Polyphenolic Estimation and Antioxidant Activity of Some Vegetables of J &K India-A Correlation Study

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Abstract

The total antioxidant activities of seven different vegetables were measured by FRAP method together with their estimation of total phenolic content by Folin-Ciocalteau method. Coriander and tomato belonged to high anti-oxidant activity group. In the medium group; capsicum, carrot and onion scored anti-oxidant activity of 5.65, 3.46 and 4.24 µ M Fe2+ /g FW respectively. Cucumber and radish were identified as vegetables of low anti-oxidant activity with a score of 1.12 and 1.96µ M Fe2+ /g FW respectively. Amongst the vegetables under study, three groups of vegetables could be identified which contained high, medium and low phenols. Coriander, tomato and capsicum were found to have high phenolic content ranging from 16.35 to 19.75 mg CE/100g FW. Carrot, onion and radish belonged to medium group with phenolic content ranging from 13.22 to 13.39 mg CE/100g FW. Cucumber was a solitary vegetable where total phenols were found to have as low as 10.82 mg CE/100g FW. The relationship which emerged on the basis of correlation between anti-oxidant activity and total phenolic content was found to be positive and strong. On the basis of regression analysis, it was found that independent variable, total phenols and dependent variable, anti-oxidant activity is linearly related.

Key words: Antioxidants, Polyphenols, Correlation, Regression.

I. Introduction

Agro-climatic regions of J&K, India are bestowed with diverse environmental conditions conducive for growth of numerous vegetables. With the increase in income and improvement in lifestyle, per capita vegetable consumption is increasing steadily with an associated increase in demand. Vegetables are being consumed both in cooked and raw form. The protective action of fruits and vegetables has been attributed to the presence of antioxidants. Numerous studies have conclusively indicated that majority of antioxidant activity flows from phenolic compounds (Kaur and Kapoor, 2002). Phenols are present in abundant quantities in plants. Lignin is a good example of ubiquity of phenolic compounds in plant kingdom. It is now evident that biosynthesis and productivity of phenolic compounds are regulated by variety of external and biological factors (Salvini et al., 1998; Navarro et al, 2006). Chemically, plant phenolic compounds are extremely heterogeneous and may range from simple monomers to very large polymers. According to Loomis and Battaile (1966) phenolic compounds belong to either one of two biochemical groups: (1) the flavanoid compounds (including condensed tannins), or (2) the group of compounds where the 6-carbon ring has a 1 or 3 carbon side chain and their derivatives, e.g. caffeic acid, gallic acid, hydrolysable tannins, tyrosine and lignin. Epidemiological studies have shown that consumption of food rich in phenolics can slow the progression of various debilitating diseases (Landbo & Mayor, 2001). Therefore, mostly, the current focus is on the anti-oxidant action of phenolics. The anti-oxidant activity of phenolics is mainly because of their redox properties. Oxygen derived free radicals have played a major role in the pathogenesis of a number of degenerative diseases. These free radical molecules are released during the normal metabolic process of oxidation and thus can lead to cancerous changes, accelerate the aging process etc. Recently phytochemicals in fruits and vegetables have attracted a great deal of attention mainly concentrated on their role in preventing diseases caused as a result of oxidative stress. Oxidative stress, which releases free oxygen radicals in the body, has been implicated in a number of disorders including cardiovascular malfunction, cataracts, cancers, rheumatism and many other auto-immune diseases besides ageing and contributes to heart disease and degenerative diseases such as arthritis (Cross, 1987). Antioxidants that inhibit enzyme-catalyzed
oxidation include agents that bind free oxygen (*i.e.*, reducing agents), such as ascorbic acid (Vitamin C) and agents that inactivate the enzymes, such as citric acid and sulfites which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Rice-Evans *et. al.*, 1997).

Before the turn of current century, special attention has also been accorded to edible vegetables that are rich in plant secondary metabolites responsible for induction of detoxifying enzymes (e.g. glutathione-S-transferase, quinone reductase, and epoxide hydrolase), which inactivate reactive carcinogens by destroying their reactive centers or by conjugating them with endogenous ligands, thereby triggering their elimination from the body (Talalay, 1992).

