PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF ONION (Allium cepa L.) EXTRACTS

Mohamed A. El-Saied*, S.S. El-Saadany, H.T. Hefnawy and A.I. El-Sayed
Biochem. Dept., Fac. Agric., Zagazig Univ., Egypt

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ABSTRACT: Phytochemical screening and antioxidant activities in different solvent extracts of onion was carried out. The extracts were subjected to various chemical Test for phytochemical constituents. Total phenolic contents were evaluated using Folin Ciocalteu method and their antioxidant activity was assayed through «in vitro» radical scavenging activity using DPPH- assay, FRAP and ABTS. The phytochemical screening of this study indicated the presence of steroids, flavonoids, alkaloids, saponins, and catechic tannins. The average total polyphenol content of hydroethanolic extracts was significantly (P<0.05) higher than in the hexane, ethyl acetate and dichloromethane extracts. The order of effectiveness (IC₅₀) of the plant extracts the potent inhibitors was hydroethanolic extract, followed by Dichloromethane, ethyl acetate while the least was the hexane extract. When using (DPPH, ABTS and FRAP). This shows that onion organic solvent extracts especially the hydroethanolic extracts may be a potent source of natural antioxidant and can be used in the management of diseases associated with oxidative stress is justified

Key words: Flavonoid, tannins, saponin, phytochemical, onions, antioxidant, extraction solvent, flavonoids

INTRODUCTION

Plants have been used to treat chronic and infectious diseases since antiquity. The biological activities of plants have been attributed to the presence of various secondary metabolites such as alkaloids, glycosides, phenols, flavonoids, coumarins and volatile oils etc., Sharma et al. (2014). Phenolic compounds are known for their antioxidant properties and play a vital role in the prevention and management of many chronic diseases such as cancer, diabetes, cardiovascular and neurodegenerative diseases Slimestad et al. (2007). Currently natural antioxidants are gaining popularity due the belief that they are safer and provide more health benefits than the synthetic antioxidants which have numerous health hazards Prakash et al. (2007). Thus plants containing phenolic compounds are potential reservoir for the discovery of effective and safe antioxidants.

Onion (Allium cepa L.) is the most widely cultivated species of the genus Allium Makris and Rossiter (2001). The plant portion commonly used is the bulb, which is utilized as a food ingredient to give flavour and aroma to a great variety of dishes. Onions are an important source of several phytonutrients such as flavonoids, fructooligosaccharides (FOS), and thiols and other sulfur compounds, recognized as important elements of the Mediterranean diet Boots et al. (2008). In fact, onions contain high levels of phenolic compounds, which have antioxidant properties besides beneficial effects against different degenerative pathologies cardiovascular and neurological diseases, dysfunctions based on oxidative stress) Santas, et al. (2010). Onion, is widely planted in Egypt and, is the seasoning vegetables eaten by many east and west people, which are rich in flavonoids Prakash et al. (2007).

* Corresponding author: Tel.: +20112257683
E-mail address: dr.m.adel00@gmail.com
**Allium cepa** commonly known as onions, has been valued for its medicinal qualities by many cultures around the globe. Numerous health benefits have been attributed to the vegetable, including prevention of cancer and cardiovascular disorders. Sequel to this, studies on specific compounds found in onion bulbs have been carried out (Block, 1985). Onions have a unique combination of three families of compounds that are believed to have effects on human health. They include fructans, flavonoids and organosulphur compounds. Fructans a polymer of fructose, may help maintain gastrointestinal health by sustaining beneficial bacteria (Eja et al., 2007) have focused on a flavonoid—quercetin, which is found at particularly high levels in onions. It functions as an antioxidant, deactivating molecules that are injurious to cells in the body (Block, 1985).

