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Research Article

STUDIES ON *INVITRO* ANTIOXIDANT PROPERTIES OF *BRASSICA* VEGETABLES

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ABSTRACT

This study was undertaken to assess the antioxidant capacity of extract of five different vegetables namely *Brassica oleracea var. capitata* (White cabbage), *Brassica oleracea* var. *capitata.f.rubra* (Red cabbage), *Brassica oleracea var*. *botrytis* (Cauliflower), *Brassica rapa var.rapa* (Turnip), *Brassica oleracea* var. *gongylodes* (Knoll khol). The freshly prepared extract of *Brassica* (Turnip, Knol khol, Cauliflower, Red cabbage and White cabbage) vegetables was taken for the present study. The fresh vegetables were purchased from local markets and the analyses were carried. From the above said vegetables 500gms were taken and about 200ml of fresh juice was used for analysis. Red cabbage was found to have the highest antioxidant activity. Compared to other vegetables, *Brassica* vegetables have higher antioxidant potential which makes them very interesting crops from the consumer's point of view. Here, we will provide an overview of the role of Total phenols, Carotenoids, Proline, Protein present in *Brassica* vegetables in relation to antioxidant properties and human health. As conclusion *Brassica* vegetables contain bioactive substances with a potential for reducing the physiological as well as oxidative stress and this could explain the suggested cancer preventive effect of these plants as well as protective role on other major diseases.

Keywords: Antioxidants, Brassica vegetables, Protein and Total phenol.

INTRODUCTION

Antioxidants are molecules that retard or prevent the oxidation of other compounds. Antioxidants are believed to control and reduce the oxidative damage in foods by delaying or inhibiting the oxidation process caused by reactive oxygen species, thus enhancing the shelf-life and quality of the products as well as protecting the biological systems. Antioxidants like Vitamin C and Vitamin E, carotenoids, flavanoids, selenium, coenzyme Q10 and B complex must be supplied in the diet. Antioxidant enzymes, convert reactive oxygen species into nonreactive oxygen molecules (Lee *et al*., 2004). The most important antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) (Jinghua *et al*., 2007).

Brassica foods are very nutritive, providing nutrients and health-promoting phytochemicals such as vitamins, carotenoids, fiber, soluble sugars, minerals, glucosinolates and phenolic compounds. (Podsedek, 2007) and (Jahangir, 2009). In a study focused on *B. oleracea*, found a comparable antioxidant activity between redcabbage and brussels sprouts, which were higher than that for white and savoy cabbages. Authors suggest that the highest antioxidant activity of red cabbage compared to the other green and white cultivars may be due to the presence of different antioxidant components such as phenolic compounds (Podsedek et al., 2006). Asparagus and broccoli extracts, as well as their juices, showed no significant difference in total phenolics content. (Ting Sun et al., 2007). Globally, about 85% of the traditional medicines used for primary healthcare are derived from plants. The family *Brassicaceae* (*Cruciferae*) consists of 350 genera and about 3,500 species, and includes several genera like

Camelina, Crambe, Sinapis, Thlaspi and *Brassica.* The genus *Brassica* is the most important one within the tribe Brassiceae, which includes some crops and species of great worldwide economic importance such as *Brassica oleracea* L., *Brassica napus* L. and *Brassica rapa* L. The same species can be utilized for several uses according to different forms or types. The genus is categorized into oilseed, forage, condiment, and vegetable crops by using their buds, inflorescences, leaves, roots, seeds, and stems. *Brassicaceae* vegetables represent an important part of the human diet worldwide, are consumed by people all over the world and are considered important food crops in China, Japan, India, and European countries. The following are the vegetables used for our study,

S.No	Common name	Botanical name	Parts used
1	White cabbage	*Brassica oleracea* var. *capitata*	Terminal leaf buds
2	Red cabbage	*Brassica oleracea* var. *capitata.f.rubra*	Terminal leaf buds
3	Cauliflower	*Brassica oleracea var. botrytis*	Inflorescences
4	Turnip	*Brassica rapa var.rapa*	Roots
5	Knol khol	*Brassica oleracea* var. *gongylodes*	Stem

Considering the antioxidant effects of *Brassica* vegetables, the aim of the present study is to establish the protective role of *Brassica*. The present study is focussed with the following main objective to access the antioxidant properties of *Brassica* on cellular metabolism process under the following major heading, estimation of Total phenols, proline, carotenoids and protein.