Furheen Amin, S. M. Wani, Adil Gani, F. A. Masoodi / International Journal of Engineering Research and Applications (IJERA) ISSN: 2248-9622 www.ijera.com
Vol. 3, Issue 2, March -April 2013, pp.595-603

Among crucifers (e.g. broccoli, cabbage, cauliflower, etc.) this inducer activity is principally because of highly reactive thiocyanates. The anti-carcinogenic activity of isothiocyanate, a sulphoraphane present in broccoli, was demonstrated in a rat mammary tumor model (Zhang et al., 1994). Glucosinolates, which are thought to be anticarcinogenic, are very stable precursors of isothiocyanates, typically present in crucifers at very high levels and their hydrolysis by myrosinase is a prerequisite for observed biological activity (Williamson et al., 1998; Fahey & Stephenson, 1999). Antioxidant compounds in food play an important role as a health protecting factor. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative diseases. Antioxidant compounds like phenolic acids, Polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. The study has been carried out to determine values of anti-oxidant activities and total phenolics of selected vegetables and to work out relationship between these.

II. Materials and methods

2.1 Chemicals

The chemicals used for estimation of total polyphenols and antioxidant activity were obtained from Hi-media and Merck.

2.2 Sample preparation

Seven vegetables were procured fresh from the local vegetable markets of Srinagar. These were cleaned, washed and chopped into small pieces. Onions having dead and dry skins were processed after removal of their skins, carrot and raddish were processed after removal of their leaves, capsicums were processed after complete removal of their placenta and seeds, tomatoes were processed after removal of their calyx. Only edible portions of vegetables were weighed and homogenized using a blender for 3 minutes at high speed.

2.3 Determination of total phenolic content

Total phenolics were determined according to Folin-Ciocalteau procedure (Singleton & Rossi, 1965) with slight modifications. Phenolic substances all absorb UV light; this property can be used to determine phenolics by spectral analysis. Samples (2g) were homogenized in 80% aqueous ethanol at room temperature and centrifuged in cold at 10,000 g for 15 minutes and the supernatant was saved. The residue was re-extracted twice with 80% ethanol and supernatant were pooled out into evaporating dishes and evaporated to dryness in a water bath at temperature of 40oC. Residue was dissolved in 5mL of distilled water. One-hundred □ L of this extract was diluted to 3mL with water and 0.5mL of Folin-Ciocalteau reagent was added. After 3 minutes, 2mL of 20% of sodium carbonate was added and the contents were mixed thoroughly. The colour was developed and absorbance measured at 650nm in a UV spectrometer after 60 minutes using catechol as a standard. The results were expressed as mg catechol/100g of fresh weight material.

2.4 Determination of antioxidant activity (AOX) by Ferric reducing antioxidant power (FRAP)

Total antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1999). FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant system. The principle underlying the assay is that at low pH, reduction of ferric tripyridyl triazine (Fe^{3+} TPTZ) complex to ferrous form Fe^{2+} (which has an intense blue colour) can be monitored by measuring the change in absorption at 593nm. The reaction is non specific, in that any half reaction that has lower redox potential, under reaction conditions, than that of ferric ferrous half reaction, will drive the ferrous (Fe^{3+} to Fe^{2+}) ion formation. The change in absorbance is therefore, directly related to the combined or "total" reducing power of the electron donating antioxidants present in the reaction mixture. FRAP reagent consisted of 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) in 40 mM HCl, 20 mM ferric chloride and 300 mM sodium acetate buffer (pH 3.6) in the ratio of 1:1:10 (v/v/v). A 100 □ L extract was added to 3 ml of FRAP reagent and mixed thoroughly. After standing at ambient temperature (20 C) for 4 min, absorbance at 593 nm was noted against reagent blank. Value was expressed in µM FRAP/g fresh weight material. FRAP value of sample was measured in terms of µM Fe^{2+}/g fresh weight material as per the calculations indicted as follows:

FRAP value of Sample (µM) = (Change in absorbance of sample from 0 to 4 minute / Change in absorbance of standard from 0 to 4 minute) X FRAP value of standard.

Procedure for preparation of Blank, Standard and Test solution.

Solutions	Blank	Standard	Test
Sample			100µl
Standard(Ascorbic acid)		100µl	
Working FRAP soln.	3000 µl	3000 µl	3000 µl

2.5 Statistical analysis

Data generated in course of the present study was examined in terms of coefficient of variation (CV) and subjected to correlation and regression analysis and least square fit [Steel and Torrie, 1981].

