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# DPPH scavenging and reducing power properties in common vegetables

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**ABSTRACT**

A study was carried out to determine the antioxidant activity of aqueous extracts of ten selected common vegetables viz., *Brassica oleracea* var. *capitata*, *Lycopersicon esculentum, Daucus carota, Raphanus sativus, Momordica charantia, Allium cepa, Dioscorea alata, Brassica oleracea* var. *gongylodes, Luffa acutangula* and *Benincasa hispida* by *in vitro* models like DPPH and reducing power assay at different concentrations. The DPPH activity was highest in *Brassica oleracea* var. *capitata* and least in *Raphanus sativus* in terms of $IC_{50}$. The reducing power was found to be high in *Brassica oleracea* var.*capitata and least in Benincasa hispida.*

**Keywords:** antioxidant, DPPH scavenging, reducing power, vegetables

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## INTRODUCTION

It is ironic that oxygen, an element indispensable for life can, under certain situations, have deleterious effects on the human body. Most of the potentially harmful effects of oxygen are due to the formation and activity of a number of chemical compounds, known as reactive oxygen species, which have a tendency to donate oxygen to other substances [1]. Many such reactive species are free radicals. Free radicals are chemical species possessing one or more unpaired electrons and usually make a molecule more reactive than the corresponding non-radical. The molecule acts as an electron acceptor and essentially 'steals' electrons from other molecules. Free radicals are referred to as oxidizing agents since they cause other molecules to donate their electrons [2]. They are produced continuously in cells, either as accidental byproducts of metabolism or deliberately. The most common cellular oxygen free radicals are superoxide radical ($O2^{-}$), hydroxyl radical ($OH^{\cdot}$) and nitric oxide ($NO^{\cdot}$) [3]. Other molecules, such as hydrogen peroxide ($H_2O_2$) and peroxynitrate ($ONOO^{\cdot}$) are not free radicals themselves but can lead to their generation through various chemical reactions.

An average cell utilizes 1013 molecules of $O_2$ per day. It is estimated that 1% of respired molecular oxygen will form ROS, thus approximately 1011 ROS are produced by each cell in a day. Cells normally employ a number of defence mechanisms against damage induced by free radicals [3-4].

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidant defenses fall in to two main categories, those whose role is to prevent the generation of free radicals and those that intercept any radicals that are generated. Various animal studies have shown that antioxidants delay or protect against the oxidative damage produced by the free-radical reaction and a protective role against ailments mediated by free radicals is now well established [5].

There are increasing evidences that increased consumption of fruits and vegetables and intake of certain non-nutrients that are present in foods reduce the risk of various pathological events such as cancer [6-7], and cardio- and cerebro-vascular diseases [8]. The vegetables are rich sources of many nutrients and antioxidant vitamins. Therefore, the objective of the present study was to determine the antioxidant activity of aqueous vegetable extracts using *invitro* antioxidant models.

## MATERIALS AND METHODS

### Plant materials

Ten different commonly consumed vegetable in tropical India were selected. Samples of fresh vegetables were purchased from a local market of Shimoga – Bhadravathi, Karnataka, when they were most available, during the year of 2009. The vegetable comprised of Kohlrabi

(*Brassica oleracea* L.var. *gongylodes* L.), Radish (*Raphanus sativus* L.), Ridge gourd (*Luffa acutangula* (*Roxb.*)L.), Cabbage (*Brassica oleracea* L. var. *capitata* L.), Ash- gourd (*Benincasa hispida (thunb)*), Carrot (*Daucus carota* L.), Elephant yam (*Dioscorea alata*), Tomato (*Lycopersicon esculentum mill.*), Onion (*Allium cepa* L.), and Bitter gourd (*Momordica charantia* L.) which were authenticated by the taxonomist from the Dept of Botany, Sahyadri Science College, Shimoga.

