

**Ascorbic acid, Carotenoids, Total Phenolic content and Antioxidant activity of various genotypes of *Brassica Oleracea acephala***

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**Abstract**

The disease preventing potential of naturally occurring substances in the diets is a major area of scientific interest. Recently antioxidants and secondary metabolites have attracted a great deal of attention for their effect in preventing disease due to oxidative stress, which leads to degeneration of cell membranes and many pathological diseases including cancer. In the current study, green leafy vegetable extracts of six genotypes of kale (*Brassica oleracea acephala*) were evaluated for total phenol, carotenoids, Vitamin C content and antioxidant activities. Ascorbic acid ranged from 142 mg per 100g in Wappal hakh to 164 mg per 100-g fr wt in knol khol. Wild genotypes Wappal and Pumb, had significantly high phenolic content (285 and 227 mg per 100 g fr wt) and possessed highest antioxidant activities (840 and 780 umol FRAP petr g fr wt) than cultivated genotypes. A positive and strong correlation ( $R^2=0.807$ ) between total phenolic content and antioxidant activity suggests that kale especially "Wappal and pumb" have enormous potential to enhance the antioxidant potential of our daily food supply.

**Key words:** kale; *Brassica Oleracea* ; phenols; antioxidant activity

**Introduction**

Recently special attention has been paid towards edible plants, especially those that are rich in secondary metabolites (frequently called phytochemicals) and there is now increasing interest in antioxidant activity of such phytochemicals present in the diet. Antioxidants are important in prevention of pollution damage of plants, disease prevention in both plants and animals and play a very important role in the body defense system and reactive oxygen species (1). These naturally occurring compounds present in fruits and vegetables act by scavenging harmful free radicals, which are also implicated in the most common cancers and other degenerative diseases including poor brain function etc (2). The mechanism involved for their antidegenerative and anticancerous activity besides retarding oxidative degradation, lies in the fact that such compounds are responsible for induction of enzymes that detoxify carcinogens and also block the formation of cancer by deactivating at least 30 types agents that may cause cancer (3). In contrast, the synthetic forms of these compounds have been seen to have entirely different role to play with most of them possessing toxic and carcinogenic effects (4). Therefore, the potential of these photochemical constituents of plant material for the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists and food manufactures as consumers move towards functional foods with specific health effects (5).

Recent reports suggest that cruciferous vegetables act as good source of natural antioxidants due to high levels of carotenoids, tocopherols, and ascorbic acid (6) and convincing epidemiological evidence shows these compounds may help to protect the human body against damage by reactive oxygen and nitrogen species. Foremost are their antioxidative effects, manifested by the ability to scavenge free radicals (7) or to prevent oxidation of low-density lipoproteins (8). However, recent research indicates that in addition to carotenoids, tocopherols, and ascorbic acid most of the antioxidative effect in plants is mainly due to presence of phenolic compounds, which have not yet been characterized very well in vegetables (9). Phenolic compounds are secondary metabolites, which have been associated with flavour and colour characteristics of fruits and vegetables and are gaining considerable attention because of their potent antioxidant and health promoting properties (10). Thus, growing evidence of their health benefits warrants their presence in varieties of fruits and vegetables and their quantification with special reference to cruciferous vegetables. Edible green leaves of *Brassica oleracea acephala* is commonly known as Kale or "Saag" in most parts of India. Previously kale has been reported to possess the highest antioxidant activity, among 32 different vegetables studied (11). It is among the widely consumed vegetables and provides significant proportion of antioxidants, carotenoids, calcium, and vitamin C. However to our knowledge there has been no systemic study on

the antioxidant constituents of Kale. Therefore, the aim of the present study was to evaluate some important Kale varieties new potential source of natural antioxidants and to develop a correlation between total phenolic content and antioxidant activity.

## Materials and method

**Chemicals** -All chemicals and reagents used in the study were of analytical grade and mostly purchased from Sigma chemicals (India).

