

COMPARATIVE EVALUATION OF ANTIOXIDANT PROPERTIES OF METHANOL EXTRACTS OF *Allium cepa* bulb, *Allium cepa* bulb peels and *Allium fistulosum*

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ABSTRACT. Medicinal plants have protective effects against many physiological diseases because of their phytochemical components which are better antioxidants. This study was aimed at comparing the *in vitro* antioxidant activity, phenolic and flavonoid content of the methanol extracts of *Allium cepa* bulb, *Allium cepa* bulb peel and *Allium fistulosum*. The antioxidant activity was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), iron chelating activity and molybdate ion reduction assays. The total flavonoid and total phenolic content (TPC) were carried out using aluminium chloride and Folin-Ciocalteu assay respectively. The results showed that *A. cepa* bulb peel has the highest phenolic content and the antioxidant activity while the *A. fistulosum* showed the least. Furthermore, the total flavonoid content and metal iron chelating activity was highest in *A. cepa* bulb, while lowest values were obtained in *A. fistulosum* and *A. cepa* bulb peel respectively. The results suggest that the *A. cepa* bulb peel, *A. cepa* bulb and *A. fistulosum* extracts can be used as sources of natural antioxidants. However, *A. cepa* bulb peel may be the most potent.

Keywords: *Allium* species, antioxidant activity, phenolics, flavonoids.

INTRODUCTION

Modern consumers are becoming more health conscious and more aware of food nutritional value. Among the nutrients, antioxidants are popular due to their ability to prevent many physiological diseases or illnesses e.g. neuronal degeneration (GONZÁLEZ-SARRÍAS *et al.*, 2017). Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (ROS) or free radicals, which are harmful by-products generated during aerobic activity of normal cells (WOLF, 2005). Increasing the intake of dietary antioxidant is believed to assist in maintaining an adequate antioxidant status and therefore, the normal physiological function of living system. Many fruits and vegetables are potentials for decreasing risk effect of several chronic diseases, such as cancer, coronary heart disease and many more (TEPE *et al.*, 2005). Onion (*Allium cepa* Linn.) and Scallions (*Allium fistulosum* Linn) are members of the Liliaceae family and belongs to the genus *Allium* which consists of

