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Antioxidant activity and phytochemical screening of five species of *capsicum* fruits

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Antioxidants are compounds that help to inhibit many oxidation reactions caused by free radicals. The oxidants activity of five extracts from five varieties of pepper fruits (*Solonaceae*) were evaluated using the ferric thiocyanates method. The total phenolic content of the extracts was determined spectrophotometrically as tannic acid equivalent. The reducing power was also investigated. The antioxidant activity (% oxidation inhibition) ranged from as high as 78.46% in red bird pepper (*Capsicum frutescens*) extracts to as low as 10.11% in green pepper fruits (*Capsicum annum*) extract. Antioxidant activity correlated significantly and positively with total phenolic content ($R^2 = 0.78$), while there was no correlation between total antioxidant activity and reducing power ($R^2 = -0.46$), neither between reducing power and total phenolic content ($R^2 = -0.56$). The phytochemical screening revealed the presence of tannins, flavonoids, saponins, phenols, alkaloids and volatile oils. Pepper fruits studied possess potential health benefits by inhibiting lipid peroxidation hence their use as a value added ingredient. The results indicate that reducing power does not fully characterize the antioxidant activity of the *capsicum* species studied.

Keywords: Antioxidant, peroxidation, phytochemicals.

INTRODUCTION

Pepper belonging to the genus *Capsicum* is a vegetable that comes in an exciting range of colours like green, red and yellow. It is used as a spice, circulatory stimulants, analgesic and obviously, a vegetable (Manson, *et al.*, 1991). They are rich in carotenoids and as good sources of phenolic compounds with antioxidant properties (Perucka and Meterska, 2001). Reactive oxygen species (ROS) which include free radicals and non free radicals are various forms of activated oxygen generated in the body (Yildirim *et al.*, 2000; Gulcin and Oktay, 2002). They have attracted increasing attention because of their role in cellular injury and ageing processes (Lai and Chou, 2000). Overproduction of ROS due to exposure to pollutants, cigarette smoking, UV-rays, radiations and toxic chemicals (Valko *et al.*, 2006) results in a weakened body defence system creating the need for phytochemicals through dietary supplementation. Epidemiological studies have suggested positive associations between the consumption of phenolic-rich,

foods or beverages and the prevention of diseases. The effects have been attributed to antioxidant components such as phenolics, flavonoids and phenylpropanoids among others (Odunkoya *et al.*, 2005). Several studies have reported that plants have antioxidants and represent important source of natural antioxidants. The study is aimed at investigating the antioxidant activity, phenolic content and percentage reducing power as well as phytochemicals in five species of *Capsicum* fruits.

MATERIALS AND METHODS

Plant Materials

All the fresh pepper fruits were purchased from the market in Minna, Niger State, Nigeria and identified in the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria.

Preparation of Extracts

The fruits were picked and debris removed to show only

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edible portions and cut into small pieces. The materials were weighed and homogenized with distilled water using an electric blender. The homogenate was boiled for 3mins and allowed to stand at room temperature for 24hrs before filtration. The filtrates were diluted to produce extract needed for the assay and preserved in the refrigerator for use.

Antioxidant Activity

The antioxidant activity was determined using the ferric thiocyanate method described by Kikuzaki and Nakatani, 1993 with slight modification. 2.0mls of 200mg/L extract, 2mls of 2.5% (w/v) oleic acid in 95% ethanol, 4mls of 0.05M of phosphate buffer (pH 7.0) and 2mls of distilled water were mixed in a 10ml test-tube covered with aluminum foil and fastened with rubber band. A blank sample was prepared using 4ml of distilled water, 2ml of 2.5% (w/v) oleic acid in 95% ethanol and 4ml of 0.05M phosphate buffer (pH 7.0). The tubes were placed in a water bath at 37°C and kept in the dark cupboard to accelerate oxidation. 0.1ml of the mixture was added to 9.7ml of 75% ethanol and 0.1ml of 30% (w/v) ammonium thiocyanate. After 5mins, 0.1ml of 0.02M Ferrous chloride solution 3.5% (v/v) HCl was added and stirred. The peroxide formed was determined by reading absorbance at 500nm at intervals of 24hrs. α -Tocopherol was used as standard antioxidant.

