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Evaluation of Total Phenol and Flavonoid Content, Antioxidant and Iron Chelation Activities of Ethanolic Extracts of Green Beans

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ABSTRACT

Green beans contain high concentration of polyphenols, carotenoids, ascorbic acids, tocopherol and flavonoids which fight against oxidative damage. Free radicals have been implicated in the pathogenesis of most diseases. Thus, the consumption of dietary antioxidants from vegetables and fruits is beneficial in preventing these diseases. The present study was undertaken to evaluate antioxidant potential of ethanolic extracts of four different species of green beans. Ethanolic extract of *Phaseolus vulgaris* (french bean), *Vicia faba* (broad bean), *Cyamopsis tetragonoloba* (cluster bean) and *Vigna unguiculata* (cowpea) were studied for antioxidant properties by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, H_2O_2 decomposition, reducing power ability assay. Total phenolic content was measured by Folin Coilteau reaction and flavonoid contents were determined by $AlCl_3$ assay. Efforts were made to study the iron chelation. Total phenol content ranged from 17.00 ± 0.004 to 53± 0.007 µg GAE/mg while total flavonoid ranged 22 ± 0.003 to 36 ± 0.006 µg QE/ mg. All the extracts scavenged DPPH radical, decomposed H_2O_2 and had significant reducing potential. The IC_{50} for DPPH scavenging activity was highest for *Vigna unguiculata* as 2.27 ± 0.19 mg/ml while lowest for *Vicia faba* as 0.18± 0.02 mg/ml. All the extracts chelated Fe^{2+} form of iron but not Fe^{3+}. A positive and strong correlation (r^2 = 0.9) between total phenolic content & antioxidant activity suggests that green beans have enormous potential to enhance antioxidant capacity of our daily food supply and may provide health benefits in oxidative stress as neutraceuticals.

Keywords: Biological properties, phytochemicals, neutraceutical, oxidative stress, DPPH scavenging, green beans.

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INTRODUCTION

The role of free radicals in pathogenesis of various clinical conditions is widely accepted[1]. The biological and physiological damage induced due to free radical mediated oxidative stress can be counteracted by antioxidants. During the last few decades, much attention has been focused on the use of the natural antioxidants and plant extracts, because of possible ill health effects generated by the use of synthetic antioxidants [2,3,4,5].

Diet containing vegetables, fruits, spices and medicinal plants with increased levels of essential vitamins and nutrients (e.g. vitamin E, lycopene, vitamin C, bioflavonoids, thioredoxin etc.) provide a rich source of compounds like antioxidants that can be used in functional foods[6]. Higher intake of foods with functional attributes including high level of antioxidants may be the right effort in prevention as well as cure of various free radical borne diseases.

Vegetables are important protective foods and serve as good source of antioxidants apart from nutritive addition of vitamins, dietary fibres and mineral contents. In addition to vitamins and provitamins in fruits and vegetables, the presence of bioactive plant components, often called phytochemicals, has been considered of crucial nutritional importance in the prevention of chronic diseases, such as cancer, cardiovascular disease and diabetes [7].

Green beans are important part of human diet. They are rich in various nutrients such as vitamin A, vitamin C, vitamin K, manganese, iron, potassium, folate, calcium, magnesium and thiamine. They act as an anti-diabetes and weight-loss food because they are digested slowly, having a stabilizing effect on blood sugar, which promotes satiety and helps to prevent food cravings. Plus they contain soluble fibre, which lowers cholesterol levels[8].

In search for effective, natural compounds with antioxidative property, the present study was carried out to evaluate ethanolic extracts of four varieties of green beans for total phenol content, flavonoid content. The antioxidant activity of extracts was measured by total reducing power and radical scavenging activity (DPPH assay). As iron plays an important role in generation of free radicals, therefore study was extended to evaluate iron chelation property.