Onion (**Allium cepa** L.) is one of the oldest and most frequently cultivated food plants highly valued for its pharmacological properties, such as antioxidant, antimicrobial and antitumor ones, reduction of cancer risk and protection against cardiovascular diseases (Ly et al., 2005). Though it is not specifically considered as a medicinal herb, the onion has shown health promoting effects based on its secondary metabolites, such as flavonoids to which the strong antioxidant properties of onion have been attributed (Lachman et al., 2003).

The present study, therefore, aimed to analyse the biochemical constituents of onion including antioxidant vitamins (A, C and E), phytochemicals (alkaloids, flavonoids, saponins, tannins, and glycosides), minerals (calcium, iron, manganese, copper and magnesium) and proximate compositions of Allium cepa (Nuutila et al., 2003).

**MATERIAL AND METHODS**

**Plant Material**

**Chemicals and Reagents**

The reagents used for the study included concentrated H$_2$SO$_4$, petroleum ether, NaOH, Boric acid solution, Anhydrous Na$_2$SO$_4$, CuSO$_4$, distilled water, HCl, Wagner’s reagent, Meyers reagent, Ferric chloride solution, Fehling’s solution, Ethanol, Benzene, Ammonia solution, Methanol, Chloroform, Ethylacetate, Eja et al. (2007) Tannic acid, Folin-Denis reagent, Na$_2$CO$_3$, Lead acetate, Baljet reagent. All other chemicals and reagents used were of analytical grade and purchased from standard manufacturers.

Samples Collection the **Allium cepa** (Onions) was purchased from a local market at Zagazig, Egypt. Crude extracts were prepared by Soxhlet extraction with solvents of increasing polarity: hexane, dichloromethane, ethyl acetate and water-ethanol (1:4 V/V) for 8 hours.

**Chemical composition**

The samples were analyzed for chemical composition (moisture, proteins, fat, carbohydrates and ash) using the Association of official Agricultural Chemists (AOAC) procedures. The leaves of the plant were dried for the estimation of ash, proteins, fiber, fat and total carbohydrates.

**Determination of total carbohydrate**

The percentage of total carbohydrates was calculated by the difference method according to this equation: 

\[ \text{Total Carbohydrates} = \text{Total Dry Weight} - \left( \text{Total Ash} + \text{Total Protein} + \text{Total Fat} + \text{Total Fiber} \right) \]

**Determination of Minerals**

The method applied for the assessments of mineral concentration in samples after digestion was by using the Atomic Absorption Spectrophotometric (AAS) technique (Analyst 200, Perkin Elmer, Waltham, MA, USA) as described in [AOAC], 2019. All tests were repeated 3 times, the mean and standard error of the mean was calculated (n = 3).

**Determination of Antioxidant Vitamins**

The assessment of antioxidant vitamin(s) was conducted using standard methods described by Rutkowski et al. (1998)

**Pungency Analysis**

Pungency of onions was determined as enzymatically (alliinase) produced pyruvate (EPY) by colourimetric analysis according to Schrimmer and Weston (1961) with slight modifications. Onion bulbs were sliced in half longitudinally: 50 g were homogenized by
Ultra-Turrax blender (T25, IKA Werke, Staufen, Germany) with 50 mL of distilled water for the determination of total pyruvate alliinase produced. Another 50 g of onions were pretreated with 50 mL of 5% trichloroacetic acid solution to inactivate the alliinase in order to quantify pyruvate basal level. Both mixtures were left at room temperature for 15 min and filtered with Whatman filter paper (grade 1) and 10 mL of the filtrate was diluted ten times with bidistilled water. One milliliter of sample was placed in a reaction tube with 1 mL of 2,4-dinitrophenyl hydrazine (DNPH) solution (0.0125% DNPH in 2 M HCl) and 1 mL of bidistilled water. Reaction tube was vortexed and insulated in a water bath at 37°C for 10 minutes. After the incubation time, 5 mL of 0.6 M NaOH was added to the tube and allowed to stand for 5 min. The DNP hydrazine derivative of pyruvate was measured using PerkinElmer Lambda 25 UV-Vis spectrometer at 420 nm. Enzymatically (alliinase) produced pyruvate (EPY) in each sample was calculated from the difference of total and basal concentration of pyruvate. A blank sample was prepared with 2 mL of water and 1 mL of DNPH; standards were prepared replacing onion sample with 1 mL of sodium pyruvate solution, ranging from 20 to 100 μm.

