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Research Paper

Phytochemicals in Some Common Vegetables and Their Correlation with the Antioxidant Activity

Home Science

KEYWORDS : Antioxidants, free radicals, Vegetables, Total phenolic content, vitamin C, correlation

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ABSTRACT

The present investigation was undertaken to determine the phytochemicals and antioxidant activity of some common vegetables viz. Carrot, Tomato, Cabbage, Cucumber, Radish, Spinach, Bitter gourd and Bottle gourd. These phytochemicals are proved to be important factors in body defense system activity against reactive oxygen species. Antioxidants are helpful in combating the free radicals in body that cause cellular damage. The total phenolics were determined spectrophotometrically according to the Folin Ciocalteau procedure and ranged from 33.28 to 69.66 mg/100g Gallic acid equivalent(GAE), Ascorbic acid ranged from 4.59 to 67.21 mg/100g and the free radical scavenging activity was determined using 2,2,-diphenyl-2-picrylhydrazyl(DPPH) that ranged from 22.31 to 50.22 per cent. The correlation between antioxidant activity and total phenols and vitamin C was found to be linear. The results indicated that such vegetables containing high phenolics and vitamin C may provide a source of dietary anti-oxidants that have preventive and therapeutic roles in a number of human diseases.

Introduction

Fruits and vegetables have had conferred on them the status of functional foods (Hasler, 1998). Free Radicals (FRs) are the atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. These radicals can start a chain reaction and can react with important cellular components such as DNA or the cell membrane. This may lead to improper cell functioning or death of cells. Generation of oxygen radicals are associated with cellular and metabolic injury, and accelerating aging, cancer, cardiovascular diseases, neurodegenerative diseases, and inflammation (Halliwell and Gutteridge, 1999). To prevent free radical damage the body has a defence system of antioxidants which can safely interact with FRs and terminate the chain reaction before vital molecules are damaged (Osman et al., 2009).

Research has provided evidence of an inverse association between plant constituents rich diet and chronic and degenerative diseases incidence, showing that high dietary intake of antioxidative compounds would help maintaining adequate antioxidant status and proper physiological functions of the body tissues (Kanazawa, 2011). Fruits and vegetables contain a wide variety of biologically active, non-nutritive compounds known as phytochemicals. The daily consumption of minimum 400 g of fruit and vegetables for the prevention of heart disease, cancer, type II diabetes and obesity have been recommended by WHO and FAO in 2003 (Isabelle et al., 2009). In view of the study reported above there is a need for comprehensive measurement of antioxidants in fruits and vegetables. Present study was aimed to measure the total phenolic content, vitamin C and antioxidant activity of eight different vegetables commonly grown and consumed in India.

Materials and methods

Plant Materials

Eight species of vegetables (Table 1) were used. All the vegetables were purchased from the local market, Ludhiana, Punjab. The vegetables were washed, air dried, and crushed into paste form.

Preparation of Methanolic Extracts

Five grams of crushed paste of each species was refluxed in 50 ml methanol for 2 hrs followed by filtration. The refluxing procedure was repeated and filtrates were pooled. Organic solvents (used in single or mixed forms), especially polar ones, are suitable for extraction of biologically active plant ingredients (Ferrero et al., 2007). In this study, methanol was the most highly polar organic solvent for extraction.

Determination of Total Phenol Content

Total phenolic content was estimated by the Folin–Ciocalteu method (Singleton and Rossi, 1965). Two hundred microlitres of diluted sample were added to 1 ml of 1:10 diluted Folin–Ciocalteu reagent. After 4 min, 800 µl of saturated sodium carbonate (75 g/l) was added. After 2 h of incubation at room temperature, the absorbance at 765 nm was measured. Gallic acid (0–500 mg/l) was used for the standard calibration curve. The results were expressed as gallic acid equivalent (GAE)/g.

Determination of Antioxidant Activity

Antioxidant activity was determined by the 2,2,-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (RSA) method of Brand-Williams et al. (1995) with some modifications. The methanol extracts of vegetables used were chosen as an appropriate concentration for assessing antioxidant activity(%AA) based on preliminary studies. An aliquot of 2 ml of 0.1 mM DPPH radical in methanol was added to a test tube containing 1 ml of vegetable extract. Instead of methanolic extract of plants, pure methanol was used as the control. The reaction mixture was vortexed and left to stand at room temperature in the dark for 30 min before absorbance at 517 nm was measured. Pure methanol was used to calibrate the spectrophotometer. BHT was taken as a standard at a fixed concentration of 5mg/ml. Antioxidant activity was expressed as percentage inhibition to the DPPH radical determined by:

$$\%\,AA = \frac{\text{Control OD (0 min)} - \text{Sample OD (30 min)} \times 100}{\text{Control OD (0 min)}}$$

