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Comparative evaluation of antioxidant property in methanol extracts of some common vegetables of India

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ABSTRACT

The aim of this study was to assess the antioxidant potential of methanol extracts of ten selected common vegetables viz., Brassica oleracea var. capitata, Lycopersicon esculentum, Daucus carota, Raphanus sativus, Momordica charantia, Allium cepa, Amorphophallus campanulatus, Brassica oleracea var. gongylodes, *Luffa acutangula and Benincasa hispida. In this work, in vitro models namely DPPH, reducing power assay and total antioxidant capacity were used at different concentrations. The DPPH activity was highest in Brassica oleracea var. capitata and least in Benincasa hispida in terms of IC_{50}. The reducing power was found to be high in Raphanus sativus and least in Brassica oleracea var. capitata, and total antioxidant capacity was highest in Brassica oleracea var. capitata and least in Amorphophallus campanulatus.*

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

INTRODUCTION

A free radical is a molecule with one or more unpaired electrons in the outer orbital. These free electrons are referred to as oxidizing agents since they cause other molecules to donate their electrons [1]. Many of these free radicals, in the form of reactive oxygen and nitrogen species, are an integral part of normal physiology. An over production of these reactive species can occur, due to oxidative stress brought about by the imbalance of the bodily antioxidant defense system and free-radical formation [2]. Reactive oxygen species (ROS) such as superoxide radical ($O2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), peroxyl radical ($ROO^{\bullet}$) and nitric oxide radical ($NO^{\bullet}$), attack

biological molecules, such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury associated with aging, atherosclerosis, carcinogenesis [3] and may lead to the development of chronic diseases related to the cardio and cerebrovascular systems [4].

Antioxidants are free-radical scavengers which can provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species [5]. The most commonly used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), Propylgallate (PG) and butylated hydroquinone. However, these synthetic antioxidants have side effects such as liver damage and carcinogenesis [6-7]. Therefore, there is a need for isolation and characterization of natural antioxidant having less or no side effects, for use in foods or medicinal materials in order to replace synthetic antioxidants.

Vegetables contain high amounts of known antioxidants such as polyphenols, vitamin C, vitamin E, carotene, and lycopene. The consumption of vegetables has been inversely associated with morbidity and mortality from degenerative diseases [8-12]. It is not known which dietary constituents are responsible for this association, but antioxidants appear to play a major role in the protective effect of plant foods [13-16].

MATERIALS AND METHODS

Plant materials

Ten different commonly consumed vegetables in tropical India were selected. Samples of fresh vegetables were purchased from a local market of Shimoga – Bhadravathi, Karnataka,India when they were most available, during the year of 2009. The vegetables 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* (*thumb*.)], Carrot (*Daucus carota* L.), Elephant yam (*Amorphophallus campanulatus*), 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, edible parts of each fresh vegetable were washed under running tap water and with distilled water to remove surface impurities. Exactly 500g of vegetables were collected and weighed. The vegetables were minced using a mixer grinder and finely macerated. After homogenization, macerates were extracted in 500 ml of methanol for 7 days at room temperature with intermittent shaking. After incubation, the whole extracts were filtered through filter paper and were maintained in the dark. 300 ml fresh methanol was then added and the mixture was refluxed for 90 min. The yield of crude extracts obtained from solvent was noted. The extract was stored in desiccators for maximum of 3 days and later preserved in a deep freezer (-20^0C) for further analysis.

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) were purchased from HIMEDIA, India.

Ascorbic acid, monobasic and dibasic sodium phosphate, potassium ferric cyanide, ferric chloride, sulphuric acid, sodium phosphate, ammonium molybdate were 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

Standard phytochemical screening tests were performed to identify the different constituents present in methanol extracts of ten different vegetable extracts [17-19].

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

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

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

Where, A_0 is the absorbance of the control
A_1 is the absorbance of test samples.

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

Reducing power assay

The reducing power of the extracts was evaluated according to Oyaizu, [20]. Different amounts of methanol extracts were perched in methanol 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^{o}C 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 methanol (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 indicated increased reducing power. All the tests were performed in triplicates and the results expressed as mean ± SE.