III. Results and discussion

3.1 Phenolic content and anti-oxidant activities

Folin-Ciocalteau's method allows the estimation of all flavonoids, anthocyanins and all nonflavonoids phenolic compounds, that is, of all the phenolics present in the samples. Table 1 and Fig 1 report the amount of total phenols quantified and antioxidant activity in each vegetable. Coriander scored highest value in terms of total phenols which was closely followed by tomato and capsicum, whereas cucumber scored the least. Amongst the vegetables under study, three groups of vegetables could be identified which contained high, medium and low polyphenols. Coriander and tomato were found to have high phenolic content of 19.72 and 17.34 mg CE/100g FW respectively. Carrot, onion and radish belonged to medium group with phenolic content ranging from 13.22 to 13.77 mg CE/100g FW. Cucumber was a solitary vegetable where total phenols were found to have as low as 10.82 mg CE/100g FW. Typical phenols that possess anti-oxidant activity are known to be phenolic acids and flavonoids, the major classes of phenolic compounds occurring widely in the plant kingdom especially in fruits and vegetables (Wojdylo et al, 2007). Analogous to total phenols, three groups of vegetables having high, medium and low anti-oxidant activity were identified. Coriander and tomato belonged to high antioxidant activity group. Coriander, in correspondence to its highest total phenols, scored maximum value of 14.22 µM Fe^{2+}/g FW which was closely followed by tomato with anti-oxidant activity score of 11.25 µM Fe^{2+}/g FW. In the medium group; capsicum, carrot and onion scored anti-oxidant activity of 5.65, 3.46 and 4.24 µM Fe^{2+}/g FW respectively. Cucumber and radish were identified as vegetables of low anti-oxidant activity with a score of 1.12 and 1.96 µM Fe^{2+}/g FW respectively. The amount of total phenols and anti-oxidants, are, by and large, with certain exceptions, within the range of values reported by several workers (Chu et al, 2000; Lee, 1992; Anese et al;1999; Pellegrin et al, 2003) but they are lower than those reported by (Kaur and Kapoor, 2001, 2002; Kahkonen et al, 1999; Prior and Cao, 2000). Environmental factors such as differences in light, season, climate, and temperature conditions on the one hand and production, optimal extraction factors and genotype on the other may have contributed to the differences in total phenols and anti-oxidant activities of various vegetables (Kalt, 2005; Rababah et al, 2010). Variability measured in terms of coefficient of variation (CV) was found to be in tune with minimum and maximum range of values in total phenols and anti-oxidant activity as CV value was numerically higher in the latter (75.99) than in the former (18.60).

3.2 Relationship between anti-oxidant activity and total phenols

Correlation worked out on the basis of relation between mean values, over replicates, of total phenols and anti-oxidants in various vegetables turned out to be strong with a Correlation coefficient value of 0.9519 which was statistically highly significant ($p < 0.01$). This correlation is represented in the scatter diagram in Fig. 2. The strong correlation regarding the intensity and the level of association between two data series of values obtained for FRAP and the polyphenols content, can be explained by the reducing character of polyphenols, that is higher than those of vitamin C (Luminita et al,2006). Since correlation was strong and highly significant, a regression analysis was performed. On the basis of regression analysis (Fig 3), it was found that independent variable x [total phenols] and dependent variable y [anti-oxidant activity] are linearly related. Regression analysis revealed very high coefficient of determination value ($R2= 0.9062$) indicating thereby high prediction power of the first order linear regression equation as far as determination of anti-oxidant activity on the basis of total phenol is concerned. These results, with regard numerous vegetables and plant material, are in agreement with various workers (Pellegrin et al, 2003; Jacobo-Velazquez and Cisneros-Zevallos, 2009). Because plant foods contain many different classes and types of antioxidants, knowledge of their total antioxidant capacity (TAC), which is the cumulative capacity of food components to scavenge free radicals, would be useful for epidemiologic purposes.

IV. Conclusion

Epidemiologic studies have demonstrated an inverse association between consumption of fruits and vegetables and morbidity and mortality from degenerative diseases. The antioxidant content of fruits and vegetables may contribute to the protection they offer from disease. Because plant foods contain many different classes and types of antioxidants, knowledge of their total antioxidant capacity (TAC), which is the cumulative capacity of food components to scavenge free radicals, would be useful for epidemiologic purposes. To accomplish this, antioxidant capacity of selected vegetables was determined which will result in a complete and versatile database of total antioxidant capacity of different choices or combination of food items. The relationship which emerged between anti-oxidant activity and total phenolic content highlights the potentiality of the phenols as a descriptor of the diet.

