Journal of Global Biosciences
ISSN 2320-1355
Volume 6, Number 1, 2017, pp. 4680-4688
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Research Paper

IN VITRO ANTIOXIDANT AND PHYTOCHEMICAL PROPERTIES OF ETHANOLIC EXTRACT OF WHITE AND PURPLE ONIONS (*Allium cepa)*

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Abstract

The antioxidant activity of the two species of onion was evaluated using DPPH (2,2-Diphenyl-1-picryl hydrazyl) radical scavenging activity, reducing power, nitric oxide radical inhibition activity, hydroxyl radical scavenging activity and Folin-Ciocalteau method for the determination of total phenolic content. The phytochemical screening of white and purple onion showed the presence of alkaloids, saponins, flavonoids, terpenoids, volatile oils and carbohydrate while tannins, pesin, phlobatanins, anthraquinones and cardenolides were absent in the two ethanolic extracts of onion. Balsm, cardiac glycosides and phenols are present only in the ethanolic extract of purple onion whereas, sterol and glycosides were found to be present in the ethanolic extract of white onion. Total phenolic content was 0.855±0.17mg/ml and 1.022±0.36 for white and purple onion extracts respectively. This present study revealed that ethonolic extract of purple onion (*Allium cepa*) showed relatively high hydroxyl scavenging activity, reducing power and DPPH radical scavenging assay when compared to the ethanolic extract of white onion (*Allium cepa*). Whereas nitric oxide inhibition activity is relatively high in the ethanolic extract of white onion (*Allium cepa*) when compared to the ethanolic extract of purple onion (*Allium cepa*). This present study revealed that free radical scavenging activity of DPPH ranges from (1.9% – 99.5%) in purple onion extract and (34.5% - 84.1%) in white onion extract. This therefore, justifies the therapeutic activity of onions against array of diseases and their antioxidant properties that can prevent number of diseases. With these health benefits, there is need to emphasis a diet rich in indigenous vegetables to promote health and prevent diseases.

INTRODUCTION

Since ancient times onions (*Allium cepa, L.*) have been an important dietary resource and have also been of interest for medical purposes (Rose *et al*., 2005). Traditionally, onions and plants belonging to the *Allium* genus have been used as an herbal remedy for a wide range of ailments due to their pharmacologic effects (Yin and Cheng, 1998; Rose

et al., 2005). Onions is known for being a good natural source of flavonoids mainly represented by the flavonolsquercetin and kaempferol which are present as their glycosides (Fossen *et al*., 1998). Several epidemiological studies have associated the consumption of flavonoids with a reduction of the risk of chronic diseases including cancer, diabetes and coronary heart problems (Hirvonen *et al*., 2001; Kosmider and Osiecka, 2004). In addition, many flavonoids have been reported to possess antibacterial and antifungal properties (Rauha *et al*., 2000). Onions can be considered as a good source of natural additives to retard food deterioration (Navas *et al*., 2006). However, the application of thiosulfinates and volatile compounds for food preservation is limited because of their biochemical instability (Benkeblia, 2004). These properties focus attention on the more stable flavonoids as additives to enhance food shelf-life by inhibiting microbial spoiling and oxidative deterioration, due to their antimicrobial and antioxidant properties (Ramos *et al*., 2006; Naz *et al*., 2008). Several fruits and vegetables like onions have been demonstrated to contain antioxidants and colorants to prevent lipid peroxidation in food and help the human body to reduce oxidative damages (Wang *et al*., 2000).

Aims and objectives

To determine the free radical scavenging activities, phytochemicals present and total phenolic contents of white and purple onion extracts.

MATERIALS AND METHODS

Solvents and reagent used in the study were of analar grade and unless otherwise stated, were sourced from Zayo-Sigma, Abuja, Nigeria.