MATERIALS AND METHODS

MATERIALS

The freshly prepared extract of *Brassica* (Turnip, Knol khol, Cauliflower, Red cabbage and White cabbage) vegetables was taken for the present study. The fresh vegetables were purchased from local markets and the analyses were carried out. From the above said vegetables 500gms were taken and about 200ml of fresh juice was used for analysis.

METHODS

ESTIMATION OF TOTAL PHENOLS

Phenolic compounds are slightly water-soluble and form complexes with protein by hydrogen bonding. It includes simple phenols, phenolic glycosides, flavanoids, phenolic acids or phenyl propanoids, phenolic quinines and polyphenolic compounds like lignins, melanins and tannins. They are highly susceptible to enzyme oxidation by specific phenolase. Phosphomolybdic acid and phosphotungstic acid present in the Folin-Ciocalteau reagent is reduced by phenolic hydroxyl group. Blue colour is developed in alkaline condition, which is measured at 660 nm.

ESTIMATION OF PROLINE

Proline is a basic amino acid found in high percentage in basic proteins. Free proline is said to play a role in plants under stress conditions. Though the molecular mechanism has not yet been established for the increased level of proline, one of the hypotheses refers to breakdown of proteins into amino acids and conversion to proline for storage. Many workers have reported a several-fold increase in the proline content under physiological and pathological stress conditions. Hence, the analysis of proline in plants has become routine in pathology and physiology divisions of agricultural sciences. During selective extraction with aqueous sulphosalicylic acid, proteins are precipitated as a complex. Other interfering materials are also presumably removed by absorption to the protein-sulphosalicylic acid complex. The extracted proline is made to react with ninhydrin in acidic conditions (pH 1.0) to form the chromophore (red colour) and read at 520nm.

ESTIMATION OF CAROTENES

Carotenes belong to tetraterpene group. Carotene is the pro-vitamin of Vitamin A. Carotenes are extracted in acetone and then taken up in petroleum ether. Carotene has absorption maximum at 453 nm. The molar extinction coefficient of carotenes are used for finding the concentration.

ESTIMATION OF PROTEIN BY LOWRY'S METHOD

The method developed by Lowry *et al.* is a sensitive method for the estimation of proteins in solution and gives moderately constant value and hence largely followed. Protein content of biological extracts including enzyme extract is usually assayed by this method. The color developed by the reduction of the phosphomolybdic- phosphotungstic acid components in the Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the color developed by the Biuret reaction on the protein with alkaline cupric tartarate is measured in Lowry's method.

EXPERIMENTAL DESIGN
The experiments were conducted at laboratory level and the analysis was a completely randomized design. Three replications were taken for the experiment.

STATISTICAL ANALYSIS
Statistical analysis was conducted using AGRES software.

RESULTS AND DISCUSSION
Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. A free radical attack on the membrane usually damages a cell to the point that it must be removed by the immune system. If, free radical formation and attack are not controlled within the muscle during exercise, a large quantity of muscle could easily be damaged. Protection from damage occurs through the action of excellent defense system consisting of antioxidants, some endogenously produced and some provided through dietary intake. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, and also by acting as oxygen scavengers. However, antioxidant supplement may be used to the help the human body reduce oxidative damage.

Estimation of Protein
Proteins contain amino acids linked by peptide bonds. Differences in the number of amino acids result in different types of proteins. There are more than three methods for the estimation of proteins. In table1 the data on protein content statistically differed at all the treatments. Among the five treatments T5 (red cabbage) showed highest protein content of 53.85mg/g, which was followed by T4 (white cabbage) and T3 (cauliflower) with the value of 43.87mg/g and 30.84mg/g respectively. The lowest value of 20.69 mg/g was recorded in T2 (knolkhol) treatment.

Estimation of Total phenols
Phenolic compounds are slightly water-soluble and form complexes with protein by hydrogen bonding. They are highly susceptible to enzyme oxidation by specific phenolase.The experiment results of total phenolics content were not significantly differed among the five treatments.