MATERIAL AND METHODS

Plant Material

The four varieties of beans (whole fruit) namely-*Phaseolus vulgaris*, *Vicia faba*, *Cyamopsis tetragonoloba* and *Vigna unguiculata* were taken from local vegetable market of New Delhi, India. The samples were identified and authenticated by Dr. D.K. Awasthi, Reader, Department of Botany, M.M. (P.G.) College, Modinagar, affiliated to CCS University, Meerut, India.

Chemicals

Gallic acid and aluminium chloride hexahydrate were procured from Titan Biotech Ltd, Bhiwadi, India; folin-ciocalteu's phenol reagent was obtained from Sisco Research laboratories, Pvt. Ltd., Mumbai, India; Quercitin dihydrate and DPPH(1,1-diphenyl-2-picrylhydrazyl) were purchased from HIMEDIA laboratories Pvt. Ltd., Mumbai, India; Potassium thiocyanate and 2,2′-bipyridyl, potassium ferricyanide were obtained from Central Drug House Pvt. Ltd., New Delhi, India.

Preparation of extract

The pods of plant material were dried under shade and then grinded coarsely in a mechanical blender. 40 g of coarse powder was filled in the thimble extracted successively with ethanol (60-80º C) as solvent in Soxhlet extractor for 24 hours**.** The ethanol extracts were concentrated under reduced pressure by Buchi type rotator evaporator and kept in a vacuum dessicator for complete removal of solvent. Then they were preserved at 4º C in airtight bottle until further use[9].

Determination of total phenolic content

Total phenolic content was determined by the Folin-Ciocalteau method [10]. In brief, 0.1 ml of each extract was mixed with 4.9 ml distilled water, 0.5 ml of Folin Ciocalteu reagent was added to the mixture. After 5min of incubation, 5 ml of 7% of aqueous Na_2CO_3 solution was added. The mixture was allowed to stand for 30 minutes and the absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Systronics, model no. 2202). The standard curve was prepared by gallic acid (0.1mg/ml) in methanol: water (50:50, v/v). Total phenolic content was expressed as of gallic acid equivalent µg GAE/mg of extract.

Determination of flavonoid content

Aluminum chloride method was used for flavonoid determination [11]. Briefly, 0.1ml of each extract was mixed with 1.9ml distilled water, then 0.1 ml 10% aluminium chloride-hexa hydrate, 0.1 ml 1M potassium acetate and 2.8 ml of distilled water were added. The reaction mixture was incubated at room temperature for 40 minutes. The absorbance of the reaction mixture was measured at 415nm. Quercitin(0.2mg/ml) was used as a standard. Total flavonoid content was expressed as µg QE/mg of extract.

Reducing power assay

The reducing power was assayed as described by with some modifications[12]. Different concentrations of each extract (as per protocol) in distilled water was mixed with 2.5 ml phosphate buffer (50mM, pH 7.0), 2.5 ml 1% potassium ferricyanide. The mixture was then incubated at 50^0C for 20 minutes. Then 2.5 ml 10 % TCA was added to the mixture, which was

then centrifuged at 3000 rpm for 10 minutes. Finally, 1.25 ml from the supernatant was mixed with 1.25 ml of distilled water and 0.25 ml $FeCl_3$ solution (0.1%, w/v). The absorbance was measured at 700 nm. Ascorbic acid was used as the standard.

H_2O_2 Decomposition

Hydrogen peroxide decomposition activity was determined by the method of Aebi H[13]. Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer (pH 7.4). 0.1mg/ml of extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 240 nm as a time scan for 15 min against a blank solution containing phosphate buffer without hydrogen peroxide.

DPPH scavenging assay

The method of Mensor *et al.*[14] with slight modification was used. 1ml of 0.3mM DPPH in methanol was added to 2 ml of each sample solutions (concentrations as per protocol) and allowed to react at room temperature in the dark for 30 minutes. The blank was prepared with 2 ml sample solution and 1ml methanol while the negative control was 1ml DPPH solution and 2ml methanol. The decrease in absorbance was measured at 517nm.