**Phytochemical screening**

The preliminary phytochemical screening tests were carried out to identify the useful constituents by standard methods (Onwukeame et al., 2007).

**Determination of Total Phenolic Content**

The amount of total phenolic contents was determined according to Folin-Ciocalteu method as described by Lister and Wilson (2001). Briefly, 0.5 ml of sample solution was mixed with 2.5 ml of Folin-Ciocalteu reagent diluted with distilled water at 1:10, followed by the addition of 4 mL of Na₂CO₃ (7.5%, W/V). The mixture was then incubated in a water bath at 45°C for 30 min and the absorbance was measured at 765 nm using a UV-Vis spectrophotometer against blank sample. The standard curve of Gallic acid is obtained under the same conditions as above using a range of concentrations (0-200 mg/l). The total phenolic content was expressed as Gallic acid equivalents (mg GAE/g extract).

**Determination of Flavonoids Content**

Flavonoid contents were measured using a modified colorimetric method. 0.25 mL of extract solution was added to a test tube containing 1.25 mL of distilled water. Sodium nitrite solution (5%, 0.075 mL) was added to the mixture and maintained for 5 min. Then, 0.15 mL of 10% aluminum chloride was added. After 6 min, 0.5 mL of 1 M sodium hydroxide was finally added. The mixture was diluted with 0.275 mL of distilled water. The absorbance of the mixture at 510 nm was measured immediately in comparison to a standard curve prepared by quercetin. The flavonoid contents were expressed as mg quercetin equivalent (QE)/g of extract Yildirim et al. (2001).

**Antioxidant Activity (AA)**

**Free radical scavenging activity (DPPH)**

The free radical scavenging activity of the plant extracts was measured by 1.1-diphenyl-2-picryl-hydrazil (DPPH), according to with some modifications. Briefly, 0.2 mM solution of DPPH in ethanol was prepared and 0.5 ml of this solution was added to 2.5 ml of plant extract, allowed to stand at room temperature for 30 min, and then absorbance was read at 517 nm against blank samples. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. IC50 value was determined from the plotted graph of scavenging activity against the different concentrations of Thymus extracts, which is defined as the total antioxidant capacity of the extracts. Ascorbic acid was used as reference compound.

**Determination of Reducing/Antioxidant Power (FRAP)**

The ferric ions (Fe³⁺) reducing antioxidant power (FRAP) method was used to measure the reducing capacity of the plant extracts with a slight modification. Various concentrations of plant extracts from the stock solutions and the standard (ascorbic acid) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1% W/V). The mixture was incubated at 50°C for 20 min. Then 2.5 ml of trichloroacetic acid (10% W/V) was added to the reaction mixture. Afterwards, it was
centrifuged at 1000 g for 10 min. The upper layer of the solution (2.5 ml) was mixed with deionised water (2.5 ml) and ferric chloride (0.5 ml 0.1% w/v). The absorbance was measured at 700 nm at the reaction time of 30 min. The reducing power of the extracts was represented as ascorbic acid equivalent (mg AAE/ g of extract). Studies on products of browning reaction prepared from glucoseamine.

**ABTS radical scavenging assay**

The scavenging activity of extracts against ABTS radical was determined by following the method described by Miller *et al.* (1993). Briefly the stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate (K$_2$S$_2$O$_8$) in equal volumes were allowed to stand in the dark for 12-16 h at room temperature. Prior to assay, ABTS solution was diluted in ethanol to give an absorbance of 0.700 ± 0.02 at 734 nm. 2 ml of the resulting solutions was allowed to react with 200μl of the plant extracts with different concentrations, reaction mixture was vortexed and absorbance was measured at 734 nm after 30 min. The same was done for the ascorbic acid standard (oxo-3-gulofuranolactone acid) of various concentrations (1 – 100 μg/ml). The amount of sample necessary to decrease the absorbance of DPPH by 50% (IC50) was calculated graphically.