**Sample collection and preparation**- A total of 48 freshly harvested green leafy cruciferous vegetable samples belonging to six different taxa of genus *Brassica oleracea* were procured from local market as well as from various fields of SKUAST (K), Shalimar, Srinagar, Kashmir at fresh market stage. Eight samples of each genotype viz Khanyari, Kawdari, GM Dari varieties (*Brassica Oleracea* var. *acephala*), knol kohl (*Brassica Oleracea* var. *gongilodes*) and two wild type kale varieties viz Wappal and Pumb (*Rheum emodi*) were included in the study. Samples were placed in polyethylene bags and transported under refrigerated conditions to Division of Post Harvest Technology, SKUAST (K), Shalimar Campus, Srinagar within an hour. Samples received were washed under running tap water to remove the adhering dirt and then stored under  $-80^{\circ}\text{C}$  until analyzed. Analysis was completed within a month of sample collection. All measurements were conducted in triplicates.

**Determination of ascorbic acid** - Ascorbic acid was quantitatively determined according to 2, 6-dichlorophenolindophenol dye method (12). The ascorbic acid of fresh samples 10g was extracted by grinding in a suitable medium with a small amount of sand and using 3% metaphosphoric acid (v/v) as a protective agent. The extract was made up to a volume of 100ml mixed and centrifuged at 3000g for 15 min at room temperature. Ten milliliters was titrated against standard 2, 6-dichlorophenolindophenol dye, which was already standardized against standard ascorbic acid. Results were expressed as  $\text{mg}100\text{g}^{-1}$  on fresh weight (fw) basis.

**Determination of Total carotenoids** - Total carotenoids  $\text{mg}100\text{g}^{-1}$  were determined by a modified method of Ranganna (12) using acetone and petroleum ether as extracting solvents and measuring the absorbance at 450nm. Preparation of sample extract - Two grams of sample was thoroughly crushed and homogenized in mortar pestle with 10 ml of 80% ethanol. The extract was centrifuged at 10000g for 15 min at  $4^{\circ}\text{C}$ . The pellet following was centrifuged and the resulting supernatant was combined with initial extract. Triplicate supernatant extractions were made for each sample. The ethanolic extract volume was reduced in the evaporator to 20ml. The same extracts were used for the estimation of total phenolics, and FRAP assay.

**Total phenolic compound analysis** - Total soluble phenols in ethanol extracts were determined with Folin-Ciocalteu reagent using catechol as a standard (13). Results were expressed as  $\text{mg}100\text{g}^{-1}$  wet weight catechol as equivalents.

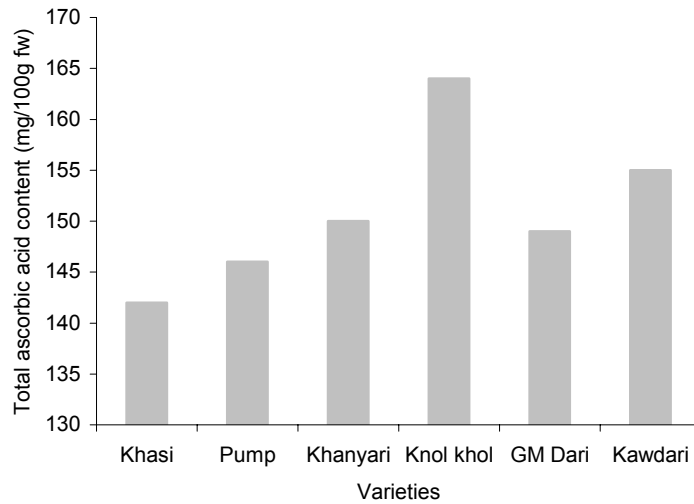
**Determination of total antioxidant activity** - Antioxidant activity was measured using Ferric reducing antioxidant power (FRAP) assay. Reagents and equipment used were as described elsewhere (14). FRAP assay was determined based on the reduction of  $\text{Fe}^{3+}$ -TPTZ to a blue coloured  $\text{Fe}^{2+}$  TPTZ (14). The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ and 20 mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in a ratio of 10:1:1, at to  $37^{\circ}\text{C}$ . FRAP reagent (3 ml) was pipetted into test tubes. A total of 100  $\mu\text{l}$  of sample and 300  $\mu\text{l}$  of distilled water was then added to the same test tubes, and incubated at  $37^{\circ}\text{C}$  for 4 min. Each sample was run in triplicate. Absorbance was measured at 593 nm. FRAP value was calculated according to the equation (14)

$$\text{FRAP (mM)} = 0-4 \text{ min of } \Delta A_{593 \text{ nm}} \text{ of test sample} / 0-4 \text{ min of } \Delta A_{593 \text{ nm}} \text{ of standard sample} \times [\text{standard}] \text{ mM}$$