% AA (antioxidant activity) was calculated using the formula:

$$\% \text{ AA} = \left\{100 - \left\{\frac{[(\text{As} - \text{Ab}) \times 100]}{Ac}\right\}\right\} \qquad (1)$$

Where, A_s was the absorbance sample, A_b was the absorbance of the blank and A_c was the absorbance of the control. Ascorbic acid (1mg/ml) was used as the standard.

Metal chelation

Fe^{2+} chelation: The concentration of ferrous ion was estimated by 2,2′- bipyridyl-Fe^{2+} complex. The reaction mixture contained 0.01g of $FeSO_4$, 1 ml of 1mM NaCl (pH 7.0) and different concentration of extracts as per protocol. Reaction mixture was incubated for 30min at room temperature. At the end of incubation time, 2 ml of 2, 2′-bipyridal was added and absorbance of Fe^{2+} 2,2′-bipyridal complex was measured at 525 nm in final volume of 3 ml[15].

Fe^{3+} chelation: Iron (III) reacts with thiocynate and gives an intensely red colour compound which remains in true solution. In the spectrometric determination a large excess of thiocyanate should be used, since this increases the intensity and also the stability of the colour [15]. 0.01g of $FeCl_3$ was incubated with different concentrations of extracts for 30 min at room temperature. At the end of incubation time 1ml potassium thiocyanate was added. Absorbance was measured at 450 nm in final 3ml solution. For both forms of iron EDTA-Na_2 was used as the standard.

$$\% (Fe^{2+} / Fe^{3+}) \text{ chelation} = \left[\frac{Ac - As}{Ac} \times 100\right] \quad (2)$$

Where,

A_c is the absorbance of the control

A_s is the absorbance of the sample

Calculation of 50% Inhibitory Concentration (IC_{50})

The concentration (mg/ml) of the fractions that was required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at six different concentrations of the extracts. Percentage inhibition (I %) was calculated using the formula.

$$I\ \% = \left[\frac{Ac - As}{Ac} \times 100\right] \quad (3)$$

Where A_c is the absorbance of the control and A_s is the absorbance of the sample. IC_{50} was calculated graphically.

Statistical analysis

IC_{50} values were computed for various assay of each extract. Results are presented as mean ± SD of six independent experiments. Statistical analyses were performed by Student's t-test (Microsoft Excel). The values of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSIONS

% Yield

Values in Table 1 indicate the % yield of ethanolic extract. The maximum yield was obtained for *V. faba* followed by *P. Vulgaris, V. unguiculata* & *C. tetragonoloba.*

Table 1: %Yield of various ethanolic extracts

S.No.	Sample	Yield (%)
1.	*Phaseolus vulgaris*	08.75
2.	*Vigna unguiculata*	06.47
3.	*Cyamopsis tetragonoloba*	04.50
4.	*Vicia faba*	11.01

Total phenols and flavonoids

The ethanolic extracts of four varieties of green beans were studied for their contents of total phenols and flavonoids. Table 2 shows the total phenolic content of extracts measured by Folin-Ciocalteau reagent in terms of gallic acid equivalent (GAE). The highest phenolic content was observed in *Vicia faba* and lowest in *Vigna unguiculata*.

The flavonoid content of extracts was calculated as quercetin equivalent (QE). Total flavonoids content was also highest in *Vicia faba* and lowest was obtained in *Cyamopsis tetragonoloba.*

Vicia faba possessed highest content of phenols as well as flavonoids, while in case of *Phaseolus vulgaris* content of phenols and flavonoids were almost equal.

Table 2: Total phenolic and flavonoid content of ethanolic extracts

Extracts (50µlof 10mg/ml)	Total phenolic content (µg GAE/mg of extract)	Total flavonoid content (µg quercetin equivalent/mg of extract)
P.vulgaris	22 ± 0.020*	22 ± 0.003*
V. ugniculata	17 ± 0.004*	24 ± 0.005*
C.tetragonoloba	29 ± 0.003*	20 ± 0.006*
V.faba	52 ± 0.007*	36 ± 0.006*

*Each value is expressed as mean ± standard deviation (n= 6); $P < 0.05$)