**RESULTS AND DISCUSSION**

The result of the proximate composition of *Allium cepa* showed significant difference (P < 0.05) (Table 1). The moisture contents of the spices was 86.67% in *Allium cepa*. The values showed that the onion relatively wet (moisture contents higher than 12%) and would not be stored for a long period of time without microbial and biochemical spoilage. The moisture content of food can be used as an index of its keeping quality. Water is an important medium for most biochemical reactions, food samples with water content of >12% are more prone to high biochemical activities and usually have short shelf life.

Nutritionally, ash aids in the metabolism of protein, carbohydrate and fat. Carbohydrate content ranged from 9.89% to 20.46% in the spices. Carbohydrate provides energy to cells in the body, particularly the brain, and the only carbohydrate-dependent organ in the body. These spices could be supplementary for carbohydrate need in the diet. These spices are consumed in very small amount as food ingredients, and their contribution to nutrition in menu may not be as high as is the case with staple food items. However, among many rural consumers who use these spices copiously in various local dishes, the spices can make a considerable nutritional contribution in menu.

Onion had low contents of most of the minerals investigated in this study (Table 2). The most abundant mineral in onion was calcium which ranged from 122.40 mg/100 g in onion to 83.83 mg/100 g. The onion was significantly (P < 0.05) higher source of calcium which also was comparatively a higher source than onion. There was no significant difference (P>0.05) in minerals between the two spices except in calcium. Calcium is needed for regulating most internal organs, including the heart and liver. It is needed for most physiological functional integrity, involving normal functioning of heart muscles, the skeletal system and cell membrane, blood clotting, nerve signal transmission and regulation of enzymes and hormones. Deficiency of Ca in the body leads to malfunctioning of organ systems. Next in the hierarchy of mineral contents in the spices was magnesium. (3.14 mg /100 g). Magnesium is needed for normal functioning of the body. It activates the enzymes necessary for carbohydrate metabolism.

**Yields of Extract**

The extraction yield of various solvent. The values of extraction yield varied from 0.66% for the hexane extract to 5.35% for the hydroethanolic extract. The extraction yield increased in the following order: water/ethanol >
Table 1. Proximate analysis of Allium cepa

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Allium cepa (%)</th>
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<tbody>
<tr>
<td>Moisture</td>
<td>86.67 ± 0.33</td>
</tr>
<tr>
<td>Ash</td>
<td>0.67 ± 0.33</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>0.58 ± 0.01</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>0.73 ± 0.01</td>
</tr>
<tr>
<td>Protein</td>
<td>1.46 ± 0.01</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>8.60 ± 0.02</td>
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Table 2. Determination of some mineral contents in Allium cepa

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Allium cepa (mg/100 g)</th>
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</thead>
<tbody>
<tr>
<td>Manganese</td>
<td>0.012 ± 0.002</td>
</tr>
<tr>
<td>Calcium</td>
<td>122.40 ± 0.459</td>
</tr>
<tr>
<td>Copper</td>
<td>0.001 ± 0.0002</td>
</tr>
<tr>
<td>Iron</td>
<td>0.026 ± 0.003</td>
</tr>
<tr>
<td>Magnesium</td>
<td>3.15 ± 0.006</td>
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ethyl acetate > Dichloromethane > Hexane. Few researches have been reported about the extraction yield of onion extracts. Variation in the various extracts yield may be due to the polarities of different compounds present in the plants, and such differences have been reported in the literature concerning fruit seeds, three Mentha species, Red Clover plant Moroccan macro algae species and Moroccan Flowers and seeds Burdock (1998). The highest yield in the sequential extractions was achieved with polar solvents.