Reducing Power Assay: (Oyaizu, 1986)

2.0mls of each sample was added to 2mls of 0.2M phosphate buffer (pH 6.6) and 2.0ml of 1% potassium ferricyanide. The mixtures were incubated at 50°C for 25mins. 2.0ml of 10% trichloroacetic acid was added to each reaction mixture and centrifuged for 10mins. 2.0ml of upper layer solution was mixed with 2.0ml distilled water and 0.5ml FeCl₃. After 10mins, the absorbances were read at 700nm.

Determination of Phenolic Content (Ranganna, 1997)

Tannic acid equivalent method was used. Calibration curve was prepared by mixing a solution of tannic acid with 5ml Folin-Dennis reagent, 75mls of distilled water and 10mls of 0.7M sodium carbonate. Absorbances were recorded at 720nm and standard curve drawn. 50ul of extract was made up to 7.5ml by distilled water, then 1.0ml reagent and 1.0ml of sodium carbonate were mixed and volume made up to 10mls with distilled water. Absorbance was recorded at 710nm. The tannic acid equivalents were then calculated.

Phytochemical Analysis: Trease and Evans (1989)

Tannins

A portion of the extract was dissolved in water and clarified by filtration. 10% Ferric chloride solution was then added to the resulting filtrate. The bluish colour indicates presence of tannins.

Alkaloids

0.5g of the extract was stirred in 5.0ml of 1% HCl on steam bath and filtered while hot. Few drops of distilled water were added and 1.0ml of the filtrate was treated with few drops of Wagner's reagent. A reddish brown precipitate indicates presence of alkaloids.

Cardiac Glycosides

0.5g of the extract was dissolved in 2.0ml glacial acetic acid containing a drop of Ferric chloride solution followed by 2ml of Conc. H₂SO₄. A brown ring formation at interphase indicates presence of deoxy sugars.

Flavonoids

2.0mls of dil NaOH was added to 2.0ml of the extract. The appearance of a yellow colour indicates presence of flavonoids.

Saponins

1.0ml distilled was added to 1.0ml extract and shaken vigorously. A stable persistent froth indicates the presence of saponins.

Phenols

Equal volumes of extracts and FeCl₃ were mixed. A deep bluish green solution indicates presence of phenols.

Anthraquinones

0.5g of extract was shaken with 10ml of benzene and filtered, 10% of ammonia solution was added to filtrate and the mixture shaken. The formation of a pink, red or violet colour on the ammoniacal phase indicates anthraquinones.

Reducing Sugars

3.0ml of extracts was dissolved in 5ml of distilled water followed by Fehlings A and B solution, it was then boiled. A red precipitate indicates a reducing compound.

Protein

0.5g of the extract was added to 10ml distilled water and the mixture was left to stand for 3hrs and filtered. The 2ml portion of the filtrate was added to 0.1ml Millon's reagent. A yellow precipitate indicates presence of protein.

Carbohydrate

0.5g of the extract was shaken vigorously with water and then filtered. To the aqueous filtrate, few drops of Molisch reagent were added, then 1.0ml Conc H₂SO₄ to form a layer of aqueous layer. A brown ring at interphase indicates carbohydrates.

Volatile Oils

The extract was dissolved in 90% ethanol and drops of FeCl₃ were added. A green colour formed indicates presence of volatile oils.

Steroids

0.5g of extract was dissolved in 3ml of chloroform and filtered. Conc. H₂SO₄ was carefully added to the filtrate. A reddish brown colour at interphase indicates a steroid ring.

Amino Acids

Few drops of ninhydrin reagent were added to 1.0ml of extract. Appearance of purple colour shows the presence of amino acids.

RESULTS

Table 1 shows results of antioxidant activity, phenolic activity and percentage reducing power while table 2 shows the phytochemical screening results.

