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NUTRITIONAL CONTENT, PHYTOCHEMICALS AND IN-VITRO ANTIOXIDANT ACTIVITIES OF ETHANOL EXTRACT OF RED AND WHITE ONION

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ABSTRACT

Since antiquity onions (*Allium cepa, L*) have been cultivated across the globe as an important source of food and medicine. The study evaluated the nutritional content, phytochemistry and invitro antioxidant activities of ethanol extract of red and white onion bulbs. Red and white onion bulbs were purchased from a Local market in Maiduguri. Ethanol extracts were prepared using homogenized bulb. The proximate analysis, flavonoids, tannin, and phenol contents of the extracts as well as the antioxidant activities (total antioxidant activity, reducing power, and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities) were evaluated using standard procedures. The ash and carbohydrate contents of the two extracts were similar. However, the fat and protein contents were higher in white onion (2.44% & 1.05%) compared to the red onion (1.00% & 0.13%) while the moisture content was higher in red onion (16.10%) compared to the white onion (13.60%). The flavonoids, tannin, and phenol content as well as the total antioxidant activity was significantly higher ($p<0.05$) in the red onion relative to the white. White onion had a better reducing power activity compared to the red while the red had higher DPPH free radical scavenging activity compared to the white. Conclusively, our findings revealed that both red and white onion contains varying quantity of phenolic compounds with strong reducing power and DPPH free radical scavenging activity. However, red onion was shown to have higher antioxidant activity relative to the white.

Keywords: Antioxidant, flavonoid, phenol, onion, reducing power

INTRODUCTION

Since antiquity onions (*Allium cepa, L*) have been cultivated across the globe (Africa, America, Asia, and Europe) as an important source of food and medicine (Kavalcova et al., 2015). Onion is rich in vitamins, carbohydrate, minerals (selenium, calcium, and iodine), as well as bioactive compounds such as rutin, volatile sulfur, quercetin, cepaenes, benzoic acid, and anthocyanins (Benitez et al., 2011; Lee et al., 2015; Kavalcova et al., 2015). The phytochemistry and constituent of onion is reported to vary in different varieties (red, white, and yellow) of onion (Mahmood et al., 2021). Previous studies reported that continuous consumption of onion reduces the risk of developing heart disease, several type of cancers, neurodegenerative disease and metabolic disorders (Aoyama and Yamamoto, 2007; Singh et al., 2009; Kumar et al., 2022; Ijeoma et al., 2023). The role of onion as food and medicine is attributed to the high protein and carbohydrate and the presence of biologically active compounds (Ligouri et al., 2017).

Onion grows well in a loose water-logged loamy or clay soil with slightly acidic pH (6-6.5). It is the second most cultivated crop after tomato with a cultivation area of over 3.4 million hectares of land world-wide (Shokri et al., 2022; Aksay and Yavuzaslanoglu, 2023). The annual global onion production is estimated at over 89 million tons with China, India, and USA being the top three producers and contributing to over 52 percent of the world onion (FAO, 2020; Adeoti et al., 2021; Ochar and Kim, 2023). Onion is ranked among the top 20 onion producers in the world with Kaduna, Kano, Sokoto, Katsina, and Borno contributing to over 80 percent (Adeoti et al., 2021). The onion production is believed to significantly increase in the coming years. However, lack of adequate storage facilities has increases onion post -harvest losses and limiting the amount of nutrients available in onion for the world's populace (Yeshiwas et al., 2023). Hence, evaluating the phytochemicals and nutritional content of different onion varieties will help producers in choosing the variety with maximum nutrients and phytochemicals. The current study evaluated the nutritional content, phytochemistry and invitro antioxidant activities of ethanol extract of red and white onion bulbs.

MATERIALS AND METHODS

Plant authentication

Red and white onion bulbs were purchased from a Local market in Maiduguri, Nigeria and authenticated at the Herbarium, Faculty of Pharmacy, University of Maiduguri, Nigeria (UMM/FPH/AMA/001).

Extraction. The bulbs were homogenized with a blender and dissolved in ethanol in 1:1 ratio for 48 hours. The mixtures were filtered and the filtrate was evaporated in an oven at 45 ^oC to get the ethanol extract of the red and white onion bulbs.