**Phytochemical Screening**

From the phytochemical analysis, it has should in all extracts remarkable presence of steroids, flavonoids and alkaloids. Others metabolites and bioactive compounds were identified such as saponosides, catechin tannins. They are present in water/ethanol extracts, while they are absent in the other extracts, Coumarin, protein, and hydrolysable tannin are absent in all the extracts (Table 3).

The presence of flavonoids in all extract is likely to be responsible for the free radical scavenging activity observed. Flavonoids are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers.

All onion extracts were also revealed to contain steroids, which are known to produce an inhibitory effect on inflammation and alkaloids that have been reported to exert analgesic, antispasmodic and antibacterial activities. The phytochemical screening results of the extracts are consistent with the results reported by Eloff (1998), for onion from Egypt.

**Total Phenolic and Flavonoid Content**

Phenolic is a kind of polyphenols that can be divided into tannin, propanoid and flavonoid. Phenolic compounds are known as powerful chain breaking antioxidants, which may contribute directly to the antioxidative action. These compounds are very important constituents of plants and their radical scavenging ability is due to their hydroxyl groups.
Table 3. The extraction yield of four solvent extracts

<table>
<thead>
<tr>
<th>Extract</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>0.66</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>1.79</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>2.48</td>
</tr>
<tr>
<td>Water/ethanol</td>
<td>5.35</td>
</tr>
</tbody>
</table>

The content of phenolic compounds in various extracts was determined from regression equation of calibration curve of Gallic acid and expressed as milligrams equivalent of Gallic acid per gram of dry extract (mg GAE/g). Flavonoids content was expressed as milligrams equivalent of Quercetin per gram of dry extract (mg QE/g). Total phenolic and flavonoid contents are shown in Table 3. From these results, Hydro-alcoholic extract showed high phenolic and flavonoid compounds (403.01 ± 3.63 mg GAE/g and 267.8 ± 2.6 mg QE/g respectively) followed by Dichloromethane extract (243.06 ± 0.80 mg GAE/g of phenolics and 159.6 ± 2.6 mg QE/g of flavonoids). Ethyl acetate extract showed lower values of phenolic content (129.08 ± 2.02 and 114.4 ± 1.2 respectively). Lowest phenolic and flavonoid content was seen in Hexane extracts (97.90±6.45 and 92.13±1.006, respectively).

These results clearly show that the solvent influences the extractability of the phenolic compounds. The phenolic extracts of plants are always a mixture of different classes of phenols, which are selectively soluble in the solvents. The use of an alcoholic solution provides satisfactory results for the extraction process. The use of mixture alcohol and water present the advantage of modulating the polarity of alcohol solvents, also adding that solubility of polyphenols depends mainly on the hydroxyl groups, the molecular size and the length of hydrocarbon. Hydro alcoholic solvents are the best solvents for extraction of phenolic compounds from onion plant. Ethyl acetate and Hexane are inefficient solvents for extraction of total phenols from plant part studied.

Our results are almost similar to those reported by Yingming et al., (2004) for onion from Egypt, the lower polarity solvents, particularly hexane and diethyl ether showed much lower ability in extracting the phenolic compounds as compared to the polar solvents.

**Antioxidant activity**

The activity of the antioxidants is dependent on the compounds capable of protecting the organism system against the potential harmful effect of oxidative stress. In this study, the antioxidant capacity of extracts from onion was assessed by three different assays: Ferric Reducing Antioxidant Power (FRAP), DPPH scavenging activity and ABTS assay. IC50 of ABTS and DPPH scavenging activities of each extracts were compared to IC50 of Ascorbic Acid.

**DPPH scavenging activity**

The DPPH method was evidently introduced nearly 50 years ago it is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate the antioxidant capacity. The parameter IC50, is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity. Some plant extracts and essential oils were examined in relation to their IC50 value, while others were tested for their antioxidant capacity.