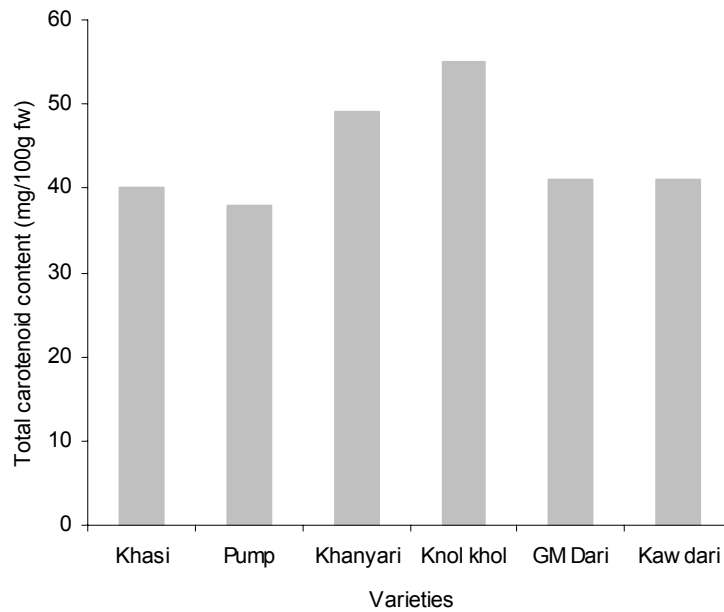
**Statistical analysis** - Three replicates of each sample were used for statistical analysis. Analysis of the data was performed on the original data by one-way analysis of variance (ANOVA) or regression analysis. Differences at  $P < 0.05$  were considered significant. The data was also analyzed with the help of Fisher least differential technique through S-PLUS (2000) Software package.

## Results

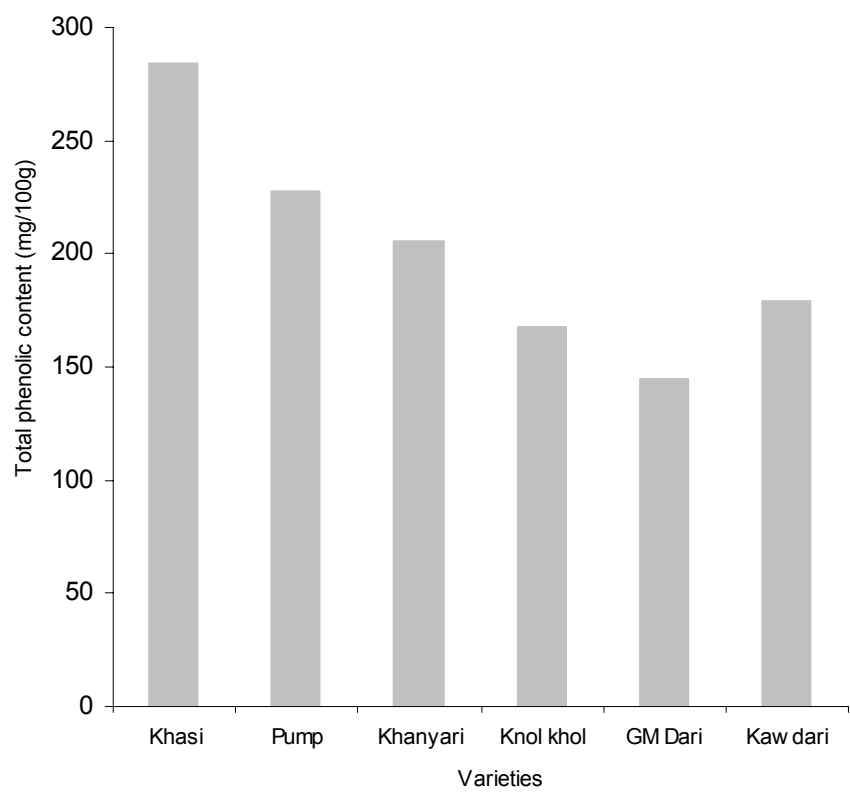
From the current investigations, no significant variation was observed in ascorbic acid content in kale genotypes, which ranged from 142 for Pumb to 164 for Knol khol  $\text{mg}100 \text{ g}^{-1}$  of fw (Fig 1). Similarly, in all the kale genotypes except Knol khol, no significant variation was observed for total carotenoid content that ranged from 38 for Pumb to