Proximate analysis

The ash, moisture, fat, protein, fiber, and carbohydrate contents of the extracts were evaluated as follows; for the ash content, 5g of the extract was placed in a muffle furnace at 550 ^oC for 180 min. It was removed, cooled in a desiccator and weighed. For moisture content, 5g of the extracts was placed in an oven at 105 ^oC for 180 min. It was removed, cooled in a desiccator and weighed. For protein content, 7 ml of sulfuric acid and sodium sulfate were added to 1 g of the extract for digestion. The mixture was placed in a heating mantle and the temperature was gradually increased from 30-100 ^oC until a color change from black to clear or neon green was observed. The digest was cooled and diluted with 150 ml of distilled water. 20 ml of NaOH was added, then the solution was distilled and the distillate collected in Boric acid. The

distillate was titrated with 0.1 ml sulfuric acid until the formation of pink color.
For fat content, 2 g of the extract was placed in a thimble and the thimble was placed in an extractor a little above a round bottomed flask containing petroleum ether. The flask, condenser, and extractor were fixed (Soxhlet apparatus) and placed in a heating mantle to biol for 3-5 hours. The solvent was evaporated into the flask containing the extract and the flask was weighed to get fat content. For crude fiber content, 3 g of defatted extract was mixed with 200 ml of boiling sulfuric acid (1.25%). The mixture was attached to a condenser and allowed to boil for 30 min. the content was left to settle for 1 min and filtered. The residue was transferred to a flask containing 200 ml of boiling NaOH and allowed to boil for 30 min. The hydrolyzed mixture was filtered after resting for 1 min. the residue was washed with boiling water, HCl, boiling water again, and petroleum ether. The residue was then placed in a crucible and transferred to a furnace at 550 ^{o}C for 180 min and allowed to dry. Crude fiber content (%) = 100(A-B/C). where A= crucible weight with residue (g), B= crucible weight with ash (g), and C= sample weight (g). The carbohydrate content was calculated as; Carbohydrate content= 100 - (ash, moisture, fat, protein, & fiber contents).
Phytochemical analysis
The total flavonoids, tannin and total phenol contents of the two extracts were evaluated as described in our previous study (Manye et al., 2023). Briefly, the total flavonoid was estimated by mixing 1 ml of the extract with distilled water (200 µL). Five percent sodium nitrite (150 µL) was added and incubated for 5 min. Ten percent aluminium chloride (150 µL) was then added and allowed for 6 min. Four percent sodium hydroxide (2 mL) and distilled water were added to make 5 mL. The mixture was allowed to stand for 15 min at room temperature and then measured spectrometrically at 510 nm. Total flavonoid was expressed as mg of quercetin equivalent (mg QE/g extract) on a dry matter basis using the standard curve.
The tannin content was estimated by treating the extract (500 µL) with polyvinyl pyrrolidone (100 mg) and distil water (500 µL), incubating the solution at 4 °C for 4 hours and centrifuging at 5000 rpm for 5 min. Phenolic content of the supernatant was measured at 725 nm and expressed as free phenols on a dry matter basis. Tannins content in mg Gallic acid equivalent (mg GAE/g extract) was calculated as =Total Phenols-Free phenols.