**Preparation of extracts**

After selection, each fresh vegetable was washed under running tap water followed by washing with distilled water to remove the surface impurities. Exactly 500g of vegetables were collected and weighed. The vegetables were minced using a mixer grinder and finely macerated. After homogenization, it was extracted in 500 mL of chloroform water (1.25ml $CHCl_3$ and volume is makeup to 500ml distilled water) for 7 days in the room temperature with intermittent shaking. After incubation, the whole extracts were filtered through filter paper. The filtrate is maintained in dark. To the marc, 300 mL fresh solvent was added and refluxed for 90 min. The whole extracts were again filtered and both the filtrate is mixed together and concentrated. The yield of crude extracts obtained from solvent were noted, stored in desiccators for maximum of 3 days; later preserved in a deep freezer ($-20^0$C) for further analysis.

**General Chemicals and Instruments**

All chemicals and solvents used in the study were of analytical grade. 2, 2-diphenyl-1-picryl hydrazyl (DPPH), methanol, trichloro acetic acid (TCA) are purchased from Himedia, India. Ascorbic acid, monobasic and dibasic sodium phosphate, potassium ferri cyanide, ferric chloride is procured from Sd fine chem. Ltd, India. UV-Vis Spectrophotometer (Elico SL 159, India), centrifuge (Remi RM12C, India), deep freezer ($-20^o$C, Modern Industrial Corporation, India), vacuum rotary evaporator (Shivam Instruments, India), weighing balance (Sartorius, India) and pH meter (Systronics, India) were the instruments used for the study.

**Phytochemical analysis**

The preliminary qualitative phytochemical studies were performed for testing the different constituents present in aqueous extracts of ten different vegetable extracts [9-11].

**DPPH free radical scavenging activity**

DPPH free radical scavenging assay was measured using DPPH free radical test, by employing the method of Wong *et al.* 2006 [12]. The different concentrations of each of the extracts were prepared in methanol and were added to 3ml of 0.1mM methanolic solution of DPPH. The tubes were shaken vigorously and allowed to stand for 30 min at room temperature

in dark. Changes in absorbance of samples were measured at 517 nm. A control reading was obtained using methanol instead of the extract. Ascorbic acid was used as the standard.

Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula,

$$\% \quad \text{Inhibition} \quad = \quad \left[ \frac{(A_0 - A_1)}{A_0} \right] \quad X\ 100$$

Where, $A_0$ is the absorbance of the control (without test samples)
$A_1$ is the absorbance of test samples.

All the tests were performed in triplicates and the results were reported as $IC_{50}$, which is the amount of antioxidant necessary to decrease the initial DPPH• concentration by 50%.

**Reducing power assay**

The reducing power of the extracts was evaluated according to Oyaizu, 1986 [13]. Different amounts of aqueous extracts were perched in aqueous solvent and diverse with 2.5 ml of 0.2M phosphate buffer (pH 6.6), and 2.5 ml of 1% $K_3Fe(CN)_6$. This mixture was incubated at 50$^oC$ for 20 min, 2.5 ml of 10% TCA was added to the blend and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was assorted with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicates increased reducing power. All the tests were performed in triplicates and the results were pooled and expressed as mean ± standard error (SE).

## RESULTS AND DISCUSSION

**Qualitative phytochemical analysis**

All the ten aqueous vegetable extracts showed the presence of carbohydrates, proteins, amino acids, glycosides, flavonoids, tannins & polyphenols. *Brassica oleracea* var. *capitata*, *Lycopersicon esculentum, Raphanus sativus, Allium cepa, Dioscorea alata, Brassica oleracea* var. *gongylodes, Luffa acutangula* and *Benincasa hispida* revealed the presence of additional alkaloids whereas the latter was absent in other vegetables *viz. Daucus carota and Momordica charantia.* Analysis also revealed that none of the vegetables under study gave positive results for saponins in the aqueous extract **(Table 1)**.