Radical-Scavenging Activity Assay

Unlike laboratory-generated free radicals, such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by metal ion chelation and enzyme inhibition (Amarowicz et al., 2004). A freshly prepared DPPH solution exhibits a deep purple color with absorption maximum at 517 nm. This purple color generally fades/disappears when an antioxidant is present in the medium. Antioxidant molecules can quench DPPH free radicals by providing hydrogen atoms or by electron donation, conceivably via a free radical attack on the DPPH molecule, and convert them to a colorless/bleached product, 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine, resulting in a decrease in absorbance at 517 nm. The DPPH method was used to evaluate the antioxidant activity of plant extracts because it is one of the most effective methods for evaluating concentrations of radical-scavenging materials by incorporation of a chain-breaking mechanism. The DPPH radical-scavenging capacity in this study was reported after 30 min reaction time for each diluted plant extract. The EC50 value, defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals in a specified

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time period, is used to measure antioxidant activity. A smaller EC50 value corresponds to a higher antioxidant activity of the plant extract.

Determination of Ascorbic acid content
Vitamin C was determined in fresh vegetable samples by dichlorophenol Indophenol dye reduction method (Smirnoff, 2000).

Statistical Analysis
Data analysis was carried out with Systat statistical program version 16 (SPSS Inc., USA). Analysis of variance was used to evaluate phytochemical characteristics and the antioxidant activity of vegetables. Means were separated with Tukey's tests. Simple linear correlation analysis was used to measure the correlation between the results obtained for % Antioxidant activity and and total phenolics and vitamin C contents

Results and discussion
Antioxidant activity
The presence of different antioxidant components in plant tissues especially fruits and vegetables make it relatively difficult to measure each antioxidant component separately. In the present study, total antioxidant activity was calculated using DPPH. The antioxidant activity ranged between 22.31% for spinach to 50.22% for Tomato (Table 1). These results corroborated with the earlier reports (Cao et al., 1996; Wang et al., 1996; Gazzani et al., 1998). Amin et al. (2002) reported that spinach contains water soluble components that possess antioxidant activity. Ademoyegun et al. (2013) reported its activity to be 29.96%. The antioxidant activity of carrot, tomato, radish, cucumber, cabbage was found to be 67, 70, 13, 34.3 and 69.3% respectively (Kaur and Kapoor 2002). The similar results for antioxidant activity of bitter gourd were found to be 82.05% (Hamissou et al., 2013) and 17.24% for bottle gourd (Mohankumar et al., 2013).

Total phenolic content
Phenolics are aromatic secondary plant metabolites associated with color, sensory qualities, nutritional and antioxidant properties of food (Jha et al., 2011). The total phenolic content varied from 33.28 mg/100g GAE for bitter gourd to 69.66 mg/100g GAE for cabbage. The higher phenolic content of some vegetables is due to their individual phenolic components. The results of phenol analysis of 8 vegetables are given in Table 1. The similar results were found for cabbage(92.5mg/100g)and radish(54.5mg/100g) by Kaur and Kapoor(2002), 13.28mg GAE /g for bitter gourd(Hamissou et al., 2013), 42.17 mg GAE/g extract for bottle gourd(Mohan et al., 2012) and 53.19 mg GAE/g for spinach(Ademoyegun et al., 2013). The total phenolic content of tomato ranged between 10-50 mg/100g according to finding by Shen et al. (2008), 58.15mg/100g GAE for carrots (Gitanjli et al., 2004) and 48mg/100g GAE for cucumber (Ghasemzadeh et al., 2013)

Vitamin C content
Vitamin C is a water soluble vitamin required in high amount, as its loss is frequent from body. It participates in reversible oxidation-reduction system and acts as an antioxidant. The antioxidant content of the vegetables under investigation varied from 4.59 mg/100g for carrot to as high as 67.21mg/100g for bitter gourd (Table 1). The findings were found close to those of Hanif et al. (2006) who found the ascorbic acid content of cabbage and spinach to be 55 and 76 mg/100g respectively. Rahman et al. (2008) found that cucumber, bottle gourd and bitter gourd have 7, 6 and 88mg/100g ascorbic acid content respectively. The vitamin C content varied from 10.44 to 22.1mg/100g among 20 varieties of tomato tested by Bajaj et al.(1988). According to Alasalvar et al. (2001), vitamin C content in carrots varied between 1.25 and 5.33 mg/100 g, being lowest in white and highest in orange varieties of carrot. Radish has 55.29mg/100g of ascorbic acid content (Jha et al. 2011).