Table 4 showed the DPPH radical scavenging activity of onion extracts. DPPH is a stable free radical which is reduced in the presence of hydrogen donating antioxidants. The scavenging ability of different solvent extracts of onion for free radicals of 1,1-diphenyl 1-2-picyrhydrazyl (DPPH) showed remarkable scavenging activities. Hydro-ethanolic extract showed the highest scavenging activity (lowest IC50; 3.86 ± 0.07 µg/ml) followed by Dichloromethane extracts (IC50; 8.18 ± 0.07 µg/ml). The lowest activity...
was found in hexane extract (275.71 ± 11.26 µg/ml). Phenolic compounds are hydrogen donating antioxidants, thus higher radical scavenging activity of hydro-alcoholic extract may be attributed to higher amount of hydrogen donating phenolic antioxidants in ethanol extract. Ramchoun et al. (2012) found for the same plant from Tafilalet Region an IC50 of 0.48 mg/mL. A similar result was found by Ramchoun et al. (2012) for Thymus vulgaris, who conclude that the antioxidant activities of plant extracts expressed as antiradical power (ARP) are affected by solvents used for extraction.

**ABTS assay**

ABTS assay is better to assess the antiradical capacity of both hydrophilic and lipophilic antioxidant because it can be used in both organic and aqueous solvent system as compared to other antioxidant assay. This method is based on the ability of antioxidants to reduce the ABTS radical cation. In the present work, different solvent extracts of *Thymus satureoides* were evaluated for their ABTS radical cation scavenging activity. Ascorbic acid was used as standard and its IC50 values was 25.29 µg/ml. IC50 values ranged from 51.27 to 127.38 µg/ml. Hydro-ethanolic extract showed good ABTS radical cation scavenging activity with IC 50 values of 51.27 µg/ml. Ethyl acetate and dichloromethane extracts showed moderate activity and its IC 50 values were 80.09 and 85.16 µg/ml respectively. Hexane extract showed poor ABTS radical cation scavenging activity with IC 50 values of 127.38 µg/ml. Moderate to weak antioxidant activity by ABTS method was shown by some medicinal plant extracts.

**Ferric Reducing Antioxidant Power (FRAP)**

The antioxidant compounds are responsible for the reduction of ferric (Fe³⁺) form to ferrous (Fe²⁺) form. The addition of FeCl₃ to the ferrous form led to the formation of blue colored complex. So the reduction ability can be determined by measuring the colored complex at 700 nm. The reducing properties associated with the presence of compounds exert their action by breaking the free radical chain through donating a hydrogen atom. Hydroethanolic extracts of *onion* showed greater FRAP value as 233.292 (mg equivalent of ascorbic acid/g of extract). The other extracts dichloromethane, ethyl acetate, and Hexane showed FRAP value 153.457, 123.004 and 97.819 (mg equivalent of ascorbic acid/g of extract) respectively. The ability of extract to reduce iron (FRAP) suggests that they contain compounds that are electron donors, which can react with free radicals converting was them to more stable products.
and terminate radical chain reaction. FRAP assay showed positive correlation between reducing power and phenolic content in onion extracts (Table 3). So these compounds are phenolic compounds. It was reported by Rice-Evans et al. (1996) that phenolic compounds have redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. The redox potential of phenolic compounds played an important role in determining the antioxidant potential.

It is generally accepted that there is a high correlation between levels of enzymatically produced pyruvate (EPY) present in onions and the perception of pungency. The investigation of this parameter was important to estimate the potential flavour and to define the aroma characteristics of various onion. Classification of onions according to pungency was proposed as follows: low, 0–3.

**Conclusion**

Based on the results obtained in the present study, it is concluded that the hydroethanolic extracts of onion exhibit considerable antioxidant radical scavenging activity on all tested assays (DPPH, ABTS and FRAP) and they possess substantial amounts of phenolic compounds. Thus, onion can be considered as good source of antioxidants which might be beneficial for combating oxidative stress.

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