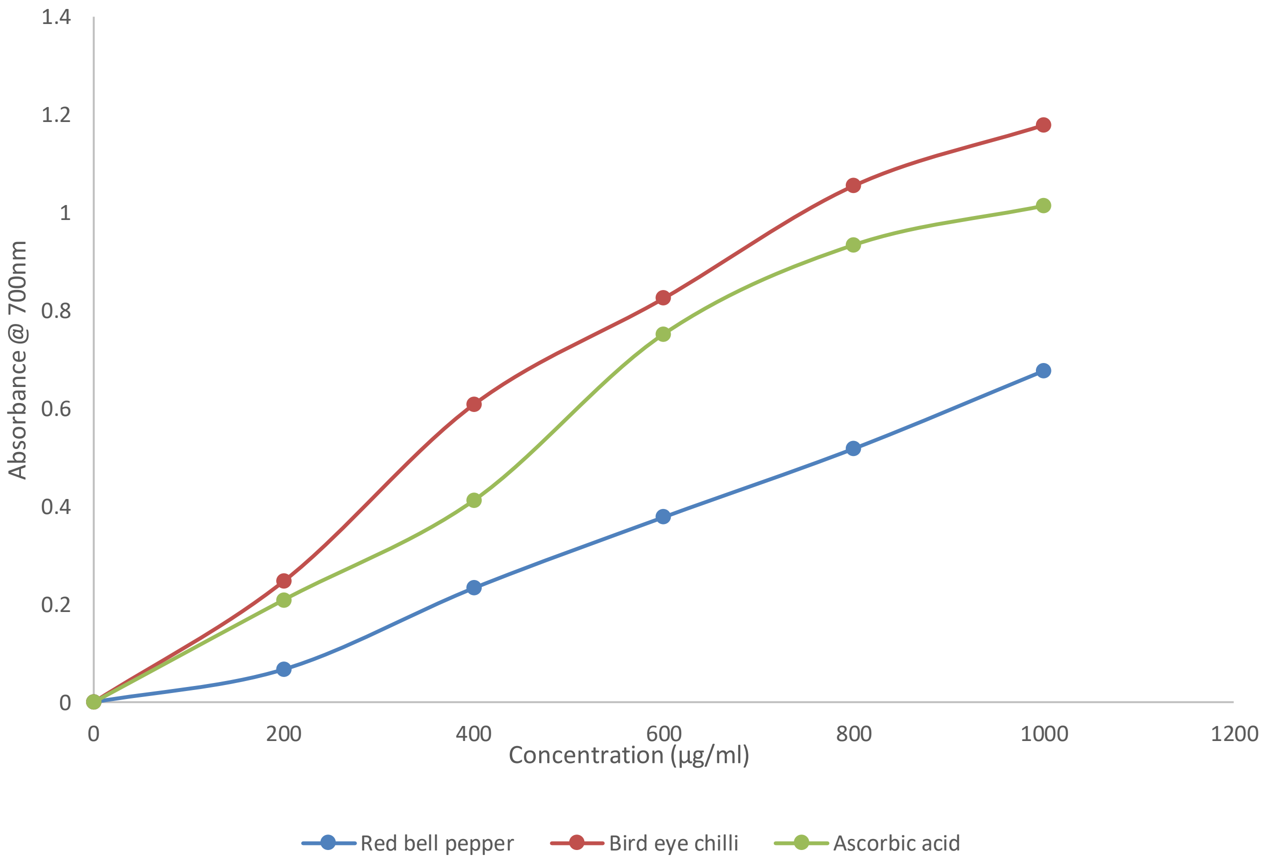

Figure 2: A graph showing the reducing power capacity of the extract of red bell pepper and bird eye chilli compared with that of a standard antioxidant, ascorbic acid.