Table 1: Antioxidant activity, total phenols and vitamin C content of the vegetables.

Vegetables	Antioxidant Activity (%)	Total Phenols (mg GAE)	Vit C content (mg/100gm)
Carrot	43.48 ± 0.91^{b}	45.48 ± 1.27^{c}	4.59 ± 0.72^{f}
Tomato	50.22 ± 0.98^{a}	43.43 ± 0.92^{cd}	17.94 ± 0.41^{e}
Radish	30.41 ± 1.07^{c}	57.65 ± 1.08^{b}	50.26 ± 0.87^{c}
Spinach	22.31 ± 0.99^{d}	66.75 ± 0.88^{a}	62.31 ± 0.73^{b}
Cabbage	43.86 ± 1.63^{b}	69.66 ± 0.93^{a}	42.19 ± 0.95^{d}
Bitter gourd	45.11 ± 1.05^{b}	33.28 ± 0.75^{e}	67.21 ± 0.81^{a}
Cucumber	48.57 ± 0.73^{a}	41.47 ± 0.87^{d}	5.84 ± 0.85^{f}
Bottle gourd	45.52 ± 0.71^{b}	40.90 ± 1.03^{d}	6.36 ± 0.74^{f}

- Values with same superscript are at par and non significantly different from each other
- Values with different superscript differ significantly ($p<0.05$)
- Values are means of triplicate batch analysis ± SE

Relationship between antioxidant activity and total phenolic and vitamin C
Antioxidants delay the oxidation process, inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions (Halliwell and Aruoma, 1991). The protective effect provided by vegetables against cancer and cardio- and cerebrovascular diseases has been attributed to antioxidant compounds (Gey, 1990).

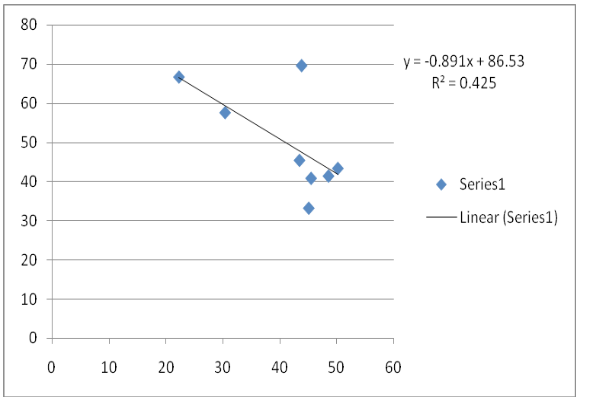

Fig.1 Relationship between total phenolic content and antioxidant activity of the vegetables

There appears to be a linear relationship between total phenolics and antioxidant activity (Djeridane et al., 2006; Kim et al., 2003). In this study, phenolics and antioxidant activity exhibited a linear distribution with correlation coefficient of R^2= 0.425(fig.1). Similarly, antioxidant activity and and vitamin C exhibited a linear relationship with correlation coefficient of R^2 =0.376(fig.2).

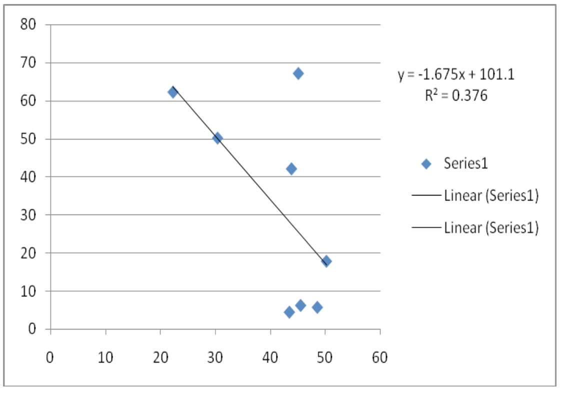

Fig.2 Relationship between Vitamin C content and antioxidant activity of the vegetables

These results agree with reports that phenolic compounds contribute significantly to antioxidant activity in other types of plants from other locations (Cai et al., 2004; Djeridane et al., 2006; Tang et al., 2004; Wong et al., 2006). Also, it can be concluded that antioxidant activity of these vegetables is not limited to phenolics and vitamin C. Activity may also come from the colored pigments like carotenoids in carrot, lycopene in tomato, chlorophyll in green leafy vegetables, other volatile oils, secondary metabolites etc which contributes rest of the antioxidant activity.

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