Total antioxidant capacity (Phosphomolybdenum method)

The total antioxidant capacity was measured by spectrophotometeric method of Prieto *et al.* [21]. At different concentration, methanol extracts were prepared in water and combined in an

eppendorf tube with 1ml of reagent solution (0.6M H_2SO_4, 28mM sodium phosphate, 4mM ammonium molybdate mixture). The tubes were incubated for 90min at 95ºC. The mixture was cooled to room temperature and the absorbance was read at 695nm against blank. The experiment was conducted in triplicates and values are expressed as equivalents of ascorbic acid in µg per mg of extract.

RESULTS AND DISCUSSION

Phytochemical Screening

All the ten vegetable extracts showed the presence of carbohydrates, proteins, amino acids, glycosides, flavonoids, steroids, tannins & polyphenols. *Brassica oleracea* var. *capitata, Lycopersicon esculentum, Raphanus sativus, Allium cepa, Amorphophallus campanulatus, Brassica oleracea* var. *gongylodes, Luffa acutangula* and *Benincasa hispida* revealed the presence of additional alkaloids whereas alkaloids were 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 methanol extract **(Table 1).**

DPPH radical scavenging activity

$DPPH^{\bullet}$ is one of the few stable and commercially available organic nitrogen radicals [22-24]. This assay is based on the theory that a hydrogen donor is an antioxidant. The antioxidant effect is proportional to the disappearance of $DPPH^{\bullet}$ in test samples. 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 [25-26]. Results were reported as IC_{50}, which is the amount of antioxidant necessary to decrease the initial $DPPH^{\bullet}$ concentration by 50%. The lower the IC_{50}, the higher is the antioxidant power [25].

DPPH radical scavenging activity of the methanol vegetable extracts were measured along with standard ascorbic acid. Methanol extracts of all the vegetables studied showed remarkable free radical scavenging activities. The IC_{50} values for methanol extracts of *Brassica oleracea* var.*capitata*, *Lycopersicon esculentum, Daucus carota, Raphanus sativus, Momordica charantia, Allium cepa, Amorphophallus campanulatus, Brassica oleracea* var. *gongylodes, Luffa acutangula* and *Benincasa hispida were* 1.38, 1.39, 1.49, 1.90, 2.29, 2.84, 3.39, 4.35, 4.59 and 4.88mg/ml, respectively while, the similar activity was 2.45µg/ml for standard **(Fig 1).** The results revealed 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 [27-28]. 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 [29]. Reductones are believed not only to react directly with peroxides but also prevent peroxide formation by reacting with certain precursors. Among the vegetables , reducing power was found to be high in *Raphanus sativus* **(Fig 2)** followed by *Daucus carota, Luffa acutangula, Brassica*

oleracea var. *gongylodes, Amorphophallus campanulatus, Benincasa hispida, Momordica charantia, Lycopersicon esculentum, Allium cepa, and Brassica oleracea* var. *capitata*

Total antioxidant capacity

Total antioxidant capacity by Phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid [21]. The methanol extracts of ten different vegetables showed very potent total antioxidant capacity. Among the vegetable extracts, total antioxidant capacity was found to be highest in *Brassica oleracea* var. *capitata* (42µg of ascorbic acid/mg of extract) followed by *Raphanus sativus* (30µg), *Allium cepa* (29µg), *Luffa acutangula* (28.5µg), *Brassica oleracea* var. *gongylodes* (26.5µg), *Momordica charantia* (26µg), *Lycopersicon esculentum* (25µg), *Daucus carota* (24.5µg), *Benincasa hispida* (22µg) and *Amorphophallus campanulatus* (19.5µg) (**Fig 3)**.

On the basis of results of the three assays *viz*. DPPH, reducing power and total antioxidant capacity of ten methanol vegetable extracts, the vegetables can be placed in the following general order. *Raphanus sativus* > *Brassica oleracea* var. *capitata* > *Daucus carota* > *Luffa acutangula* > *Brassica oleracea* var. *gongylodes* > *Allium cepa* > *Lycopersicon esculentum* > *Momordica charantia* > *Amorphophallus campanulatus* > *Benincasa hispida*.