**Fig 1 Total ascorbic acid content (mg/100gfw) in six taxa of genus *Brassica Oleracea acephala***

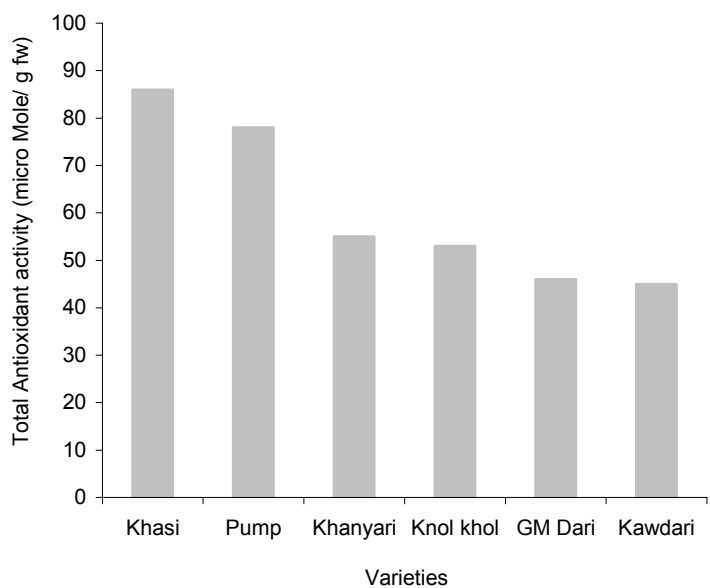


**Fig 2 Total carotenoid content (mg/100g fw) in six taxa of genus *Brassica Oleracea acephala***



**Fig 3 Total phenolic content (mg/100g fw) in six taxa of genus *Brassica Oleracea acephala***

In the current investigation, kale varieties were found to have variable and high phenolic content that ranged from



**Fig 4 Total antioxidant activity (micro mole/g fw) in six taxa of genus *Brassica Oleracea acephala***

145-284mg/100g fresh weight (Fig 3). Khasi and Pumb demonstrated 25% and 23% more antioxidant activity as well as higher phenolic contents than the other vegetable varieties selected for the present study (Fig 4). Significant differences were observed in antioxidant activity of kale varieties except GM Dari and Kawdari varieties. The correlation developed from current study between total antioxidant activity (Y) and total phenolic content (X) of Kashmiri kale varieties had a correlation coefficient of  $R^2=0.807$ . This result suggests that more than 79% of the antioxidant activity of selected kale varieties results from the contribution of Phenolic compounds (Table 1)