Reducing power assay

Figure1 shows the reducing power of ethanolic extracts of beans. An increase in absorbance indicated the possession of reducing property. Higher absorbance is indicative of high reducing power. All the extracts showed dose dependent response for reducing ability. At a concentration of 2 mg/ml, 83.35%, 79.87%, 75.15% and 65.86% reducing property was observed with *Vicia faba*, *Phaseolus vulgaris*, *Cyamopsis tetragonoloba*, *Vigna unguiculata* respectively. *Vicia faba* showed highest reducing property because of highest content of phenols as well as flavonoids, *Vigna unguiculata* showed lowest reducing property, may be due to lowest phenol content. Results were compared with ascorbic acid as standard.

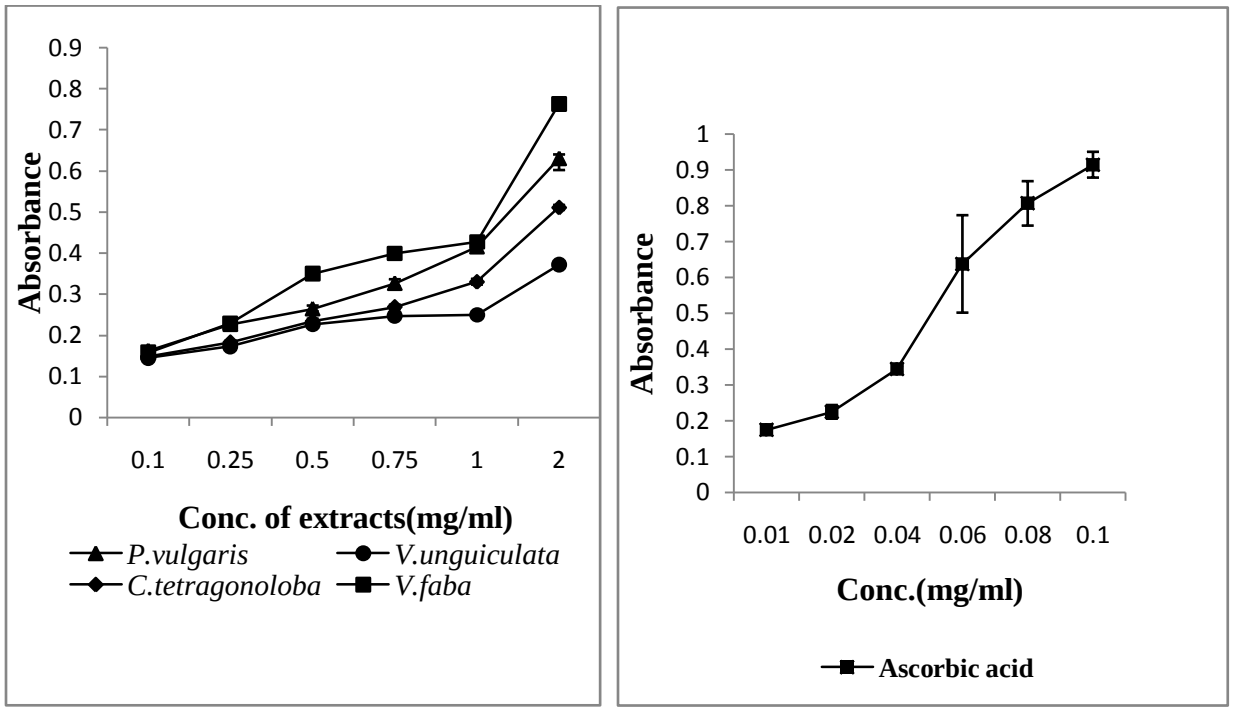

Figure1: Reducing power ability of ethanolic extracts of green beans and ascorbic acid.