Acknowledgements

The authors wish to thank gratefully Department of Biotechnology, Govt. of India for their financial support.

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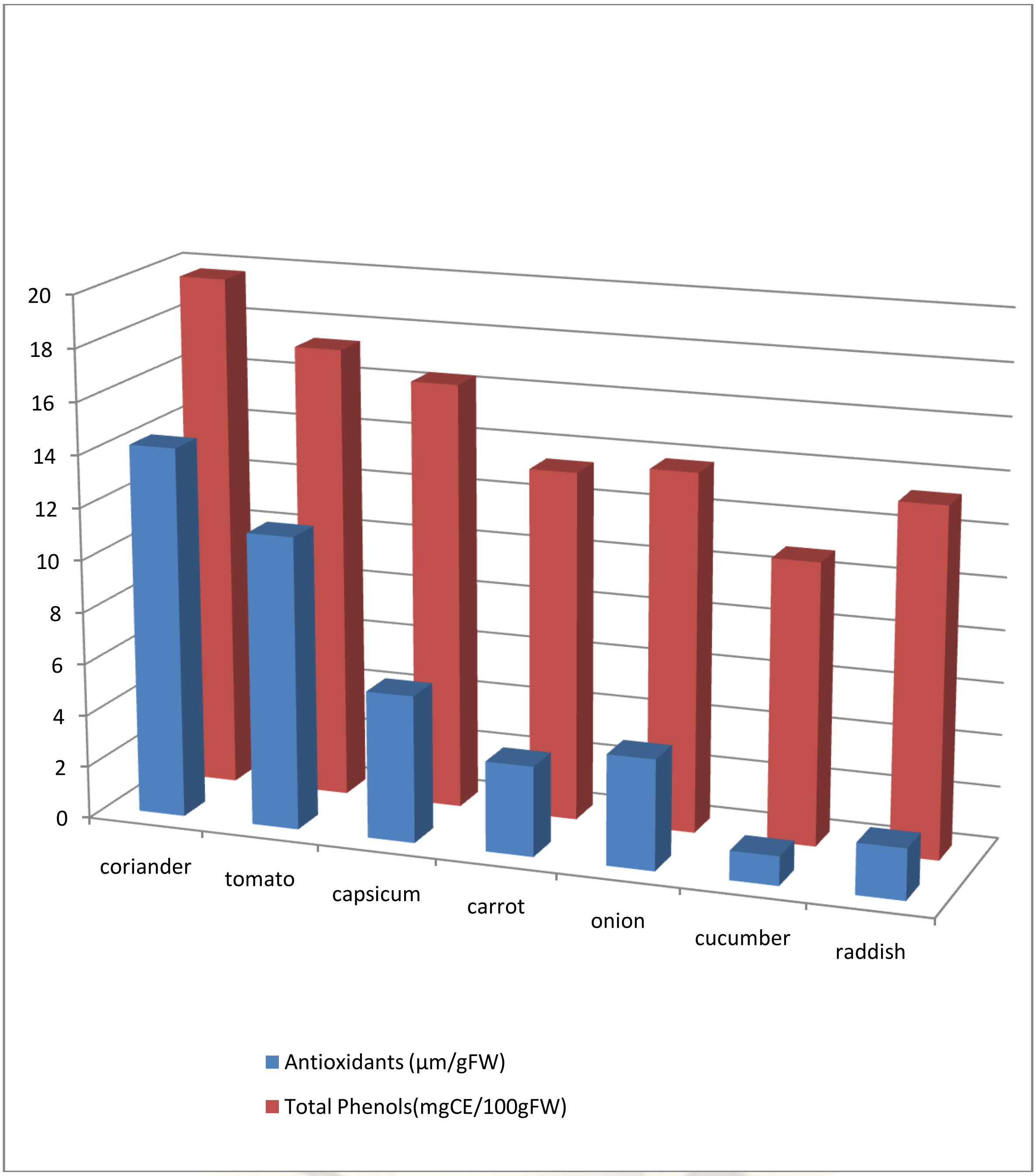

Fig.1 Total phenolic content and corresponding antioxidant status of various vegetables.

