

Evaluation of *In vitro* Antioxidant Activity of Dried Scales of *Allium Cepa* Linn Extracts

K.N.V Rao*, K. VamshiSharathNath, S. Sandhya, M. Sai Kiran, David Banji, P.Saikumar, P.Sudha

Abstract

The main characteristic of an antioxidant is its ability to trap free radicals and also reduce the risk of chronic diseases including cancer, central nervous system injury and heart diseases. Scientific information on antioxidant properties of various natural sources is still rather scarce. A variety of free radical scavenging antioxidants is found in dried scale leaves of *Allium cepa*. The purpose of this study was to evaluate antioxidant activity of dioxane, hexane, isopropyl alcohol, propane-2-ol, water, n-Butanol and ethanolic extracts of dried scale leaves of *Allium cepa* by Total Phenolic content, Reducing Power, Super Oxide Radical scavenging activity. The result suggested that these extracts can be a vital source of antioxidant phytochemicals. Further investigation may be carried out in isolation and purification of the compounds responsible for the antioxidant activity.

Keywords: Antioxidant, *Allium cepa*, Free radicals, Total Phenolic content, Reducing Power, Super Oxide Radical scavenging activity.

Introduction

Reactive Oxygen Species (ROS) as superoxide anion, hydroxyl radicals, hydrogen peroxide and Singlet oxygen are derived from normal metabolic activity/processes in the human body or from external sources such as exposure to radiation, ozone, cigarette smoking, air pollutants and industrial chemicals. (Kalyarat Kruawan and Kaew Kangsadalampai, 2006; T.Sathishkumar *et al.* 2010).

The ROS formed may cause cellular & sub-cellular damage by peroxidation of membrane lipids, by denaturing cellular proteins, & by breaking DNA strands, disrupting cellular functions. (Patra *et al.*, 2008). The ROS are major cause of human cancer and other diseases. The risk of diseases can be reduced by increased consumption of antioxidants which are abundant in food (Kalyanrat *et al.*, 2006; Ramapriya and Usha, 2010).

Another field strongly affected by ROS is the food sector, where the free radical peroxidation of the free radicals, is the predominant cause of food decay, destruction of vitamins and rancidity during storage transformation (St. Angelo, 1992).

Antioxidants widely used are mainly of synthetic origins and have recently been suspected to their toxicity and cause lipid alteration as well as carcinogenic effects. (Grillo & Dulout, 1995). Therefore, attention is focusing on the development of new, safe and cheap antioxidants of natural origin. (Shanab, 2007).

Several studies have investigated the antioxidant property of *Allium cepa*. The integrity of cells is seriously endangered, if ROS cannot be controlled by scavenging molecules and other repair mechanisms of the

cell. They have tested the ROS sensitive dye 5-(and-6) chloromethyl-2',7' dichlorodihydrofluoresceindiacetate acetyl ester (CM-H(2)DCFDA) using onion bulb scale and leaf epidermis. ROS were generated by several fundamentally different methods-externally applied hydrogen peroxide, heat shock, high light or wounding (Kristiansen KA *et al.*, 2006). Pumping of 110% of their original weight with solutions containing 5% of various ingredients (sodium ascorbate, garlic, and onion powder) into the cuts Pork loin and belly, and evaluated the physicochemical properties, and antioxidant activities during refrigerated storage at 8 degrees C. The addition of garlic and onion powder tended to increase redness (a) and yellowness (b) in both the belly lean and loin with the exception of a few cases. In both the belly and loin cuts, the content of oxidative products (volatile compounds) was reduced with the addition of garlic and onion powder. (Park SY *et al.* 2006)

In the present study, we have evaluated the antioxidant potential of the dioxane, hexane, isopropyl alcohol, propane-2-ol, water, n-Butanol and ethanolic extracts of dried scale leaves of *Allium cepa*.

Materials and Methods

Collection of Plant material

The dried scale leaves of *Allium cepa* were collected from local market, Hyderabad.

Procedure for Extraction

Dried scale leaves of *Allium cepa* were ground to coarse powder. The powder was extracted with different solvents like Dioxane, Hexane,

isopropyl alcohol, Propan-2-ol, n-Butanol, Water, Ethyl alcohol by Soxhlation for 6 hours [K.R Khandelwal, 2007, Rajeshwari Shivaraj, 2010].

Solvents and Chemicals Used: All the chemicals were purchased from MERCK, Pharma LTD Mumbai

Estimation of Total Phenolic Content

The total phenolic content of the ethanol and aqueous extracts were determined according to the Folin-Ciocalteu method.

Preparation of standard solution

Prepare 1-10 µg/ml of Gallic acid, Tannic acid. From the above concentrations take 1ml of the solution, add 1.5 ml of Folin-Ciocalteu reagent to this add 4ml of 20% Na₂CO₃ solution, Kept a side for about 30min at room temp, then measure the absorbance at 738nm, then prepare standard graph.