about 450 species. They are root vegetables that include other flavourful members like shallots, leeks and garlic to mention a few. The species *A. cepa* Linn, belong to the *Cepa* group which includes the typical bulbing onions with a single enlarged bulb (FRITSCH and FRIESEN, 2002). *A. fistulosum* species, also known as spring onions are very similar in taste and odor to the related common onion, *A. cepa*, however they do not develop bulbs, but possess hollow leaves and are referred to as bunching onions (BREWSTER, 1994). The name *A. fistulosum* comes from the fact that the leaves are hollow, ("fistulosum" means "hollow"). *Allium* species are rich in two secondary metabolites that have perceived health benefits (YOSHIMOTO *et al.*, 2015). These are the flavonoids (quercetin, anthocyanins and allixin) and the odorless organosulphur compounds, alk(en)yl cysteine sulphoxides (ACSOs). By the influence of the enzyme alliinase (E.C.4.4.1.4), ACSOs are cleaved to produce pyruvate, ammonia and a thiosulphinate (NDOYE FOE *et al.*, 2016). Thiosulphinates are the volatile organosulphur compounds and are intermediates in the formation of other sulphur volatiles, such as the disulphides. These sulphur volatiles are responsible for the flavour of onion (KEUSGEN, 2002). Onion also contain other organosulphur compounds, the gamma-glutamyl peptide that do not form sulphur volatile and sulphur-containing amino acids, which are higher in red onion bulb than the white ones. The different species of *Allium* vary in their content of the enzyme alliinase and S-alk(en)yl-cysteine sulphoxide precursors (LANZOTTI, 2006). Besides sulphur compounds, saponins, Phenolics and selenium were reported for a number of *Allium* species and may also contribute to the health benefits of these plants (KEUSGEN, 2002). In addition to the sulphur compounds found in *Allium* species, MAY-CHIEN and CHI-TANG (1992), reported the presence of 41 novel sulphur containing compounds in scallion. *Allium* plants are widely distributed over Europe, Asia and America, and they have been used for millennia as spices, vegetables and for the treatment of diseases (FRITSCH and FRIESEN, 2002; LANZOTTI, 2006; MICHAEL *et al.*, 2006). The Greeks and Romans cherished onions as curative agents (COREA *et al.*, 2005). Onion consumption combined with healthy diet can be effective in nonalcoholic fatty liver disease management therapy (EMAMAT *et al.*, 2016) while onion bulb peel has anticholesterol, antithrombotic, and insulin-sensitizing properties (CHOI *et al.*, 2015). *A. fistulosum* have also been shown to improved serum triglyceride, total cholesterol, and leptin levels in high-fat-diet-induced obese mice (SUNG *et al.*, 2015). According to BENKEBLIA (2005), *Allium* species are revered to possess anti-bacterial and anti-fungal activities. Thiosulphinate found in *Allium* species exhibit different degrees of antimicrobial activity and are found to be effective antioxidants in terms of scavenging free radicals (BENKEBLIA and LANZOTTI, 2007). During the last 20 years, *Allium* spices have been among the most studied vegetables and aroused great interest. In our previous studies, onion was found to increase the antioxidants status in rat's kidney during cyanide toxicity (OLA-MUDATHIR and MADUAGWU, 2014) and this fact is an indication of the importance of antioxidant activities of *Allium* species in diseased state. YIN *et al.* (2006), showed that scallion possess both antioxidant and antibacterial activities. However, according to LI *et al.* (2006), synthetic antioxidants such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) need to be replaced with natural antioxidants as several studies showed that a number of synthetic antioxidants were toxic and carcinogenic in animals. Onion bulb and scallion are widely consumed and have been used over the years for tackling diseases (CHEN *et al.*, 2000), while the consumption of onion bulb peels and its use for treatment of diseases have been very scanty. For this reason, the aim of this work was to compare the antioxidant levels and activities in scallion, red onion bulb and red onion bulb peels.

MATERIALS AND METHODS

The plant materials, *Allium cepa* bulb (spp. red onion bulb) and *Allium fistulosum* were obtained in February 2016 from Lafenwa market, Abeokuta, Ogun-State, Nigeria and

authenticated in the Department of Botany, University of Ibadan. The outermost cover of *A. cepa* bulbs (spp. red onion bulb) were removed rinsed in distilled water and air-dried to obtain the *A. cepa* bulb peels. 50 g of each sample (*A. cepa* bulb, *A. cepa* bulb peels and the whole plant of *A. fistulosum*) were homogenized in 250 mL of 80% methanol at 4°C using an electric blender. The extracts were stirred for 10 minutes at 4°C and filtered through Whatman No. 1 filter paper to obtain the extracts which were concentrated using a rotary evaporator at 45°C. Extract yield obtain for *Allium cepa*, *Allium cepa* peels and *Allium fistulosum* were 8.2%, 20.5% and 10.4% respectively

Chemicals

The standard chemicals like Methanol, Quercetin, Gallic acid, Ascorbic acid, Ammonium molybdate, ferric chloride, 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH), 2,4,6-tris(2-pyridyl)3-triazine (TPTZ), Hydrochloric acid, Distilled water, Sodium phosphate, Hydrogen tetraoxosulphate(xi), Acetate buffer, Sodium nitroprusside, Sodium carbonate, Folin-Ciocalteu's phenolic reagent, Aluminum chloride, Sodium hydroxide, sulphamide, EDTA, Ferrozine, Iron(II)tetraoxosulphate(xi), Potassium per sulphate were purchased from Sigma-Aldrich Chemical, United Kingdom.

