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Comparative study of scallion and dry bulb of *Allium cepa* for antioxidant activity

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Abstract
A Chemicals or organic compound that inhibitsthe oxidation of other molecules in biological system generally termed as Antioxidants. Upon oxidation of chemical compound produce free radicals, after chain of reactions that may damage cells. Either of natural or synthetic antioxidants such as ascorbic acid (vitamin C) or thiolsterminates terminates the chain reactions and prevents the oxidation of the molecules. Mainly two different groups of substances classified as "antioxidant" are:

1. Industrial chemicals that added to products for prevention of oxidation,
2. Natural chemicals added to food sand found in body tissue for beneficial health effects.

Early day's research was going on the role of antioxidants which focused on the use in preventing the oxidation of natural unsaturated fats, to prevent rancidity. Simply by placing the fat in a closed container filled with oxygen, the rate of oxygen consumption is measured for calculating the Antioxidant activity. After the discovery of vitamins A, C, and E as antioxidants revolutionized the field of biochemistry that led to the realization of the importance of antioxidants in the living organisms. After identifying the process of lipid peroxidation and prevention of the peroxidation led to the recognition of vitamin Eas antioxidants. So Antioxidants are the reducing agents that prevent oxidative reactions, generally by scavenging reactive oxygen species so they cannot damage cells.
In the present research comparatively studied the antioxidant activity of scallion and dry bulb of *Allium cepa*.

Keywords: antioxidant activity, *Allium cepa,* DPPH method, ABTS method

Introduction

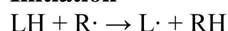
Most of the biological systems on Earth require oxygen to process major pathway in metabolism for its existence. At the stage of metabolism of complex life highly reactive oxygen species is formed that may damages living organisms by producing reactive oxygen species. Contrary, organisms prevent oxidative damage to cellular components such as DNA, lipids and proteins by maintaining complex network of antioxidant such as enzymes and metabolites that work together.

In the late 1980s and early 1990s, the relationship between the protective role of antioxidants against age and disease-induced damage to cells and biological molecules, DNAs, lipids and proteins exploited. Numerous researchers studied the mechanism (s) of action of antioxidants and identified the factors which influence their effectiveness.

The mechanism of oxidation takes place via free radical-mediated chain reaction which included initiation, propagation, branching and termination steps. The reaction may be initiated by the catalytic action of some external agents such as light, heat or ionizing radiation or by chemical activation involving metalions or metalloproteinase as catalyst.

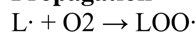
The Mechanism of oxidation[1]

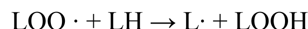
Initiation


LH + R· → L· + RH

Where LH is the substrate molecule, and with R· is the initiating oxidizing radical. The oxidation of the substrate generates a highly reactiveallyl radical (L·) that can rapidly react with oxygen to form a substrate peroxyl radical (LOO·)

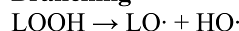
Propagation


L· + O2 → LOO·


LOO · + LH → L· + LOOH

The peroxyl radicals formed may act as chain carriers of the reaction which further oxidize the substrate to produce hydroperoxides (LOOH). This hydroperoxides in turn break down to a wide range of compounds such asalkyl formates, alcohols, aldehydes, ketones and hydrocarbons, and radicals.

Branching

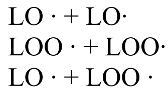

LOOH → LO· + HO·

2 LOOH → LOO · + LO· + H2O

The breakdown of substrate hydroperoxides often involves transition metal ion catalysis, in reactions similar to those involving hydrogen peroxide, yielding substrate peroxyl and subtratealkoxyl radicals.

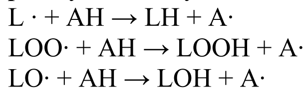
Termination

End of the reactions involve the interaction of radicals to form non-radical products:



LO · + LO·
LOO · + LOO·
LO · + LOO ·

Primary antioxidants, AH, when present in trace amounts, may interfere the initiation step by reacting with a substrate radical or by inhibiting the propagation step by reacting with peroxyl or alkoxyl radicals.



L · + AH → LH + A·
LOO· + AH → LOOH + A·
LO· + AH → LOH + A·

Secondary or preventative antioxidants are compounds that decrease the rate of oxidation which may be achieved in a several ways, including removal of substrate or singlet oxygen quenching.

Types of antioxidant [2]

The biological system of human being utilizes both endogenous antioxidants as well compounds ingested through diet, as its defense mechanism. Examples of endogenous antioxidants include enzymes and biomolecules such as glutathione peroxidase and glutathione, where as carotenoids, vitamins and polyphenolics are representative of dietary antioxidants. In general, antioxidants are categories in several ways as:

1. Enzymes, such as glutathione peroxidase, superoxide dismutase and catalase;
2. Hormones (melatonin);
3. Proteins (ferritin, albumin etc.);
4. Small molecules (carotenoids, glutathione, phenolic compounds, uric acid, tocopherol).