## Discussion

Plant breeders and food producers are increasingly identifying specific genotypes and varieties of fruits and vegetables rich in functional ingredients comprising of nutritive and non-nutritive antioxidants. Many studies have demonstrated that cruciferous vegetables contain a wide array of phytochemicals. Among these, Kale is a very popular vegetable with rich and poor alike and has been reported as a good source of lutein, zeaxanthin (carotenoid), Ca and glucosinolates (15). However most of the kale species and cultivars have not been analyzed for such important compounds and thus the comparative study of different varieties of Kale are desirable both from academic as well consumer point of view. As the samples selected for the current study were different from each other, therefore in order to compare their antioxidants and total antioxidant activities on equal basis, eight samples of each genotype were collected from diverse locations. It was clear that all the varieties were found to be excellent source of vitamin C satisfying more than 200% of RDA for Vitamin C. As all of the vitamin C rich varieties are mostly grown in open fields under direct sunlight, which support the previous findings suggesting that light intensity is involved in increasing the concentrations of ascorbic acid and glucose, the precursor to ascorbic acid in plants. High ascorbic acid in Kale (*Brassica oleracea*) thus puts it in the category of other potential vegetables high in ascorbic acid such as capsicum, bitter melon and broccoli (16, 17).

Supporting the previous reports the present study also shows kale as a good source of both Vitamin C and Carotenoids (18). Past research has been restricted only to evaluation of carotenoid, tocopherol and ascorbate content within and between subspecies of *Brassica oleracea* family, and kale has been found to have the highest levels of these three compounds followed by broccoli, brussels sprouts, cabbage and cauliflower with intermediate levels (19). However, recent investigations have shown that many phytochemicals e.g phenols, are considerably more potent antioxidants than vitamin C and vitamin E because of their structure and efficient scavengers of peroxy radicals (20). Moreover, the action of phenolic compounds can be related to their capacity to reduce and chelate ferric ions which catalyses lipid peroxidation (21). It is clear that the total free phenolic content fell in range of the content (117- 430 mg/100g) found in apple varieties (22). It was note worthy that Wappal variety recorded significantly highest value (285mg/100g fw) of total phenolic content among the six vegetables, followed by Pumb (227mg/100g fw), Khanyari (206mg/100g fw), Kawdari (179mg/100g fw), Knol khol (169mg/100g fw) and G.M.Dari (145mg/100g fw) respectively. From the statistical analysis, it was evident that there was a least variation in the total phenolic content of Knol khol and Kawdari samples, though each belonged to entirely different group. The values are typical of extractable phenolic concentration seen in other fruits and vegetables, which can vary from 2-500 mg/kg (23)

There is an increasing interest in the measurement of total antioxidant activity rather than simply the contents of antioxidant. There are several methods used to measure antioxidant activity. The FRAP ferric reducing antioxidant power assay of Benzie and Strain (14) is one of the useful assays for examining the interactions among various antioxidants by taking into account their oxidation-reduction potential. The FRAP assay measures the antioxidant effect of any substance in the reaction medium as its reducing ability and is a convenient and reproducible assay. Recently, it has been extensively used to study the antioxidant activity of hydrophilic extracts of fruits and vegetables. The antioxidant activity in Kale varieties as determined by FRAP assay was found to be significantly higher than other vegetables reported in literature (1). Statistical analysis of our data revealed significant differences in antioxidant activity of kale varieties except GM Dari and Kawdari varieties, all other demonstrated different antioxidant activities. Wappal variety recorded significantly highest value of antioxidant activity (840mM  $g^{-1}fw$ ) followed by Pumb (780 mM  $g^{-1}fw$ ), Khanyari (550 mM  $g^{-1}fw$ ), Knol khol (530 mM  $g^{-1}fw$ ), G.M.Dari (460 mM  $g^{-1}fw$ ), and Kawdari (450 mM  $g^{-1}fw$ ), respectively (Figure 4). It was evident from the statistical analysis that there is no significant variation in the antioxidant activity between GM Dari and Kawdari, Khanyari and Knol khol genotypes although GM Dari and Kawdari belonged to same group of cole crop kale. It is suggested that high antioxidant activity in the different varieties of Kale is mainly attributed to its high phenolic content. However as evident from this study, although varying in their respective antioxidant activities there is a least difference in phenolic content of Kawdari and knol kohl. The most probable reason for such results may be due to variation in the type of individual phenolic compounds possessing different antioxidant activities and role of inherent genetics, which governs the ability of cultivars to respond to biotic and abiotic stresses.