DPPH radical scavenging activity

The free radical scavenging capacity was determined using the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate) as described by BRAND-WILLIAMS *et al.* (1995). DPPH is reduced to its corresponding hydrazine in the presence of hydrogen donor such as antioxidant. The change in color from deep violet to light yellow was measured through spectrophotometry. In this method, the various concentrations of the test extracts in methanol were added to 150 µL of a solution of DPPH in a microtiter plate. After 30 min of incubation at 37°C in the dark, the absorbance was recorded at 510 nm. Ascorbic acid was used as standard and all experiments were carried out in triplicate. The decrease in the DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity of sample. The DPPH scavenging activity of the extracts was calculated and IC₅₀ (concentration of sample required to inhibit 50% of DPPH radicals) value was determined from this equation:

$\{(A_c - A_0) / A_c\} \times 100$; where A_c = absorbance of control and A_0 = absorbance of sample.

Metal chelating assay

The ferrous ion-chelating (FIC) assay was carried out according to the method of SINGH and RAJINI (2004), with some modifications. The various concentrations of the test extracts were added to 100µL of FeSO₄ solution in a microtiter plate for 5 minutes after which 100 µL of Ferrozine solution were added and incubated for another 10 minutes. After incubation, the absorbance was taken at 560 nm with a microplate reader. EDTA was used as a standard and all experiments were carried out in triplicate. The decrease of the ferrous ion chelating solution absorbance indicates an increase of the metal chelating radical scavenging activity.

Total phenolic content

The method of determining the total phenolic content was described by Singleton and ROSSI (1965) as reported by GULCIN *et al.* (2003) using the folin ciocalteu's phenol reagent which is an oxidizing reagent. 100 µL of each extract (10 mg/mL) was pipetted into three different test tubes. 900 µL of distilled water was added into the test tubes. 200 µL of Follin's reagent were pipetted into the test tubes. After 5minutes 1 mL of 7% sodium carbonate and 0.3

mL of distilled water were added to all tubes. The tubes were incubated at room temperature for 90 minutes after which the absorbance was taken at 750 nm. The results were expressed as mg gallic acid equivalent per gram dry weight extract (mg GAE/g extract). The results were averages of triplicates analyses.

Total antioxidant capacity

The total antioxidant capacity was determined by phosphomolybdate method using ascorbic acid as standard (PRIETO *et al.*, 1999). An aliquot of 25 μ L of each extract (1 mg/mL) solution was mixed with 300 μ L of TAC reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The microplate was incubated in a microplate incubator for 90 minutes. The absorbance of the mixtures was measured at 630 nm in a microplate reader. The antioxidant activity of the extracts was expressed as the number of mg equivalents to ascorbic acid (AAE)/g extract. All experiments were carried out in triplicates.

Reducing power assay (FRAP)

Reducing power was evaluated according to the method of BENZIE and STRAIN (1996). An aliquot of 25 μ L of each extract (1 mg/mL) was mixed with 300 μ L of FRAP reagent [300 mM Acetate buffer, 0.003g of TPTZ (2,4,6-Tris(2-pyridyl) 3-triazine) in 1 mL of 40 mM HCl and 18.6 μ L of 20 mM ferric chloride in 5mL of water in ratio 10:1:1]. After incubation for 90 minutes at 37°C, the absorbance was measured at 593 nm in a microplate reader. Increase in absorbance of the reaction mixture indicates increased reducing power. All the tests were performed in triplicates.

Total flavonoid content

The determination of the total flavonoid content was carried out based on the aluminium chloride colorimetric assay method as described by NEERGHEEN *et al.* (2006), using quercetin as a standard. An aliquot of 25 μ L of extract (1mg/mL) solution in methanol was mixed with 25 μ L of 5% NaNO₂ and 100 μ L of distilled water was added in microtiter plates. This was incubated for 5 min. Then 25 μ L of 10% AlCl₃ was added to all the mixtures in the microtiter plates, after which 50 μ L of 1 M NaOH was added. To the contents in the microtiter plates, 25 μ L of distilled water was added to make an overall volume of 0.25 mL. Absorbance was taken at 510 nm. All determinations were carried out in triplicate. The total flavonoid in each plant extract was determined as quercetin equivalents per gram extract (mg QE/g extract).

Statistical analysis

All experiments were carried out in triplicates; the results were expressed as the Mean \pm Standard deviation. Statistical analysis was performed using Microsoft Excel program.