The total phenolic content was estimated using the Folin-Ciocalteau reagent as follows; 20 µg of the extract was mixed with distilled water to make 1 mL. Diluted Folins-phenol reagent and distilled water in a ratio of 1:1 plus 2.5mL sodium carbonate (20%) were added to the mixture, shaken vigorously and incubated in dark room for 40 min to develop color. The absorbance was measured spectrometrically at 725 nm. A calibration curve of gallic acid was constructed and the linearity was obtained. The total phenol content was expressed as (mg GAE/g extract) using the standard curve.
In-vitro antioxidant activities
The total antioxidant activity, reducing power, and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities were evaluated follows; For total antioxidant activity, 3mL of the working solution (28 mM sodium phosphate, 4 mM ammonium molybdate, and 0.6 M sulfuric acid) was mixed with 1 ml of the extract. The mixture was incubated at 95 ^{o} C for 90 min and allowed to cool at room temperature. The absorbance of the solution was measured at 695 nm against a blank (1 ml ethanol). The antioxidant activity was expressed as gram equivalent of Gallic acid.
For the reducing power activity, the extracts at different concentrations were mixed with 1 mL sodium phosphate buffer (pH 6.6) and 1% potassium ferric cyanide (1 mL). The mixture was placed in a water bath (50 °C) for 20 min. Ten percent trichloro acetic acid (1 mL) was added to the mixture and centrifuged at 3000 rpm for 10 min. 2 ml of the supernatant and distilled water were mixed with 500 µL 1% ferric chloride and absorbance was read at 700 nm and the result was compared with ascorbic acid.
For the DPPH radical scavenging activity, 0.2 mmol/L solution of DPPH in ethanol was prepared and 500 µL of the solution was added to diverse concentrations of the extracts. The mixture was vigorously agitated and allowed for 30 min at room temperature. A control was prepared using the same procedure without the extracts and ethanol and used for the baseline correction. The changes in the absorbance of the diverse concentrations were measured spectrometrically at 517 nm. The DPPH scavenging effect was calculated as (% of inhibition) = (Control absorbance-sample absorbance/Control absorbance) x100.
Statistical analysis
GraphPad Prism version 9 (GraphPad Software) was used to analyze the data. Non-Linear Regression was used to estimate the EC50 and IC50. One-way ANOVA was used to compare the total antioxidant of the two extracts and ascorbic acid while student t-test was used to compare the phytochemical contents of the two extracts. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The results of proximate analysis were shown in Table 1. The percent of ash and carbohydrate were seen to be similar in the two extracts. The ash content of red and white onion was 4.00% and 4.80% respectively while that of carbohydrate was 78.80% and 78.15% respectively. However, the fat and protein contents were shown to be higher in white onion (2.44% & 1.05%) compared to the red onion (1.00% & 0.13%) while the moisture content was higher in red onion (16.10%) compared to the white onion (13.60%). Both the total flavonoid, phenol and tannin were shown to be significantly higher ($p<0.05$) in red onion relative to the white onion (Figure 1). A significant increase ($p<0.05$) in total antioxidant activity was observed in red onion compared to the white. However, ascorbic acid showed a significantly higher total antioxidant activity relative to both the red and white onion (Figure 1).