In the present study, an attempt was made to establish co-relationship between total Vitamin C content or total carotenoids or total phenolic content and antioxidant activity (Table 1). The results obtained for total Vitamin C content or total carotenoids and antioxidant activity were not statically significant and thus corroborated with the

earlier reports where total antioxidant activity of different fruits was actually found to be very poorly correlated with their vitamin C content (Cao, Sofic, & Prior, 1996).

**Table 1. Analysis of variance for phenolic content, antioxidant activity (AOX), ascorbic acid and total carotenoid content in kale varieties**

Source	d.f.	Total Phenols		Total Antioxidant activity		Total Ascorbic acid		Total carotenoid	
		MSS	F value	MSS	F value	MSS	F value	MSS	F value
Genotype	5	7407.9	261.88	891.52	78.67	172.32	22.47	631.34	126.26
Error	12	28.28	-	11.33	-	7.67	-	80.33	-

\*\* Significant at 1% level; MSS = Mean sum square; d.f. = Degree of freedom

In this context, recent studies have shown that phenols and polyphenols are stronger antioxidants than the vitamin antioxidant (19). The correlation developed from current study between total antioxidant activity (Y) and total phenolic content (X) of Kashmiri kale varieties had a correlation coefficient of  $R^2=0.807$ . This result suggests that more than 79% of the antioxidant activity of selected kale varieties results from the contribution of Phenolic compounds as reported earlier for Iranian *Ocimum* accessions (5). Thus from the results of simple regression analysis (SRA) it was evident that there is a significant correlation between total phenolic content and antioxidant activity. Moreover, for unit increase in phenolic content as evident from the SRA there is an increase of 0.308 in antioxidant activity. Interestingly in this study two uncommon varieties of vegetables viz Khasi and Pumb demonstrated 25% and 23% more antioxidant activity as well as higher phenolic contents than the other vegetable varieties selected for the present study (Fig 4). As some reports that have shown a low positive correlation between antioxidant activity and total phenolic content in different cultivars of vegetable (24). However, the present study shows that the total phenolic content of a crop bears strong correlation to its anti-oxidant property and could thus prove a reliable assay to identify crops with anticancerous property.

In order to cross check the above results, multiple comparison analysis (using Fisher least significant difference method) between all the six selected vegetables with respect to either total phenol content or total antioxidant activity or total vitamin C content and or total carotenoids was performed and it was observed that they differ significantly ( $p$ -value < .05) with respect to each other (Data not shown). The results obtained showed highest variation in terms of phenolic content in GM Dari and Wappal and similarly in antioxidant activity between Kawardari and khasi, in vitamin C Wappal and Knol khol and for carotenoids Knol khol and Pumb showed the maximum variation. Such results demonstrated that the three genotypes of same species *Brassica Oleraceae* var *acephala* (Kale) showed large variability with each other in both total phenolics and antioxidant activities. The most probable reason for such results may be due to the influence of different variety, harvest season, and mostly attributed to genetic and environmental conditions in which they were grown, as all these factors have a direct effect on presence of individual antioxidants (25). It was noteworthy that in almost all the varieties total phenolic content was always on higher side.

Although antioxidant efficacy of antioxidants varies considerably with genotype and maturity and without knowing the specific cultivation information for each vegetable as well as collecting the samples from different places, from our results one strong conclusion can be drawn, that the antioxidant activity of most of these vegetables is highly dependent on their total phenolic content (Table 1).

It can be concluded from the present study that green leafy vegetables belonging to *Brassica Oleracea* is promising group with rich antioxidant composition (ascorbic acid, carotenoids and phenolic content) and these green leafy vegetables possess higher potential to cope against oxidative stress and thus act as strong anticancerous as well as antidegenerative foods.. Therefore, for improving the quality and production of these vegetables further, the most import factor required is change in variety mix and supply of quality material aid to speed up breeding programs for the development of new germplasm in order to enhance the antioxidant potential of our daily food supply.

However, it is not appropriate to rely fully on only the methods used in this study due to there own limitations. Therefore, further studies and different approaches are needed to elucidate these potent natural antioxidants mostly to investigate the type of individual phenolic compounds involved in antioxidant activi

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