DISCUSSION

The study's findings give valuable insights into the biochemical character and potential health benefits of these pepper cultivars. The results suggest that both peppers contain a diverse range of bioactive compounds, including flavonoids, phenolic acids, and carotenoids, which are known to have antioxidant and anti-inflammatory properties. The use of both aqueous and ethanol solvents in the extraction process allowed for a more comprehensive understanding of the bioactive compounds present in the peppers. The ethanol extracts had higher concentrations of bioactive compounds compared to the aqueous extracts, which may be due to the ability of ethanol to extract more polar compounds with higher antioxidant activity [18]. The results obtained in the study on the total phenolic contents showed that a greater content of phenol was recorded from *C. frutescens* (bird eye) variety then followed by *C. annuum* (bell pepper). These results agree with the findings reported by [19] that total phenolic content depended on the variety; that is, the variations in the phenol in the pepper fruit extracts examined could be due to genetic difference of the varieties. But also, climatic and soil conditions at the growing area could affect the concentration of the bioactive compounds. The amount of phenol in the extract is directly related to the antioxidant properties which allow the extracts to act as antioxidants [20]. Within the realm of plant phytochemicals, phenolic compounds hold diverse physiological functions. Hence, the flavonoid and total phenolic contents serve as potential indicators of specific physiological effects associated with natural products derived from plants. The flavonoids content from this study showed greater variation among the two hot pepper cultivars evaluated. A higher content was recorded in *C. frutescens* (bird eye) than in the red bell pepper. Flavonoids are important compounds synthesized by plants as secondary metabolites and have properties such as antioxidant, antiviral, anticancer, anti-inflammatory, anti-allergic, antibacterial, therapeutic and cytotoxic properties. It is a powerful antioxidants and have free radical scavenging capacities and can prevent coronary heart diseases, inflammatory diseases and various types of cancer. Recently, flavonoid compounds have been proved that they can prevent possible antiviral activities [21].

The results from beta-carotone, showed that beta-carotene content in the hot pepper varieties depends intensely on the variety. The varieties of hot peppers evaluated showed that on average, a higher content of beta-carotene

was found in *C. annum* (red bell pepper) varieties than the content found in the *C. frutescens* (bird eye pepper). These results agree with findings reported by [22] and [24] that levels of beta-carotene in hot peppers depends on the variety and environmental conditions at the growing area, experimental conditions, extraction procedures and method used. Beta-carotene has various properties, such as pro-vitamin A, immune improvement agent and powerful antioxidant, which help to neutralize a wide range of free radicals through electron transfer process. Beta-carotene molecules also have been extensively investigated as possible cancer preventive agents [24].
The results from ascorbic acid content suggests that hot peppers are an excellent source of vitamin C. Highest amount of vitamin C was recorded from bird eye (*C. frutescens*) cultivar from Awka, Anambra State. However, both varieties evaluated contained higher amounts indicating that the selected hot pepper varieties cultivated are composed of higher levels of vitamin C. Even the lowest amount (150.03 ± 17.17 mg/100g) recorded from bell pepper variety exceeded the recommended daily amount of 60 mg/100g [25]. These results are in line with findings reported by [26], who said that some hot pepper varieties have higher amount of vitamin C as twice as the concentration found in tomatoes, apples and oranges per gram of fruit weight. Vitamin C is an essential dietary nutrient and a powerful antioxidant for various biological functions in human health and well-being. Increasing consumption of vitamin C rich foods and spices would help to strengthen body's immunity and prevent the development of many diseases [27]. Therefore, high concentration of vitamin C found in the selected hot pepper varieties indicated that the crop can be used to improve nutritional qualities of hot pepper consumers in the society. The results from lycopene showed that the concentration of lycopene was higher in red hot peppers as seen in both pepper cultivars. The compound is mostly present in most red fruits such as tomatoes and adds to antioxidant activities and maintenance of human health [28]. Lycopene is said to be non-toxic and has antioxidant, anti-inflammatory and chemotherapeutic effects, and employed in treating some cancer. Lycopene seems to be the most efficient in neutralizing singlet oxygen free radicals compared to the other common carotenoids due to its unique chemical properties. It cannot be converted to vitamin A; therefore, it is totally available for other properties such as antioxidant activities [23]. The DPPH assay results suggest that the ethanol extracts of both peppers had higher antioxidant activity compared to the aqueous extracts. The Bird eye pepper extracts had higher antioxidant activity than the Bell pepper extracts, which may be due to the higher concentration of bioactive compounds in the Bird eye pepper extracts. Presence of higher amounts of phenol, flavonoid and ascorbic acid (vitamin C) are related directly with higher antioxidant capacities of hot pepper fruit extracts assessed. These results supported the findings reported by several researchers such as [20], who stated that presence of total phenolic content in any plant extract is related to their antioxidant properties. [24] also stated that the presence of significant amount of phenol in fruit extracts contributed to high antioxidant activity of the plant extracts. In reducing power assay, the presence of antioxidants in the samples would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron; the higher the reducing power, the greater the antioxidant activity

CONCLUSION

The study results suggest that both peppers cultivars (bird eye and red bell pepper) contain a diverse range of bioactive compounds with antioxidant and anti-inflammatory properties. Further exploration of the bioactive compounds in these local cultivars holds significant potential for the advancement of native crops. These results not only enhance our understanding of the health benefits of both red bell pepper and bird eye pepper, but also offer valuable insights for the development of functional foods and dietary supplements within the food industry.

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