The consumption of foodstuffs rich in antioxidants provides protection against cancer, cardio and cerebrovascular diseases. This protection can be explained by the capacity of these active compounds to scavenge free radicals, which are responsible for the oxidative damage of lipids, proteins and nucleic acids [30-31]. Vegetables contain considerable amounts of active components, which are considered as potent scavengers of free radicals and reactive oxygen species (ROS) [32-34]. The phytochemical analysis in the study indicated the presence of secondary metabolites like polyphenols, tannins, flavonoids and alkaloids in the crude extracts. These biologically active components contained in vegetables exhibit protective effects against cell oxidation, and neutralize the free radicals by donating hydrogen or electrons, thus ending the chain reaction of the oxidation procedure. Phenolic compounds are known as powerful chain breaking antioxidants [35], may contribute directly to antioxidative action [36]. These compounds are very important constituents of plants and their radical scavenging ability is due to their hydroxyl groups [37]. Tannins are the phenolic compounds present in almost all plant foods and have ability to scavenge radicals such as hydroxyl, superoxide, and peroxyl, which are known to be important in cellular prooxidant states [38]. Flavonoids help to provide protection against the oxidation at the cellular level as antioxidants by interfering in enzyme activity, chelating of redox-active metals and by scavenging free radicals [39]. Alkaloids are, cyclic organic compounds containing nitrogen in a negative oxidation state and are pharmaceutically significant. The higher concentrations of alkaloid acts as oxygen carrying agent and serves as a pro-oxidant in the co-oxidation of linoleic acid [40].