**DPPH radical scavenging activity**

DPPH• is one of the few stable and commercially available organic nitrogen radicals [14-16]. This assay is based on the theory that a hydrogen donor is an antioxidant. The antioxidant effect is proportional to the disappearance of DPPH• in test samples. DPPH• shows a strong absorption maximum at 517 nm (purple). A freshly prepared DPPH solution exhibit a deep

purple color with absorption maximum at 517nm. The purple color generally fades or disappears when an antioxidant is present in the medium [17-18]. Aqueous extracts of all the vegetables studied showed appreciable free radical scavenging activities. The $IC_{50}$ values for aqueous vegetable extracts were 0.95, 1.39, 1.64, 1.70, 1.84, 1.95, 2.9, 3.54, 3.79, and 4.75 mg/ml for *Brassica oleracea* var. *capitata, Allium cepa, Lycopersicon esculentum mill, Dioscorea alata, Momordica charantia, Luffa acutangula, Daucus carota, Brassica oleracea* var. *gongylodes, Benincasa hispida* and *Raphanus sativus* respectively while, the similar activity was 2.45µg/ml for standard **(Fig1).** The results revealed that, dose dependent radical scavenging activity in terms of $IC_{50}$ values.

**Reducing power assay**

The reducing capacity of the extracts $Fe^{3+}$**/** ferricyanide complex to the ferrous form may serve as a significant indicator of its antioxidant capacity [19-20]. The existence of reductones are the key of the reducing power, which exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom. The reduction of the $Fe^{3+}$ **/** ferricyanide complex to the ferrous form occurs due to the presence of reductants in the solution. [21]. Reductones are believed not only to react directly with peroxides but also prevent peroxide formation by reacting with certain precursors. Among the aqueous vegetable extracts, *Brassica oleracea* var. *capitata* showed highest reducing power activity **(Fig 2)** followed by *Allium cepa* > *Momordica charantia* > *Raphanus sativus* > *Dioscorea alata* > *Daucus carota* > *Luffa acutangula* > *Lycopersicon esculentum* > *Brassica oleracea* var. *gongylodes* > *Benincasa hispida* at concentrations of 2 to 10mg/ml. The reducing power of the aqueous extract of vegetables increased with increasing concentrations of the extracts. From the two assays it can be noted that, highest antioxidant activity was shown by *Brassica oleracea* var. *capitata* and *Allium cepa* while other vegetables manifested variable activity in the two assays.

In present times, it is believed that the regular consumption of vegetables has always been associated with health benefits, but their mechanism has become clear only in the recent decades. Vegetables contain a wide variety of biologically active, non-nutritive compounds known as phytochemicals. This is often attributed to the antioxidants such as vitamin C, E, carotenoids, lycopenes and flavonoids that prevent free radical damages [22-24].These phytochemicals impart health benefits beyond basic nutrition [25]. The consumption of these vegetables may play a role in preventing human disease in which free radicals are involved, such as cancer, cardiovascular diseases and aging. However, further investigations on individual components, their *in vivo* antioxidant activity, and the different antioxidant mechanisms are warranted. [26]. Further, to elucidate a full profile of antioxidant activity against various ROS, comprehensive assays are needed.

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Table 1. Qualitative phytochemical analysis of ten aqueous vegetable extracts

| TESTS | VEGETABLE EXTRACTS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Brassica oleracea* var. | *Luffa acutangula* | *Raphanus sativus* | *Momordica charantia* | *Allium cepa* | *Lycopersicon esculentum* | *Dioscorea alata* | *Daucus carota* | *Benincasa hispida* | *Brassica oleracea* var. |
| **Carbohydrates** | + | + | + | + | + | + | + | + | + | + |
| **Proteins** | + | + | + | + | + | + | + | + | + | + |
| **Amino acids** | + | + | + | + | + | + | + | + | + | + |
| **Steroids** | - | - | - | - | - | - | - | - | - | - |
| **Glycosides** | + | + | + | + | + | + | + | + | + | + |
| **Saponins** | - | - | - | - | - | - | - | - | - | - |
| **Alkaloids** | + | + | + | - | + | + | + | - | + | + |
| **Flavonoids** | + | + | + | + | + | + | + | + | + | + |
| **Tannins and Polyphenols** | + | + | + | + | + | + | + | + | + | + |