Preparation of sample

Take 100mg of the sample in 10ml of distilled water or ethanol, take 0.1ml of the above solution to this add 1.5 ml of Folin-Ciocalteu reagent kept a side for about 5min, add 4ml of 20% Na₂CO₃ solution, measure the absorbance at 738nm.

A calibration curve using Gallic acid in a concentration range of 1-10 µg/ml was prepared. The total phenolic content of the sample was expressed as Gallic Acid Equivalents (GAE), which reflected the phenolic content as amount of Gallic acid in sample. (Wang M, Liz, 1998).

Reducing power ability

Different concentrations (20-100 µg/ml) of seven extracts were prepared and 1ml of each solution was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.8) and potassium ferri cyanide (2.5ml, 1%). The mixture was incubated at 50 °c for 20min. To this mixture, 2.5ml of 10% trichloro acetic acid (TCA) was added and then centrifuged at 3000 rpm for 10min. the upper layer of the solution (2.5 ml) was mixed with distilled water (2.5ml) and FeCl₃ (0.5 ml, 0.1%) was added and the absorbance was measured at 700nm. (Halliwel B *et al.*, 1992).

The percentage of reducing power was calculated by using the formula

$$\text{Reducing power (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} * 100$$

Where, A_{control} is the absorbance of solution without extract and

A_{sample} is the absorbance with different dilutions of drug extract,

Ascorbic acid is used as a standard.

Superoxide radical scavenging activity

Superoxide scavenging activity of the plant extract was determined by Mc Cord and Fridovich method, 1969, which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitrobluetetrazolium. 0.1ml of different concentrations of plant extract, and 0.1ml of 6 µM ethylene diaminetetraacetic acid, 0.1 ml of 50 µM nitro blue tetrazolium, 0.05ml of 2 µM riboflavin were transferred to a test tube, and final volume was made up to 3ml using phosphate buffer. Then the assay tubes were uniformly illuminated with an incandescent light (40 Watts) for 15 minutes and thereafter the optical densities were measured at 560nm. A control was prepared using 0.1ml of respective vehicle in the place of plant extract.

The percentage inhibition of superoxide production was evaluated by comparing the absorbance values of control and experimental tubes. (Wang M, *et al.*, 1998).

Calculation of percentage inhibition

The percentage inhibition of superoxide production by the extract was calculated by using the formula:

$$\text{Inhibitory ratio} = \frac{(C - T) \times 100}{C}$$

Results and Discussion

Total phenolic content in dioxan, hexane, isopropyl alcohol, propane-2-ol, water, n-butanol and ethanolic extracts of dried scale leaves of *Allium cepa* determined by interpolating the absorbance with the Gallic acid absorbance taken as the standard is found to be 3.2mcg/ml, 6.4mcg/ml, 4.4mcg/ml, 8.4mcg/ml, 5.6mcg/ml, 1.2mcg/ml, 9.6 mcg/ml

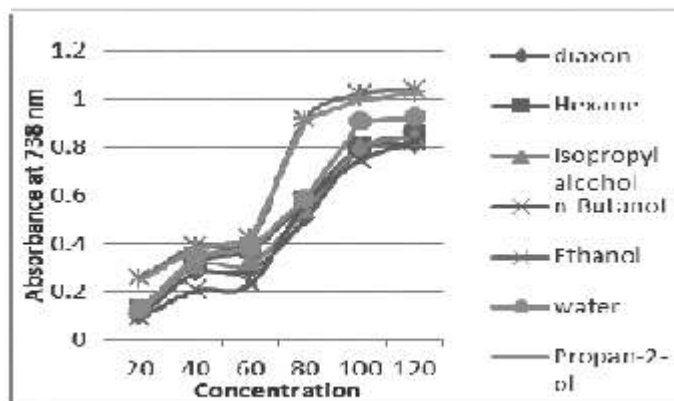
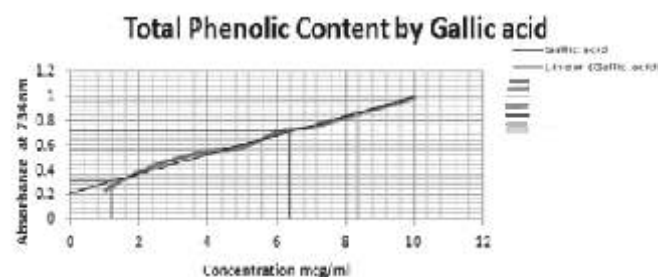


Figure 1. Total Phenolic content by Tannic acid

The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity (Awika *et al.*, 2003). Absorbance for 20mcg/ml to 100mcg/ml extracts were presented in the fig.2. In the present study, effectiveness of radical scavenging activity appeared to be more pronounced in ethanol and propan-2-ol when compared that of sample in n-butanol, dioxan, water hexane, isopropanol solutions. Moreover, method of the Folin-Ciocalteu reagent for the determining the total phenols actually measures reducing capacity of a samples. (Huang *et al.*, 2005).