RESULTS AND DISCUSSION

Antioxidants can deactivate radicals by three major mechanisms: hydrogen atom transfer (HAT), electron transfer (ET), and a combination of hydrogen atom transfer and electron transfer (Prior *et al.*, 2005). HAT measures the ability of an antioxidant to quench free radicals by hydrogen donation, thus quantifies hydrogen atom donating capacity. ET detects the ability of antioxidant to transfer one electron to reduce radicals, metals and carbonyls, thus measures antioxidant reducing capacity (HUANG *et al.*, 2005). FRAP, total antioxidant capacity and total phenolic content using Folin-Ciocalteu method are antioxidant assays that involves

ET, while DPPH assay is a combination of HAT and ET. These methods together with total flavonoid content (using aluminium chloride) and metal chelating assays were employed in this study to determine whether the onion bulb, onion bulb peel, or scallion has the highest antioxidant activity.

Total phenolic content

Phenolics are the most widely spread secondary metabolite in plant kingdom. They are natural substances in plants that can have antioxidants potential. They can have the potential to protect the body from some diseases (SINGH *et al.*, 2009; PÉREZ-GREGORIO *et al.*, 2010). Recent studies have shown that many dietary polyphenolic constituents derived from plants are more effective antioxidants *in vitro* than vitamins E or C, and thus might contribute significantly to protective effects *in vivo*. They may also be important in chelating transition metals and scavenging free radicals (MUHAMMED *et al.*, 2010). The total phenolic content of the onion extracts was examined, using Folin-Ciocalteu method and this was expressed as gallic acid equivalents. This reaction involves constituents that possess hydroxyl groups and measures reducing capacity. The methanol extract of *A. cepa* bulb peel has the highest phenolic content of 28.94 ± 2.331 mg gallic acid equivalent per gram extract than the other fractions (Tab. 1). The phenolic content of the other *Allium* fractions decreased in order *A. cepa* > *A. fistulosum* (3.41 ± 0.814 and 1.54 ± 0.172 mg gallic acid equivalent per gram extract respectively). Previous studies have shown that onion contains phenolics such as quercetin, gallic acid, ferulic acid, and their glycosides (SINGH *et al.*, 2009; PÉREZ-GREGORIO *et al.*, 2010). The phenolic compounds of this plant are responsible for their antioxidant activity (RICE-EVANS *et al.*, 1996).

The result of this study is supported by that of CHENG *et al.* (2013), where the total phenol contents in onions increase during the aging of the cells of the outer layer of onion bulbs, because it is known that the cells of the outer layer are more aged than those of the inner layer in a bulb, indicating that the onion bulb peel has the highest phenolic content. *Allium fistulosum* was also found to have the lowest total phenolics content when compared with red onion bulb and the other onion varieties (BENKEBLIA, 2005).

Total flavonoid content

Flavonoids are a class of phenolic compounds which impart bitter and astringent flavours to fruits and vegetables. They are known to scavenge particles in the body known as free radicals. The total flavonoids content was determined spectrophotometrically using aluminium chloride. The flavonoids content was expressed in terms of quercetin equivalents. *Allium cepa* bulb has the highest flavonoid content followed by *A. cepa* bulb peel, and finally *A. fistulosum* with the values of 604.63 ± 22.920 , 336.10 ± 19.250 and 237.75 ± 10.498 mg QUE/g plant extract respectively (Tab. 1). The lower value for *A. cepa* bulb peel compared to the *A. cepa* bulb suggests that the total flavonoid and their derivatives are not the main contributor to the high phenolic content in the *A. cepa* bulb peel as there are some other phenolics compounds present in the *A. cepa* bulb peel. Specifically, onion bulb has been characterized for its flavonol, quercetin and quercetin derivatives (ROLDÁN-MARÍN *et al.* 2009). The higher flavonoid content in the bulb with respect to the onion bulb peel is however in disagreement with the work of (CHENG *et al.* 2013).

Table 1. Total phenolic and flavonoid contents of the methanol extracts of *Allium cepa* bulb peel, *Allium cepa* bulb, and *Allium fistulosum*.