DISCUSSION

All the aqueous pepper extracts showed low absorbance values, which indicate a high level of antioxidant activity except the green bell pepper (Table 1). Three of the extracts exhibited strong antioxidant activity (Red bird

pepper – 78.46%, Chili pepper 42.3%, Yellow bird pepper 67.08%) surprising the activity of the commercial standard (40.04%). The other two varieties showed antioxidant activity below that of α -tocopherol. The relative antioxidant activities of red and yellow bird pepper, red bell pepper were higher (78.46%, 67.08 and 42.37%) than that of Chili and green bell pepper (36.45% and 10.11%). The antioxidant activity decreased in the order of red bird pepper < yellow bird pepper < red bell pepper < chili pepper < green bell pepper. Different antioxidants occur in plant tissues especially fruits and vegetables. Thus, it makes it difficult to measure each antioxidant component separately. Therefore, several methods have been developed in recent years to calculate the total antioxidant activity of biological samples (Odunkoya et al., 2005d). The statistical analysis showed a positive and strong linear relationship between the total phenolic content and the antioxidant activity suggesting that the antioxidant activity in these peppers is largely due to the presence of phenolic compounds. The same relationship was observed between phenolic contents and antioxidant activity in rose hill extract and some other vegetables (Odunkoya et al., 2005e, Jaranmardi et al., 2003b, Surh et al., 1998b, Okamura et al., 1993b, Gulcin and Oktay, 2002c, Sharique and Seerate, 2009a, Suskrano and Luke, 1993a and Manson et al., 1991b). Also there was no linear correlation between total antioxidant activity and reducing power. No relationship was observed between reducing power and total phenolic content. The same was observed in Ethiopian pepper and some other vegetables (Aliyu et al., 2010b, Santos et al., 2003b, Couladis, et al., 2003b and Odunkoya et al., 2005f). Recent studies have demonstrated that the antioxidant activity is correlated with the number of hydroxyl groups (Suskrano and Luke, 1993 and Odunkoya et al., 2005). The reducing capacity of compounds could serve as indicator of potential antioxidant properties (Meir, et al., 1995).

Phytochemicals such as flavonoids, saponins, tannins, alkaloids were present in the pepper extracts (Table 2). Phytochemicals especially polyphenols have received increasing attention because of their biological activities (Cho et al., 2003). They constitute a major group of compounds that acts as antioxidants (Hatano et al., 1989).

The significance of antioxidants in extracts lie in the chemical constituents that could be linked to flavonoids or tannins found in the extracts. Many flavonoids have shown strong antioxidant properties (Raj and Shalini, 1999) and quercetin has been established as a strong antioxidant principle (Thabrew et al., 1998). These results indicated that pepper containing high phenolics provides a source of dietary antioxidants and in addition imports flavour to food hence its potential use as a value added ingredient for stabilizing food against lipid peroxidation reactions.

Table 1. Antioxidant activity, phenolic content and reducing power of the aqueous extract of five species of *Capsicum* fruits

Varieties	Antioxidant Activity (% inhibition)	Phenolic Content (mg/ml) TAE	% Reducing Power
Red bird pepper	*78.46+3.42	77.83+2.81	32.50+0.00
Red bell pepper	42.37+2.69	80.00+5.86	28.33+0.44
Chili pepper	36.45+1.98	76.83+2.78	33.33+0.17
Yellow bird pepper	67.08+1.58	81.17+2.78	23.67+0.17
Green bell pepper	10.11+1.21	51.67+2.96	33.67+0.34
α -Tocopherol	40.04+2.51		

* Values are means \pm SEM of 3 determinations
TAE = Tannic acid equivalent

Table 2. Results of Phytochemical Screening of Aqueous Extract of Five Varieties of Pepper Fruits

Phytochemicals	Red Bird Pepper	Red Bell Pepper	Chili Pepper	Yellow Bird Pepper	Green Bell Pepper
	++	++	++	++	+
Flavonoids	++	++	++	++	+
Cardiac glycosides	-	-	-	-	-
Reducing sugar	-	-	-	-	-
Carbohydrates	-	-	-	-	-
Proteins	-	-	-	-	-
Alkaloids	+	+	+	+	+
Phenols	++	++	++	++	++
Volatile oils	-	+	+	-	+
Amino acids	-	-	-	-	-
Steroids	-	-	-	-	-
Saponins	++	++	+	++	+
Anthraquinones	-	-	-	-	-

++ = Abundant
+ = Moderate
- = Absent

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