Table 1: Proximate composition of red and white *Allium Cepa L.* bulb

Constituents	Red *Allium Cepa L* (%)	White *Allium Cepa L* (%)
Ash	4.00	4.80
Carbohydrate	78.80	78.15
Fat	1.00	2.40
Fiber	0.00	0.00
Protein	0.13	1.05
Moisture	16.10	13.60

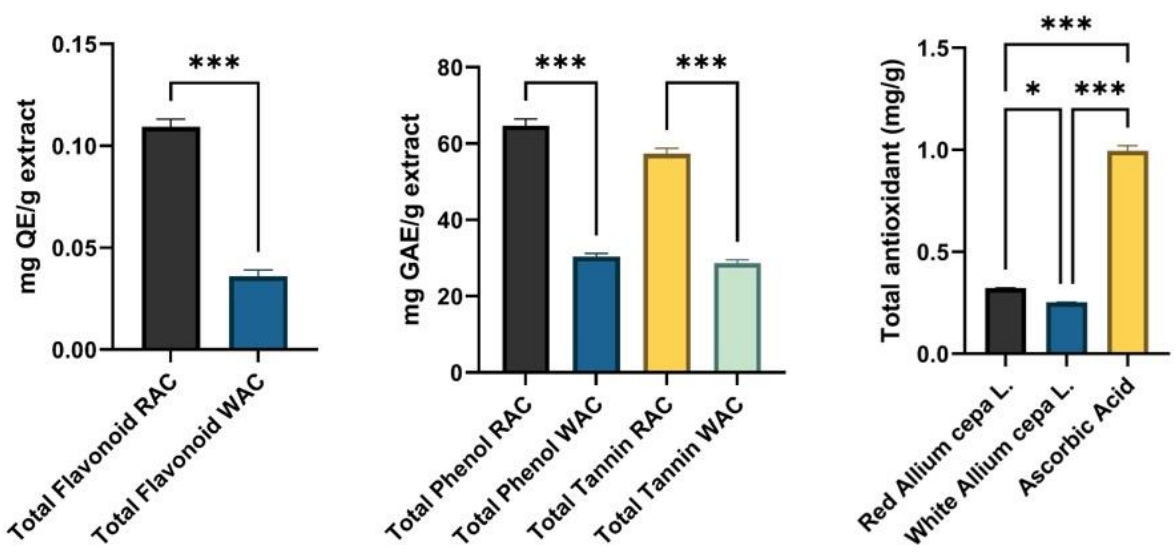

Figure 1: The total flavonoid, phenol, tannin and antioxidant of ethanol extracts of red and white *Allium cepa L.* showing significantly higher levels of all the compounds and antioxidant activity in the red onion compared to the white onion. Ascorbic acid displayed a significantly higher total antioxidant activity relative to the two extracts. *** indicates significant difference at p<0.05. Results are presented as mean±SEM, RAC= red *Allium cepa L.*, WAC= white *Allium cepa L.*

For the reducing power activity, the absorbance rate of the two extracts and ascorbic acid had a direct proportional increase with the concentration. White onion had a higher reducing power activity (EC50= 237670 µg/ml, logEC50= 5.38 µg/ml) compared to the red onion (EC50= 367701 µg/ml, logEC50= 5.57 µg/ml). The reducing power activity of ascorbic acid was higher (EC50= 202303 µg/ml, logEC50= 5.31 µg/ml) relative to the red onion but was comparable to that of the white onion (Table 2). For DPPH free radical scavenging activity, the percentage inhibition of red onion decreases with increasing concentration ranging from (55.9-10.5%) while that of the white onion increases with increasing concentration (22.9-38.4). The red onion has a better DPPH free radical scavenging activity (IC50= 78.03 µg/ml, logIC50= 1.89 µg/ml) relative to the white onion (IC50= 50.13 µg/ml, logIC50= 1.70 µg/ml), see Table 2.

Table 2: In-vitro reducing power activity of ethanol extracts of red and white *Allium cepa L.*

Dose µg/ml	Red *Allium cepa L.*	White *Allium cepa L.*	Ascorbic Acid
300	0.053 nm	0.088 nm	0.099 nm
400	0.097 nm	0.145 nm	0.115 nm
500	0.124 nm	0.211 nm	0.189 nm
600	0.182 nm	0.264 nm	0.366 nm
700	0.201 nm	0.312 nm	0.394 nm
EC50	367701 µg/ml	237670 µg/ml	202303 µg/ml
logEC50	5.57 µg/ml	5.38 µg/ml	5.31 µg/ml

Table 3: DPPH free radical scavenging activity of ethanol extracts of red and white *Allium cepa L.*

Dose µg/ml	Red *Allium cepa L.*	White *Allium cepa L.*
50	55.9 %	22.9 %
100	49.6 %	32.1 %
150	38.0 %	32.8 %
200	34.7 %	36.3 %
250	10.5 %	38.4 %
IC50	78.03 µg/ml	50.13 µg/ml
logIC50	1.89 µg/ml	1.70 µg/ml