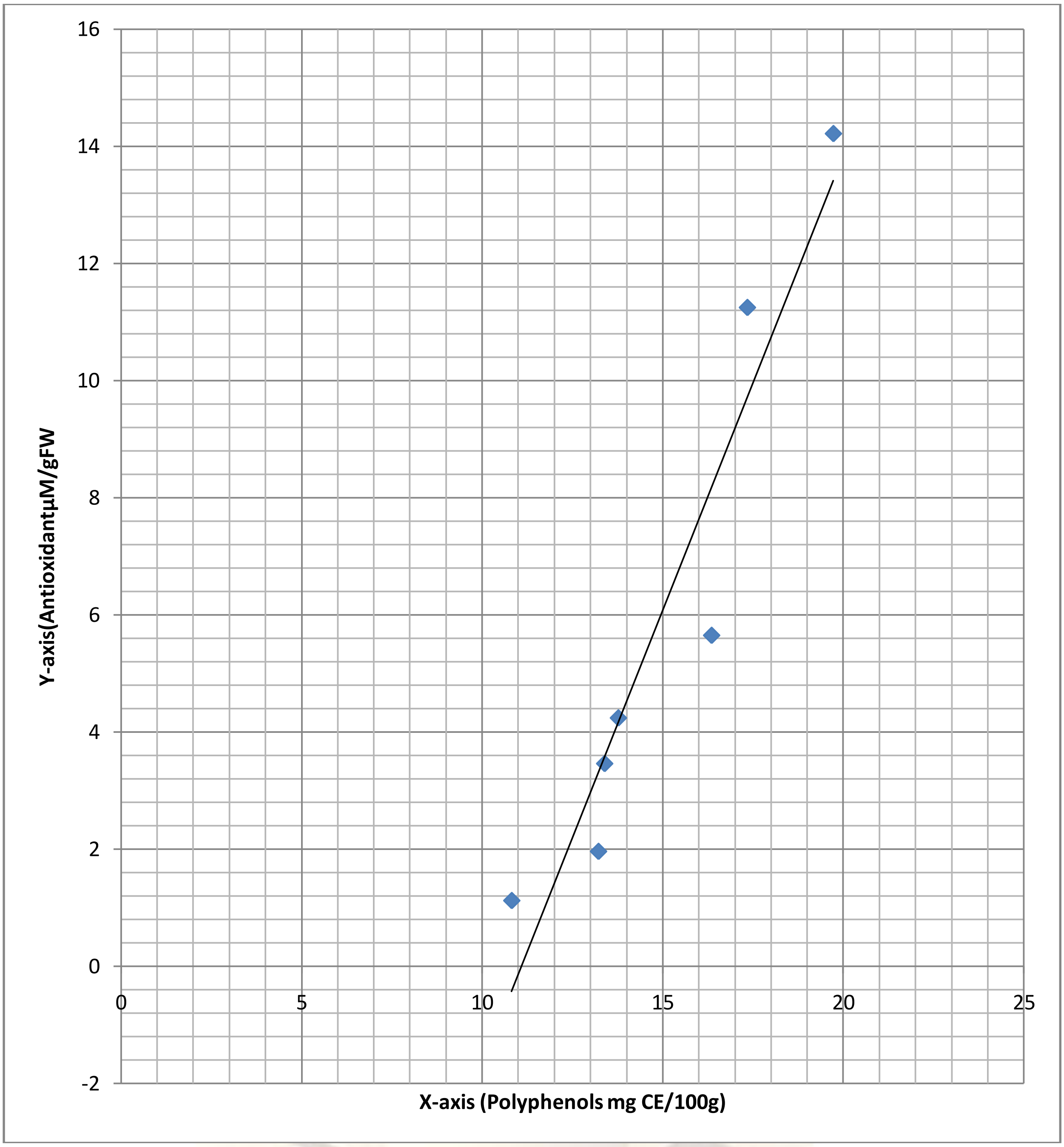

Fig.2 Relation between mean values, over replicates, of total phenols and antioxidants in various vegetables (correlation co-efficient is, r= 0.9519).