Collection and Preparation of ethanolic extract of Plant Material

The white and purple onions (*Allium cepa*) were bought in the month of April 2015 at Keffi Market, Nasarawa state, Nigeria and identified by the Ethnobotanist in the Department of Medicinal Plant Research and Traditional Medicine of the National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. A voucher specimen number NIPRD/H/8313 was deposited at the herbarium of the department. The onions were washed and cut into medium pieces. About 1000g of the chopped onions were blended with a blending machine set at high speed for some minutes. This was done repeatedly, blending for couple of times given short breaks between the processes. After blending, each blended onion samples were mixed with about 2000ml of ethanol in a bowl followed by vigorous shaken for 5 minutes and tightly covered with foil paper and stored for 72 hours. After 72 hours, it was filtered using whatman filter paper and funnel. The filtrates were evaporated to dryness by using a water bath at the temperature of 40˚C for few days and the ethanolic extracts gotten were stored in an air tight container for future use.

Qualitative Phytochemical Screening

The ethanolic extracts of *Allium cepa* were screened for some phytochemical constituents using standard procedures (Sofowora, 2002, Aiyelaagbe and Paul, 2009, Trease and Evans, 2002, Chanda and Dave, 2009).

Determination of total phenolic content

Methanolic extract (100 µL) was mixed with 1 ml of 10% Folin-Ciocalteau reagent and it was incubated at room temperature for 4 minutes. Then 2 ml of 5% Sodium carbonate was added to above mixture and vortexed. The resultant mixture was incubated in dark for 45 minutes at room temperature. Following this, the absorbance of the sample was measured at 765 nm using gallic acid (100 - 1000 µg/ml) as a standard. Results were

expressed as mg of gallic acid equivalents per gm of fresh weight of sample (Singleton *et al*., 1999)

Determination of Free Radical Scavenging Assay

Methanolic extract (100 µL) of sample was mixed with 900 µL of Tris HCl buffer (50 mM, pH 7.4) and 2 ml of DPPH (0.1 mM in methanol). The solution was incu- bated at room temperature for 30 minutes and the ab- sorbance was read at 517 nm. The percentage of DPPH scavenging activity was determined as follows, DPPH Radical Scavenging Activity (%) = [(A0−A1)/A0] where A0 is the absorbance of control and A1 is the absorbance of sample (Gyamfi *et al.,* 1999)

Measurement of Reducing Power

The reducing power of *Allium cepa* was determined using the method of (Yen and Duh, 1994). A serial dilution of the extract was performed (200, 100, 50, 25 and 12.5µg/mL) in 0.2 M phosphate buffer pH, 6.6 containing 1% ferrocyanate. The mixture was incubated at 50 ºC for 20 minutes. 10% trichloroacetic acid (TCA, 2.5 mL) was added to a portion of this mixture (5 mL) and centrifuged at 3,000 g for 10 minutes. The supernatant was separated and mixed with distilled water (2.5 mL) containing 1% ferric chloride (0.5 mL). The absorbance of this mixture was measured at 700 nm. The intensity in absorbance could be the measurement of antioxidant activity of the extract.

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging was measured by studying the competition between deoxyribose and the test compounds for hydroxyl radicals generated from the Fe^{3+}/ ascorbate/ EDTA/ H_2O_2 system. The reaction medium contains deoxyribose (2.8mM), $FeCl_3$ (0.1mM), EDTA (0.1Mm), H_2O_2 (1mM), ascorbate (0.1mM), KH_2PO_4-KOH buffer (20Mm, p^H 7.4) and the Onion extracts from (10 to 3000µg/ml) in a final volume of 1ml. After incubation for 1 hour at 37˚C, the deoxyribose degradation was measured as TBARS at 532nm (Halliwell *et al*., 1981).

Nitric Oxide Radical Inhibition Activity

The reaction mixture of 3ml contains sodium nitropruside (10mM) in phosphate buffered saline (PBS) and the Onion extracts from (10 to 1000µg/ml) were incubated at 25˚C for 150 minutes. After incubation, 0.5ml of the reaction mixture was removed and 0.5ml of Griess reagent (1% sulphanilamide, 2% H_3PO_5 and 0.1% naphylethylenediaminedihydrochloride) was added and the absorbance of the chromophore formed was evaluated at 546nm (Garret, 1964).