Discussion

The result of the current study revealed that the carbohydrate content of ethanol extract of red and white onion bulb was as high as 78.8% and 78.155 respectively. This is similar to reports from a previous study on the carbohydrate contents of different varieties of onion ranging from 73.6% in Wuyan Bijimi to 75.78% in Ex-Kudan (Karu et al., 2017). Onion is the second most cultivated crop in the world (Shokri et al., 2022). The high carbohydrate content of onion may have contributed to the consistent use of onion in different part of the world as a source of food, medicine, and spice. This study discovered a moderate moisture content in red (16.1%) and white (13.6%) onion. High moisture enhances microbial action/growth and promote food spoilage while reduction in moisture content was reported to increase shelf life of vegetables (Alegbeleye et al., 2022). Therefore, post-harvest storage might have contributed to the moderate moisture content that was reported in the current study. We hypothesized that reducing the moisture content of onion is necessary before or during storage to prevent spoilage. Hence, the higher moisture content of the red onion is associated with is faster spoilage compared to the white. The low protein (red= 0.13%, white= 1.00%) and fat (red =1.05%, white =2.30%) contents that was reported in this study might be in connection with the low dietary requirement of protein and fat. Previous studies have shown that the body needs more carbohydrate compared to fats and protein and they play a major role in human nutrition (Hauner et al., 2012). The daily recommended dietary intake of carbohydrate is 130g/day for adult and children while that of protein is 0.8g/kg (Ryan-Harshman and Aldoori, 2006). The 2020-2025 dietary guidelines for Americans offered that carbohydrate, fat and protein contains 45-65%, 25-35% and 10-30% respectively in nutritious food (Kwon et al., 2020). We suggest that the carbohydrate, protein and fat contents of onions are related to the body's requirement. Hence, onion consumption is healthy as it will provide the body's nutritional need in the right amount.

The flavonoid, tannins and phenol content were shown to be significantly higher in red onion compared to the white in the current study. Flavonoids are vital secondary metabolites found in vegetables and are reported to have numerous biological activities including preventing tumor growth (Khan et al., 2021), anti-hyperglycemic activity (Shamsudin et al., 2022), anti-inflammatory and hepatoprotective effects (Maleke et al., 2019; Suhlan et al., 2021). Despite the numerous biological activities of flavonoids, their use is limited in the medicinal, food, and cosmetic industries due to low yield in plants, low solubility in water, instable nature and low bioavailability (Liga et al., 2023). Therefore, regular consumption of onions especially the red variety can increase flavonoid uptake by tissues and cells of the body. The high flavonoid content of the red onion is also associated with its biological activities. Flavonoids are reported to protect plants from UV radiation, secrete/produce phytoalexins and lignin that prevent pathogens spread and regulate genes that produce protective metabolites, as well as producing attractive colors/fragrances in flowers to attract pollinators (Roy et al., 2022).

Tannins are polyphenols found in fruits, vegetables, and cereals. They have been used as food additives with the aim of improving feed efficacy, meat quality and health in animals (Tong et al., 2022). Other properties of tannins include ability to interact with and denature protein in herbivores, antimutagenic, and ROS reduction ability (Moura de Melo et al., 2023). However, high tannin in diet was reported to decrease nutrient bioavailability leading to weight loss and eventual death (Smulikowska et al., 2001). Phenols are also abundant in nature, mostly found in plants and essential oils. They are believed to serve as antioxidants and anti-inflammatory agents (Floris et al., 2021). We hypothesized that the presence of phenols and tannins in both red and white onion is responsible for the antioxidant activities that was noticed in the reducing power and DPPH free radical scavenging results.

Reducing power is the ability of a compound to reduce free radical and prevent oxidative stress. Hence it is used as an indicator of antioxidant activity (Manye et al., 2023). Our result showed that the EC50 of white onion was lower compared to that of red onion. EC50 is the concentration of substance that gives half-maximum response and substances with lower EC50 are more potent (Jiang and Kopp-Schneider, 2014). This suggest that white onions have a higher chance of producing stable compounds by reacting with free radicals (electron donation) to prevent oxidative stress compared to the red onion. DPPH free radical scavenging activity of a compound is the capacity of that compound to prevent oxidative stress by neutralizing DPPH free radical through electron donation (Manye et al., 2023). The current study revealed that red onion has a higher degree of neutralizing DPPH free radical compared to the white. Despite the fact that white onion had a higher reducing power activity relative to the red, the red had better DPPH free radical scavenging effect. Therefore, the higher quantity of flavonoids, tannins and phenols in red onion makes it a better antioxidant candidate compared to the white as evident in the significantly higher total antioxidant capacity relative to the white that was observed in our result.

CONCLUSION

In conclusion, our findings revealed that both red and white onion contains varying quantity of phenols, flavonoids and tannins with strong reducing power and DPPH free radical scavenging activity. However, the red onion was shown to have higher phenolic compounds compared to the white making it a better antioxidant candidate.

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