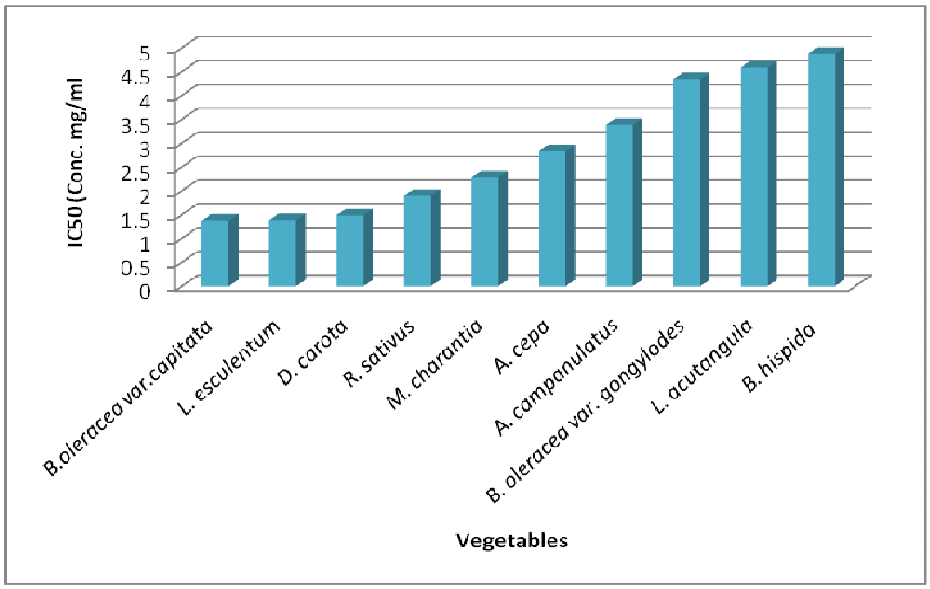

Fig 1 DPPH radical scavenging activity (IC_{50}) of methanol vegetable extracts

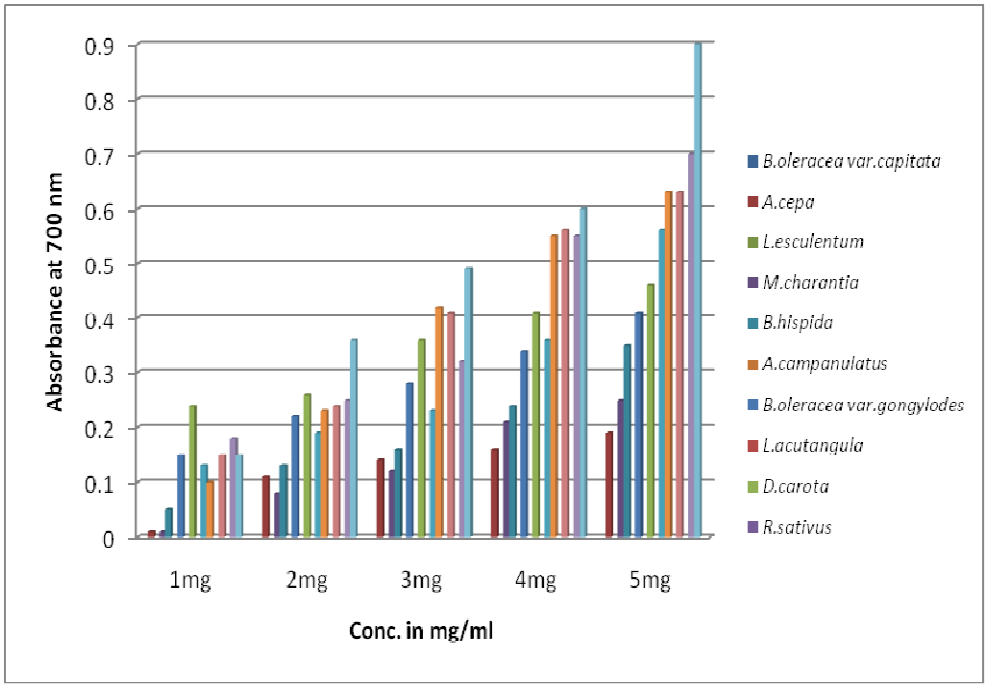

Fig 2 Reducing power assay of ten methanolic vegetable extracts

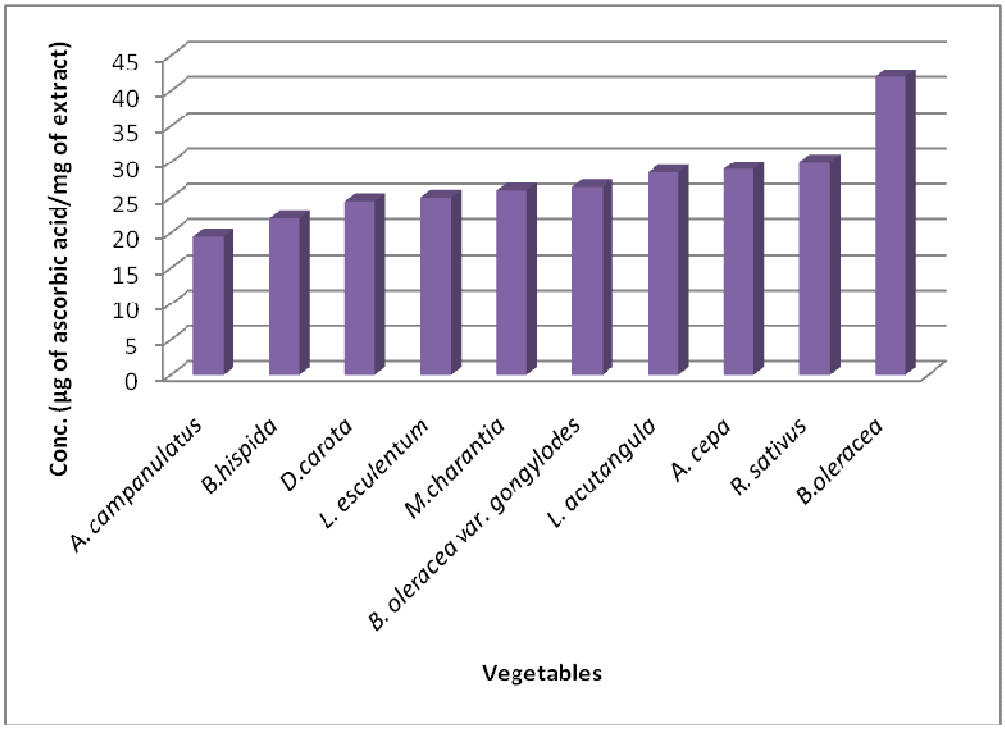

Fig 3 Total antioxidant capacity of ten methanol vegetable extracts (Equivalents of ascorbic acid)

Table 1. Qualitative phytochemical analysis of ten methanol vegetable extracts

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

In the present research program, we have attempted to rank antioxidant capacities of different vegetable extracts considering the overall results of the three assays. Similar grading has been attempted by several investigators. For example, antioxidant activity of some vegetables based on ORAC results has been previously reported by Cao *et al.* [41]. However according to Prior *et al.* [42] the major phytochemicals responsible for the antioxidant capacity can be accounted for by the flavonoid compounds and the biosynthesis of these natural products is profoundly influenced by a number of factors, such as locations, weather conditions, and harvest periods. Therefore, it is expected that the antioxidant assay values vary accordingly. Even though some kind of trend can be obtained for some vegetables, it is difficult to compare the antioxidant capacity of different vegetables due to variations in the anti oxidant capacity vs. assay methods. Therefore an index needs to be developed, which does not represent a specific antioxidant property but can rank the antioxidant capacity of the vegetables.

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