Absorbance for 20mcg/ml to 100mcg/ml extracts were represented in the fig.3. in the present study, effectiveness of radical scavenging activity appeared to be more pronounced in ethanol and propan-2-ol when compared that of sample in n-butanol, dioxan, water hexane, isopropanol solutions. Moreover, method of the super oxide scavenging, light

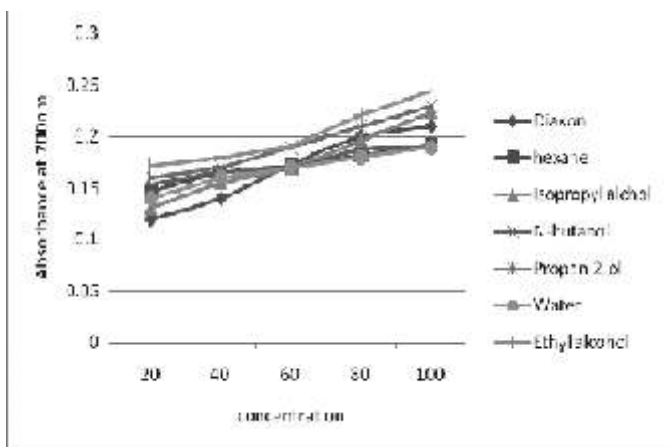


Figure 2. Reducing power of the extracts of dried scales of *Allium cepa*.

induced superoxide generation by riboflavin and the corresponding reduction of nitrobluetetrazolium, and the optical density were observed at 560nm for determining the superoxide reducing capacity of a samples.

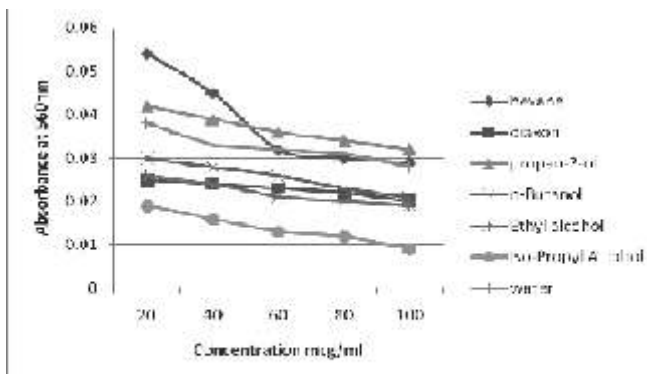


Figure 3. Superoxide radical scavenging activity of the extracts of dried scales of *Allium cepa*.

Conclusion

It is evident from the present study that the propan-2-ol and ethanolic extracts of scale leaves of *Allium cepa* could be utilized as good natural source of antioxidants in pharmaceutical industry. However, the active

compounds responsible for the antioxidant activities need to be evaluated. Therefore, it is suggested that further works may be performed on isolation and identification of the antioxidant components in scale leaves of *Allium cepa* for its industrial and pharmaceutical applications.

References

Grillo, C.A. and F.N. Dulout ,1995.Cytogenetic evaluation of butylatedhydroxytoluene .*Mutat.Res.* **345**:73-8.

Halliwel B and Guteridge JMC, Free radicals-Antioxidants and Human diseases; *Journal of laboratory and Clinical medicine*, 1992, Pg 598 - 620.

K.R Khandelwal, Practical Pharmacognosy, 17, NiraliPrakashan, Pune, 2007, 149.

Kalyanrat, K and kangsadlampai, 2006. Antioxidant activity, phenolic compound contents and antimutagenic activity of some water extract of herbs. *Thai J. Pharma. Sci.* **30**:28-35

Patra, J.K., S.K.Rath, K.Jena, 2008.Evaluation of antioxidant and antimicrobial activity of seaweed (sargassum sp.)Extract: A study of inhibition of glutathione –s-transferase activity .*Turk J. Biol.* **32**:119-125

RajeshwariShivaraj, Uma Maheswari, The Antiseptic Journal of Medicine and Surgery, 2010, 7, 34.

Ramapriya, R.and K.Usha, 2010.Antioxidant potential of the leaves and flowers of Nyctanthesarbor-tristisLinn. *Advanced Biotech.* **9**(7):40-42.

Sathish Kumar T., S.Shanmugun, M.Sampath, 2010. Investigation of Antioxidant properties of Polyalthialongifolia leaves. *Advanced Biotech.* **9**(7):43-45.

St.Angelo, A.J., 1992.Lipid Oxidation in food.ACS Symposium series 500, Washington D.C.

Shanab, M.M. S., 2007. Antioxidant and antibiotic activities of some seaweeds (Egyptainisolates).*Int.J.Agr.Biol.* **9**:220-225.

Wang M, Liz, Rangarajan M, Shavo V, Antioxidants and Phenolic compounds from sege, *Journal of Agricultural Food Chemistry* 1998,**46**:4869-4873.