Health benefits of antioxidants [3]

Apart from the discussion regarding the mechanism of antioxidants to prevent a chain of destructive chemical reactions in the body, it is not very clear about health benefit to the body. Dietary antioxidant includes beta-carotene, lutein, lycopene and selenium as well as vitamins A, C and E. Diets rich in antioxidants have been associated with several health benefits:

- Immune function
- Cardiovascular health
- Brain function
- Digestive function
- Blood sugar stability
- Prostate health
- Skin health
- Eye health

Methodology

From the literature reviewed, it shows that different authors established varied processes for the extraction and to study the antioxidant activity In vitro and *In vivo* as well. So we decided slight modification in that method so as to obtained better result as follow, the instrument used for the extraction is Soxhlet Apparatus and for the absorbance were calculated from the double beam U.V. spectrophotometer (Thermo electronics corporation). The chemical used available from the laboratory as per the method.

Extraction of plant material [4]

The outer scales of onion bulbs were separated, made free from soil, shade dried and coarsely powdered. The powdered scales (40 g) were extracted separately with 150 ml each of distilled water, 70% methanol and 70% ethanol for 30 min in Soxhlet Apparatus followed by maceration (24 hrs) in shaking incubator at 37±0.20 C. The extracts obtained were concentrated under vacuum and percentage yield with to respect to dried weight of plant material was calculated. This method is applied to both onion dry bulb and scallion.

Detection of Antioxidant Activity

1. DPPH assay [5]

The assay was performed as described by Brand-Williams *et al*. (1995) for the radical scavenging activity of the extracts in relation to the DPPH radical and modified as follows: an aliquot (0.5 mL) of methanolic solution containing different concentrations (0.3; 0.6; 0.9;1.2;1.5 and 1.8 mg/mL) was added to 2.5 mL of methanolic DPPH solution (0.1mM). The mixture was stirred gently and left to stand at room temperature in the dark for 30 min. the absorbance was measured at 517 nm for test and standard. The scavenging activity was measured as the decrease in absorbance of the samples in correlation with the DPPH standard solution. The results were expressed as % DPHH radical scavenged according to the following formula:

% DPHH radical scavenged = [(Ao-As)/Ao] ×100

2. β-carotene bleaching assay [6]

Theβ-carotene bleaching assay was performed as described by Cao *et al*. (2009) with slight modifications. β-carotene (0.01g) dissolved in chloroform (10 mL) and mixed with 40 mg of linoleic acid containing 400 mg of Tween 40 in a flask. Chloroform removed in a rotary vacuum evaporator at 40°C for 10 min. To this solution 100 mL of oxygenated distilled water was slowly added to the oily residue with vigorous stirring so as an emulsion is formed. A 4.5 mL aliquot of the emulsion was added to a tube containing 0.5 mL of extract sample solution of different concentrations. The absorbance was immediately measured at 470 nm spectro photometricaly against a blank consisting of the emulsion without β-carotene. The tubes were placed in a water bath at 50°C to promote the oxidation of the emulsion and monitored the absorbance at 470 nm spectro photometricaly every 15 min until 60 min had passed. The antioxidant activity (AA) as % Inhibition of the sample extract was evaluated in terms of the bleaching of β-carotene using the following equation:

% Inhibition = [(At-Ct) (Co-Ct)] ×100

Where At and Ct are the absorbance values of the test sample and control, respectively, after a certain time (t) of incubation and C0 is the absorbance value for the control, measured at the beginning of the experiments.

3. Hydrogen peroxide scavenging (H_2O_2) assay [7]

Human beings subjected to H_2O_2 indirectly through the environment nearly about 0.28 mg/kg/day that intake mostly from leaf crops. Hydrogen peroxide after entering into the human body through inhalation of vapor or mist and through eye or skin contactis rapidly decomposed into oxygen and water. This may produce hydroxyl radicals (OH^-) that responsible lipid peroxidation and cause DNA damage in the biological system.

The ability of plant extracts to scavenge hydrogen peroxide can be estimated according to the slightly modification in method of Ruch *et al.* (1989). A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 mM pH 7.4). The concentration of hydrogen peroxide is determined by using a spectrophotometer at absorption 230 nm. Hydrogen peroxide is added to extract (20–60 lg/mL) in distilled water and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The Hydrogen peroxide scavenging (H2O2) assay is calculated as follows:

% scavenged = [(Ai-At)/Ai] ×100

Where Ai is the absorbance of control and at is the absorbance of test.