Statistical Analysis

All the assays were carried out in triplicates and results are expressed as mean ± SD. The data were subjected to one-way analysis of variance (ANOVA) and the differences between various concentrations were determined by DMRT test using SPSS software.

RESULTS

Table 1: phytochemical constituents of ethanolic extracts of white and purple onions.

PHYTOCHEMICALS	WHITE ONION EXTRACT	PURPLE ONION EXTRACT
Alkaloid	+	+
Saponin	+	+
Flavonoid	+	+
Terpenoid	+	+
Tannin	-	-
Phenol	-	+
Sterol	+	-
Glycoside	+	-
Cardiac glycoside	-	+
Pesin	-	-
Balsm	-	+
Volatile oil	+	+
Phlobatanin	-	-
Carbohydrate	+	+
Antraquinone	-	-
Cardenolides	-	-

Keys: Present = (+), Absent = (-)

In Vitro antioxidant assay

Table 2: Total phenolic content of ethanolic extracts of white and purple onions (mg of GA/g of extract)

WHITE ONION EXTRACT	PURPLE ONION EXTRACT
0.855 ± 0.17	1.022 ± 0.36

Values were expressed as mean ± standard deviation (n = 3) at absorbance of 765nm.

Table 3: Reducing power assay of ethanolic extracts of white and purple onions

CONCENTRATION (mg/ml)	PURPLE ONION EXTRACT	WHITE ONION EXTRACT
0.1	0.148 ± 0.14	0.146 ± 0.22
0.2	0.158 ± 0.19	0.162 ± 0.26
0.3	0.173 ± 0.38	0.186 ± 0.29
0.4	0.194 ± 0.34	0.191 ± 0.32
0.5	0.286 ± 0.42	0.209 ± 0.46

Values were expressed as mean ± standard deviation (n = 3) at absorbance of 700nm.

Table 4: Nitric oxide radical inhibition activity of *Allium cepa* extracts

CONCENTRATION (mg/ml)	WHITE ONION EXTRACT	PURPLE ONION EXTRACT
0.01	0.255 ± 0.44	0.442 ± 0.23
0.02	0.258 ± 0.36	0.498 ± 0.32
0.05	0.761 ± 0.48	0.954 ± 0.40
1.0	1.555 ± 0.24	1.024 ± 0.26
5.0	1.695 ± 0.38	1.048 ± 0.34

Values were expressed as mean ± standard deviation (n = 3) at absorbance of 546nm.

Table 5: Free radical scavenging activity of DPPH

CONCENTRATION (mg/ml)	% INHIBITION OF PURPLE ONION	% INHIBITION OF WHITE ONION
0.1	99.5 ± 0.02%	34.5 ± 0.01 %
0.2	96.2 ± 0.08%	60.9 ± 0.05 %
0.3	39.7 ± 0.01 %	75.2 ± 0.04 %
0.4	39.1 ± 0.06%	84.1 ± 0.04 %
0.5	1.9 ± 0.04%	52.4 ± 0.02 %

Values were expressed as mean ± standard deviation (n = 3) at absorbance of 532nm.

Table 6: Hydroxyl radical scavenging activity of ethanolic extracts of *Allium cepa*

CONCENTRATION (mg/ml)	WHITE ONION EXTRACT	PURPLE ONION EXTRACT
1.0	0.659 ± 0.36	0.851 ± 0.20
2.0	0.806 ± 0.24	1.791 ± 0.16
3.0	0.873 ± 0.18	1.838 ± 0.18

Values were expressed as mean ± standard deviation (n = 3) at absorbance of 532nm