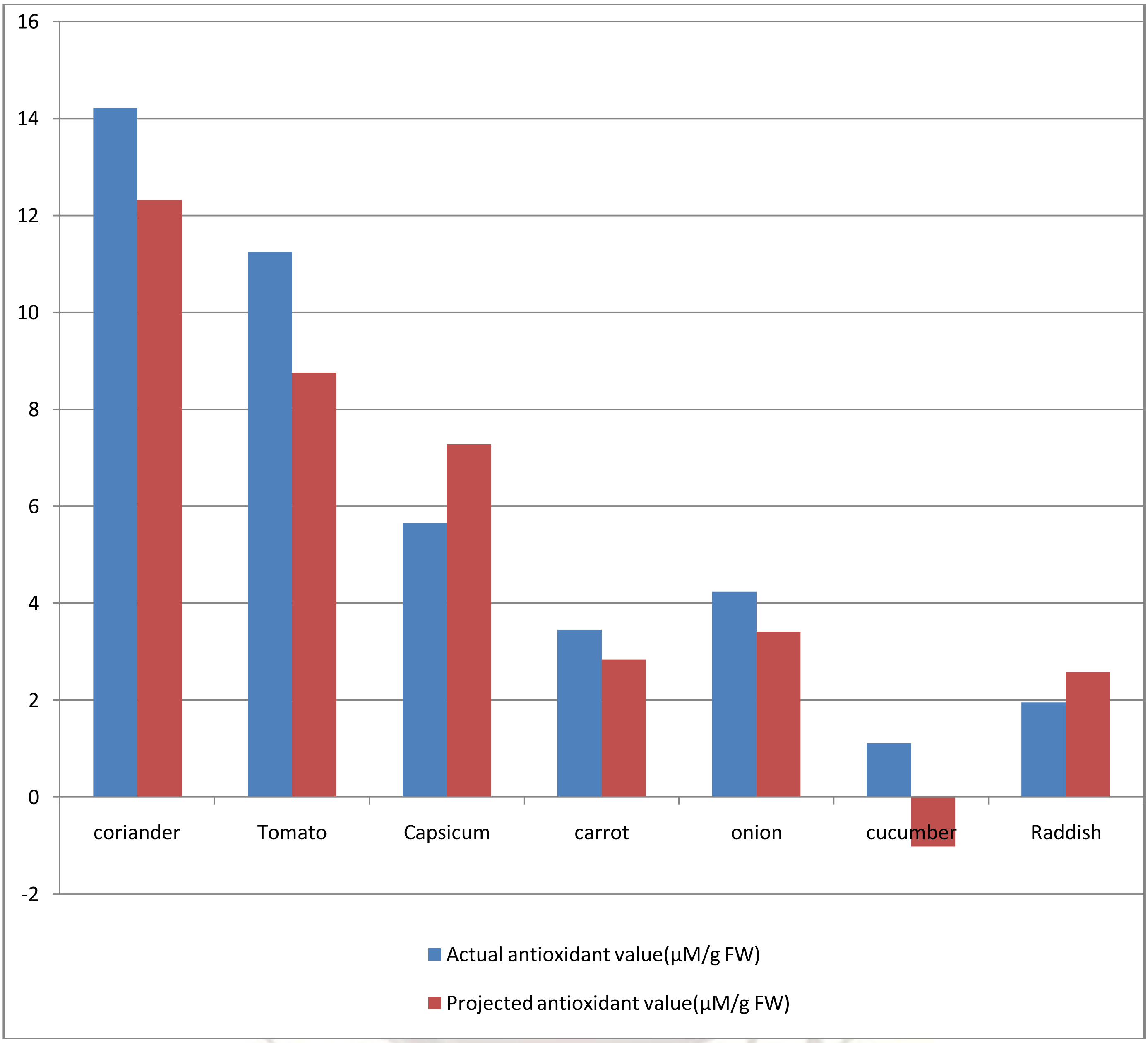

Fig 3. Projected antioxidant values of various vegetables on the basis of regression equation (Y=1.5x – 17.25) and their actual values.

Table 1. Total phenols and corresponding antioxidant activities of various vegetables.

S.No	Vegetable	Total phenols [mgCE/100g FW]	Anti-oxidant activity [μM Fe^{2+}/g FW]
1	Coriander	19.72	14.22
2	Tomato	17.34	11.25
3	Capsicum	16.35	05.65
4	Carrot	13.39	03.46
5	Onion	13.77	04.24
6	Cucumber	10.82	01.12
7	Radish	13.22	01.96
	C.V%	**18.60**	**75.99**

Table 2: Projected antioxidant values of various vegetables on the basis of regression equation (Y=1.5x – 17.25) and their actual values.

S.No	Vegetable	Anti-oxidant activity [μM Fe^{2+}/g FW]	
		Projected	**Actual**
1	Coriander	12.33	14.22
2	Tomato	08.76	11.25
3	Capsicum	07.28	05.65
4	Carrot	02.84	03.46
5	Onion	03.41	04.24
6	cucumber	-01.02	01.12
7	Radish	02.58	01.96