Results

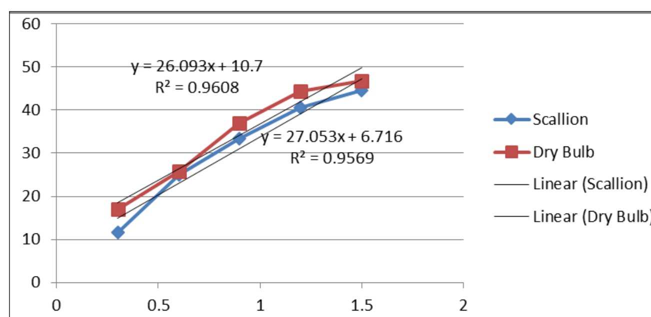
Table 1: Solvent wise antioxidant activity

S.N.	Extract	Absorbance (nm)		% DPHH radical scavenged	
		Scallion	Dry bulb	Scallion	Dry bulb
01	Blank	-	-	-	-
02	Distilled Water	0.734	0.693	6.61	11.83
03	70% Methanol	0.716	0.689	8.90	12.34
04	70% Ethanol	0.694	0.653	11.70	16.92

DPPH assay

Table 2: % DPHH radical scavenged

S.N.	Concentration (mg/mL)	Absorbance(nm)		% DPHH radical scavenged	
		Scallion	Dry bulb	Scallion	Dry bulb
01	Control (Ao)	0.786			
02	As (0.3)	0.694	0.653	11.70	16.92
03	As (0.6)	0.589	0.527	25.06	25.90
04	As (0.9)	0.523	0.496	33.46	36.89
05	As (1.2)	0.467	0.437	40.58	44.40
06	As (1.5)	0.436	0.418	44.52	46.81



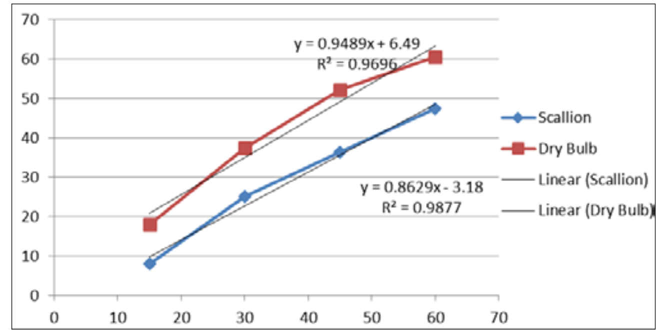
Series1: Dry Bulb, Series2: Scallion

Fig 1: % DPHH radical scavenged

B-carotene bleaching assay

Table 3: β-carotene bleaching assay

S.N.	Time (Min.)	Absorbance (nm)			% Inhibition	
		Control	Scallion	Dry bulb	Scallion	Dry bulb
01	15	0.039	0.043	0.048	08	18
02	30	0.041	0.053	0.059	25	37.50
03	45	0.045	0.061	0.068	36.36	52.27
04	60	0.051	0.069	0.074	47.36	60.52



Series1: Dry Bulb, Series2: Scallion

Fig 2

Hydrogen peroxide scavenging (H_2O_2) assay

Table 4: Hydrogen peroxide scavenging (H_2O_2) assay

S.N.	Concentration Mg/ml	Absorbance (nm) at		% Inhibition	
		Scallion	Dry bulb	Scallion	Dry bulb
01	Ai	0.349			
02	20	0.289	0.251	17.19	28.08
03	40	0.256	0.242	26.64	30.65
04	60	0.249	0.229	28.65	34.38
05	80	0.236	0.219	32.37	37.24

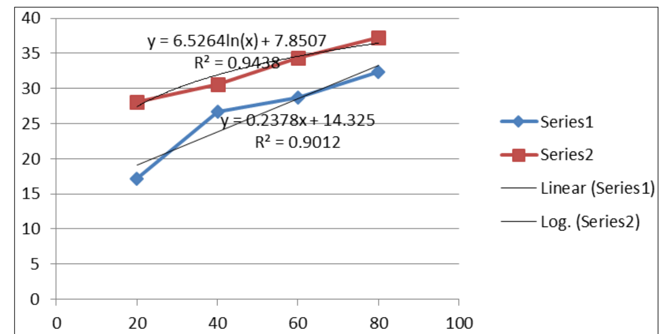


Fig 3

Conclusion

From the results obtained with the study of different assay method it has been observed that, we extracted the active antioxidant constituent in different solvent out of which, extract obtained from the 70% ethanol shows the maximum antioxidant activity than the other two solvent. So we use the extract obtained from 70% ethanol for further comparative study

The scallion shows antioxidant activity lower than that of the dry bulb.The possibility of the lower activity may be because of the net content of the antioxidant (Phenolic and flavonoid compounds) are low. The antioxidant chemicals are not completely synthesis during the biogenetic pathway.

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