DISCUSSION

The present study carried out on the plant samples revealed the presence of some phytochemicals. Table 4.1 shows the presence of alkaloid, saponin, flavonoid, terpenoid, volatile oil and carbohydrate in both extracts while tannin, pesin, phlobatanin, antraquinnone and cardenolides were absent in both extracts. Balsm, Cardiac glycoside and Phenol are present only in the purple ethanolic extract of onion whereas sterol and glycosides were present in the white ethanolic extract of onion. Flavonoids are water soluble antioxidants that prevent oxidative damage and have strong anti-ulcer activity (Del-Rio *et al.,* 1997). A reduced risk of cardiovascular disease has been reported in persons with high flavonoids intake (Huxley and Neil, 2013). Plants glycosides are used as medication for the elimination of poisons from the body (Brito Marco, 2007). Having tested that glycoside is present in the white onion extract, it can be used in the

elimination of poisonous substances from the body (Huxley and Neil, 2003). Alkaloids are most efficient therapeutically significant plant substance. Both natural and synthetic alkaloids are used as basic medicinal agent because of their analgesic, antispasmodic and bacterial properties (Njoku and Akumefula, 2007). The saponin constituent is responsible for the possession of haemolytic property. This can give the plant the traditional medicinal use as cholesterol binding agent. The biological functions of flavonoids include protection against allergies, inflammation, platelets aggregation microbes, ulcer, viruses and tumors (Okwu and Okwu, 2004). Table 2 shows the phenolic content of the ethanolic extracts of white and purple onion. The result shows that purple onion extract has more phenolic content compared to the white onion extract. Both were lower than gallic acid which is the standard. Numerous investigations of the antioxidant activity of plant extracts have confirmed a high linear correlation between the values of phenol concentration and antioxidant activity (Borneo *et al.,* 2008; Katalinić *et al.,* 2004). Phenolic compounds are the major group contributing to the antioxidant activity of vegetables, fruit, cereals and other plant-based materials. The antioxidant activity of the compounds is partly due to one electron reduction potential that is the ability to act as hydrogen or electron donors (Chan *et al.,* 2007). Atoui *et al.* (2005) mention that the antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. From the results in Table 3, reducing power of the ethanolic extracts of both the white and purple onions increases as the concentration of the extracts increases at absorbance of 700nm. Based on these results, purple onions extract has more reducing power than white onion extract. Reducing properties are generally associated with the presence of reductones (Duh *et al.,* 1999). Which have been shown to exert antioxidant action by breaking free radical chain by donating a hydrogen atom (Gardon, 1990). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. From Table 4, Nitric oxide radical inhibition increases in both extracts as the concentration of increases from 0.01 to 5.0 mg/ml at absorbance of 546nm. From these results, white onion has more inhibiting power than purple onion. Nitric oxide is an unstable free radical involved in many biological processes which is associated with several diseases. It reacts with oxygen to produce stable product nitrate and nitrite through intermediates and high concentration of nitric oxide can be toxic and inhibition of over production is an important goal (Wang *et al.,* 2005). From the results in Table 5, the scavenging activity of purple and white onion extracts on DPPH radicals increased as the concentration increased in the range of 0.1- 0.5mg/ml and it was remarkable, especially in the case of purple onion extract than the white onion extract. DPPH assay is a primary antioxidant activity test that determines the free radical scavenging activity of the onion extracts. Prakash *et al*. (1999) reported that the DPPH antioxidant activity for onion varied from 13.6% to 84.1%. Other studies showed that the radical scavenging activities in onion were 20% - 90% (Nuutila *et al*., 2003). The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow (Braca *et al.,* 2001; Shazia 2013). In Table 6, hydroxyl radical scavenging activity was observed to be increasing as concentration of extracts increases. But the hydroxyl radical scavenging activity was found to be higher in purple ethanolic extract of onion than the white onion extract. Hydroxyl radical is the most reactive of the Reactive Oxygen Species (ROS) which induces severe damage in adjacent biomolecules

(Gutteridge, 1984). The hydroxyl radical can cause oxidative damage to DNA, lipids and proteins (Spencer *et al.,* 1994). Results of the present study revealed that ethonolic extract of purple onion (*Allium cepa*) showed relatively high hydroxyl scavenging activity, reducing power and DPPH radical scavenging assay when compared to the ethanolic extract of white onion (*Allium cepa*). Whereas nitric oxide inhibition activity is relatively high in the ethanolic extract of white onion (*Allium cepa*) when compared to the ethanolic extract of purple onion (*Allium cepa*). Therefore white and purple ethanolic extracts of onion are relevant in neutralizing free radicals and in preventing the formation of peroxides.

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