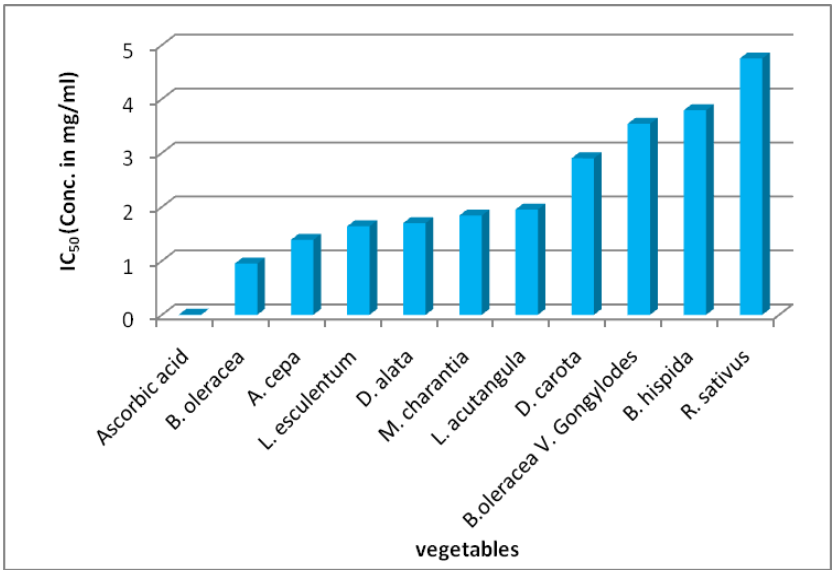


Fig 1 DPPH radical scavenging activity ($IC_{50}$) of ten aqueous vegetable extracts

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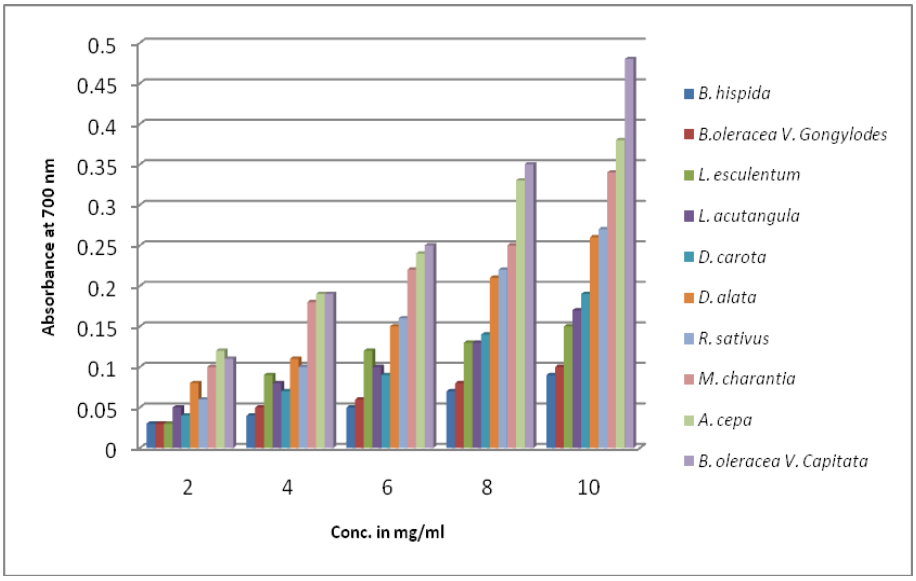


**Fig 2 Reducing power assay of ten aqueous vegetable extracts**

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