Estimation of Proline
Proline is a basic amino acid found in high percentage in basic proteins. Free proline is said to play a role in plants under stress conditions. The data on proline content were analysed statiscally and significant differences were noticed at all the treatments. The highest proline content of 3.45 μg/g was shown in T5 (red cabbage).This treatment was followed by T4(white cabbage) and T3(cauliflower) with the value of 3.44 μg/g and 3.40 μg/g respectively. The least value of 2.68 μg/g of proline content were recorded in knoll khol.
Most of the antioxidant potential in plants is due to the redox properties of Phenolic compounds which allow them to scavenge reactive oxygen spieces (ROS) and reform their resistance to environmental stresses (Hodges 2003). Generally white cabbage one of the most popular Brassica vegetables, is the poorest source of vitamin C among the group of crops studied (Podsedek 2007). Carotenoids which occur in the chloroplast membranes also help prevent oxidative damage from ROS (Hodges, 2003). Antioxidants are very important to inhibit free radical reactions, and may therefore protect cells against oxidative damage (Kurilich et al. 2002, Soto-Zamora et al. 2005). Increase in proteins and nucleic acids prior to sprouting has been observed in many vegetables (Macdonald and Osborne, 1988). Protein synthesis is required for sprouting, and nucleic acid synthesis for sprout elongation (Madison and Rappaport, 1968; Alam et al., 1994).

SUMMARY AND CONCLUSION
Based on the result of the present study, the extract of *Brassica* vegetables (Red cabbage), have shown to possess remarkable antioxidant activities. It can be concluded that vegetables belonging to the family *Brassicaceae* are rich food sources of natural antioxidants and essential nutrients (vitamins, phenolics, minerals, fibre, *etc.*) and the vegetables of this family possess a high potential to manage against oxidative stress and, thus, act as strong anticancerous as well as antidegenerative foods. Therefore, for improving the quality and production of these vegetables, breeding programs are necessary in order to enhance the antioxidant potential of our daily food supply. Therefore, the potential of these phytochemical compounds for the maintenance of health and protection against heart disease and cancer is also raising interest among scientists and food manufacturers as consumers move towards functional foods with specific health effects. An interesting aspect for future research is to clarify the genotype × environmental interactions on the different composition in plants. By combining the knowledge gained from the studies concerning the effects of different antioxidant compounds on human health, it might be possible to produce plants

with even better health properties. The vitamin C and total phenolic contents seemed to play an important role in antioxidant capacity of some vegetable juices. (Prasan Swatsitang and Ruthaichanok Wonginyoo 2008). The low Phenolic content was represented by lotus stem,yam, French beans, cabbage, broad beans, cauliflower, round melon and peas(Charanjit Kaur & Harish C. Kapoor, 2001). The results thus conform that anthocyanin rich vegetables possess strong anti-oxidant activity (Wang et al., 1997; Velioglu et al., 1998). Epidemiological data as well as in vitro studies strongly suggest that foods containing phytochemicals with anti-oxidation potential have strong protective effects against major disease risks including cancer and cardiovascular diseases (Steinberg, 1991; Block et al., 1992; Ames et al., 1993; Hertog et al., 1993Byers & Guerrero, 1995; Knekt et al., 1997; Elliot, 1999; Kaur & Kapoor, 2001). The protective action of fruits and vegetables has been attributed to the presence of anti-oxidants, especially anti-oxidant vitamins including ascorbic acid, a-tocopherol and beta carotene (Gey et al., 1991; Willet, 1994; Kalt & Kushad, 2000; Prior & Cao, 2000). Mushroom, white cabbage and cauliflower (Gazzani et al., 1998), garlic, broccoli, kidney and pinto beans (Vinson et al., 1998), beans, beet and corn (Kahkonen et al., 1999) have been reported to have high anti-oxidant activity.

Table 1: Effec of *invitro* antioxidant properties of *Brassica* vegetables

Treatments	Protein (mg/g)	Total Phenolics (mg/g)	Proline (mg/g)	Carotenoids (mg/g)
Turnip	21.90	5.640	2.70	2.04
Knolkhol	20.69	5.666	2.68	0.79
Cauliflower	30.04	5.680	3.40	2.62
White cabbage	43.87	5.691	3.44	4.33
Red cabbage	53.85	5.714	3.45	4.35
Mean	**34.0700**	**5678.2000**	**3.1340**	**2.8244**
SEd	**0.3333**	**NS**	**0.1057**	**0.1684**
CD (0.05)	**0.7066**	**NS**	**0.2241**	**0.3571**

(All values are means of three dependent determinations, n=3, analysed in triplicate)

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