Plant material	Total phenols (mg gallic acid equivalent/g plant extract)	Total flavonoids (mg quercetin equivalent/g plant extract)
<i>A. cepa</i> bulb peel	28.94 ± 2.331	336.10 ± 19.250
<i>A. cepa</i> bulb	3.41 ± 0.814a	604.63 ± 22.920a
<i>A. fistulosum</i>	1.54 ± 0.172a	237.75 ± 10.4980ab

^ap < 0.05: significantly different from *A. cepa* bulb peel

^bp < 0.05: significantly different from *A. cepa* bulb

Values are expressed as mean of triplicate determinations ± standard deviations.

DPPH radical scavenging activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) is characterized as a stable free radical due to the delocalization of free electron that gives rise to deep violet colour and characterized by an absorption band in methanol solution centered at about 517 nm. When a solution of DPPH is mixed with substrate which can donate a hydrogen atom, this gives rise to the reduced form with a loss of this violet colour (NUR *et al.*, 2012). Therefore, the use of DPPH provides an easy and rapid way to evaluate antioxidant activity of any material. The methanol extract of *A. cepa* bulb peel has the highest DPPH radical scavenging activity compared to the other test samples (IC₅₀ = 0.19 ± 0.047 mg/mL) followed by *A. cepa* bulb (IC₅₀ = 0.24 ± 0.017 mg/mL) and finally *Allium fistulosum* (IC₅₀ = 0.58 ± 0.002 mg/mL) (Tab. 2). This is in line with the findings of BENKEBLIA (2005), who reported that *Allium fistulosum* exhibited a lower scavenging activity when compared with red onion bulb. This result follows almost the same trend as that obtained for the total phenol content, supporting the view that phenols are responsible for the scavenging activity of plants (NDOYE FOE *et al.*, 2016, MUHAMMED *et al.*, 2010). Furthermore, DUH *et al.* (1999) reported similar results for *Chrysanthemum morifolium* with high relationship between phenolics content and scavenging activity of the water extracts.

Metal chelating assay

The basic principle of this in vitro antioxidant assay is based on the capacity of the plant extract to decolorize the iron-ferrozine complex. The decrease in the absorbance of the complex indicates chelating power of the extract. An extract with a high chelating power reduces the formation of this complex, which is a stable iron (II) chelate, by reducing free ferrous ion concentration, and thus decreases the extent of Fenton reaction that are implicated in many diseases (LIM *et al.*, 2006). Metal ion-chelating activity of an antioxidant molecule prevents generation of free radical and thus oxidative damage (KUMAR *et al.*, 2008).

Table 2. DPPH and metal chelating assay of the methanol extracts of *Allium cepa*, *Allium cepa* bulb peel and *Allium fistulosum*.

Plant material	DPPH free radical activity IC ₅₀ [mg/mL]	Metal chelating scavenging activity IC ₅₀ [mg/mL]
<i>A. cepa</i> bulb peel	0.19 ± 0.047	1.12 ± 0.054
<i>A. cepa</i> bulb	0.24 ± 0.017	0.11 ± 0.001a
<i>A. fistulosum</i>	0.58 ± 0.002ab	0.24 ± 0.026a

^ap < 0.05: significantly different from *A. cepa* bulb peel

^bp < 0.05: significantly different from *A. cepa* bulb

Values are expressed as mean of triplicate determinations ± standard deviations.

Low metal chelating activity was observed for *A. cepa* bulb peel with IC_{50} of 1.12 ± 0.054 mg/mL with respect to *A. cepa* bulb and *A. fistulosum* having IC_{50} of 0.11 ± 0.001 and 0.24 ± 0.026 mg/mL respectively (Tab 2). Since the ability to chelate transition metals can be considered as an important antioxidant mode of action, the antioxidant activity observed in *A. cepa* bulb which as the highest metal chelating activity may be in part as a result of this activity. Although, MUHAMMED *et al.* (2010) showed that phenol might play an important role in chelating transition metals, the relatively low phenolic content in *A. cepa* bulb and *A. fistulosum* that was observed in this study suggests that other organic compounds contribute to the high metal chelating activities in these plants. It was also reported by KHAMSAH *et al.* (2006) that antioxidant activity is not due to the phenolics only because they observed that the antioxidant activity of methanol extract of *Orthosiphon stamineus* was not solely caused by phenolic compounds.

Reducing power assay (FRAP)

Reducing power was measured by direct electron donation in the reduction of $[Fe^{3+}(CN)_6]^{3-}$ to $[Fe^{2+}(CN)_6]^{4-}$. The product was visualized by forming the intense Prussian blue color complex and then measured at 700 nm (NISHAA *et al.*, 2012). The reducing ability of a compound generally depends on the presence of reductants i.e. antioxidants (Umamaheswari *et al.*, 2008). The *A. cepa* bulb peel has the highest reducing power activity with a value of 8.59 ± 2.220 mg AAE/g of sample followed by *A. cepa* bulb and *A. fistulosum* (5.37 ± 1.185 and 3.38 ± 0.227) mg AAE/g of sample respectively (Tab. 3). This result is comparable with that of Aoyama and YAMAMOTO (2007), in which the FRAP values obtained for red onion bulb was higher than that of *A. fistulosum*. The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity (CHUA *et al.*, 2008). The observed trend in the reducing power might be as a result of that obtained for total phenolic content in which *A. cepa* bulb peel contained more phenolic compounds than *A. cepa* bulb, which also contained more phenolic compounds than *A. fistulosum*, as the phenolic content increases the ability to reduce the ferricyanide complex of Fe^{3+} to the ferrous (Fe^{2+}) form. Phenolic compounds are known to possess hydrogen donating ability (SHIMADA *et al.*, 1992), the hydrogen donated then serve as the reducing agent (DUH, 1998).

Total antioxidant capacity (Phosphomolybdate assay)

The phosphomolybdate method is based on the reduction of Mo (VI) to Mo (V). The antioxidant sample is detected by the formation of a green color phosphomolybdenum (V) (BAIG *et al.*, 2011). The quantitative antioxidant activity is expressed as mg equivalent to ascorbic acid per gram of extract (mg AAE/g extract). The total antioxidant capacity of the test samples, *A. cepa* bulb peel have the highest activity (63.91 ± 1.312 mg equivalent of ascorbic acid / g plant extract) followed by *A. cepa* bulb and *A. fistulosum* (12.94 ± 1.944 and 6.35 ± 1.698) mg equivalent of ascorbic acid / g plant extract respectively (Tab. 3). The *A. fistulosum* have also been shown to possess the lowest antioxidant capacity when compared with red and yellow onion (AOYAMA and YAMAMOTO, 2007). As the antioxidant activities of plant extracts are often explained by their total phenolic, flavonoid contents (JANG *et al.*, 2008) and reducing ability (CHUA *et al.*, 2008), this may account for the observed trend in the total antioxidant capacity, i.e. the highest total antioxidant capacity of *A. cepa* bulb peel may be due to its having the highest total phenolic, reducing ability, free radical scavenging activity and fairly high total flavonoid content (NDOYE FOE *et al.*, 2016), while the moderately high value obtained for the total antioxidant content of *Allium cepa* bulb may be attributed to its high flavonoid content and metal chelating ability.

Table 3. Reducing power assay and total antioxidant capacity assay of the methanol extracts of *Allium cepa*, *Allium cepa* bulb peel and *Allium fistulosum*.

Plant material	Reducing power assay (absorbance at 563 nm)	Total antioxidant assay (absorbance at 563 nm)
<i>A. cepa</i> bulb peel	8.59 ± 2.220	63.91 ± 1.312
<i>A. cepa</i> bulb	5.37 ± 1.185	12.94 ± 1.944a
<i>A. fistulosum</i>	3.38 ± 0.227a	6.35 ± 1.698ab

^ap < 0.05: significantly different from *A. cepa* bulb peel

^bp < 0.05: significantly different from *A. cepa* bulb

Values are expressed as mean of triplicate determinations ± standard deviations.

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