Each value in the graph represents the mean ± standard deviation (n=6); P< 0.05

DPPH scavenging assay

Antioxidants react with the stable free radical DPPH (deep violet color) and convert it to 1,1-diphenyl-2-picryl hydrazine resulting in decoloration. All the extracts significantly scavenged DPPH (Table 3). *Vicia faba* showed highest scavenging activity (IC_{50} 0.18 mg/ml) followed by *Cyamopsis tetragonoloba* (IC_{50} 0.86 mg/ml), *Phaseolus vulgaris* (IC_{50} 2.07 mg/ml), *Vigna unguiculata* (IC_{50} 2.27 mg/ml). Results were compared with ascorbic acid (IC_{50} 18.08 µg/ml)

Table 3: Antioxidant activity of ethanolic extracts

Conc.(mg/ml)	% Antioxidant Activity(AA)			
	P.vulgaris	***V.unguiculata***	***C.tetragonoloba***	***V.faba***
0.10	10.71 ± 2.53*	10.28 ± 0.44*	08.71 ± 0.43*	48.13 ± 0.39*
0.25	13.61 ± 1.17*	10.38 ± 0.50*	17.01 ± 1.12*	58.60 ± 0.59*
0.50	20.44 ± 0.88*	22.43 ± 0.51*	28.85 ± 0.63*	79.03 ± 0.76*
0.75	28.91 ± 1.33*	27.97 ± 0.60*	42.42 ± 0.35*	82.67 ± 0.60*
1.00	29.83 ± 2.33*	39.32 ± 0.64*	58.16 ± 0.64*	89.00 ± 0.60*
2.00	50.74 ± 0.68*	47.46 ± 0.97*	93.96 ± 0.24*	92.57 ± 0.48*
3.00	57.74 ± 1.32*	56.54 ± 0.50*	97.02 ± 0.80*	98.23 ± 0.41*
IC_{50}	2.07 mg/ml**	2.27 mg/ml**	0.86 mg/ml**	0.18 mg/ml**

* Each value is expressed as mean ± standard deviation (n= 6); $P < 0.05$ **P< 0.05 when compared with ascorbic acid

Table 4: Antioxidant activity of Ascorbic acid

Conc.(µg/ml)	% Antioxidant Activity(AA)
5	04.88 ± 0.693
10	20.07 ± 0.363
15	36.07 ± 0.429
20	56.92 ± 0.881
25	71.79 ± 0.952
IC_{50}	18.08 ± 0.74 µg/ml

Each value is expressed as mean ± standard deviation (n=6);

Metal chelation activity

Table 5 : Metal Chelation activity of ethanolic extracts.

Conc.(mg/ml)	Metal Chelation (%I)			
	P.vulgaris	***V.unguiculata***	***C.tetragonoloba***	***V.faba***
0.10	22.05 ± 0.59*	10.20 ± 0.35*	05.86 ± 0.37*	03.13 ± 0.40*
0.25	23.82 ± 0.39*	50.28 ± 1.25*	19.12 ± 0.30*	12.61 ± 0.39*
0.50	24.54 ± 0.43*	79.71 ± 0.40*	19.96 ± 0.39*	25.18 ± 0.34*
0.75	47.81 ± 0.91*	91.20 ± 0.41*	32.66 ± 0.38*	39.13 ± 0.79*
1.00	54.42 ± 0.45*	95.69 ± 0.39*	44.05 ± 0.29*	45.85 ± 0.40*
2.00	83.42 ± 0.42*	99.08 ± 0.34*	59.03 ± 0.22*	53.08 ± 0.19*
IC_{50}	0.82 mg/ml	0.28 mg/ml	1.68 mg/ml	1.66 mg/ml

*Each value is expressed as mean ± standard deviation (n=6); P< 0.05

***P* < 0.05 when compared with EDTA

Toxic role of iron is linked with catalytic decomposition of hydrogen peroxide (Fenton reaction) leading to formation of highly reactive hydroxyl radicals ($^{\cdot}OH$). Thus chelation of free iron blocks its ability to catalyze the formation of free radicals. All the four extracts under study chelated Fe^{2+} but no chelating activity was observed for Fe^{3+}. The IC_{50} values for Fe^{2+} are given in Table 5. It was highest for *C.tetragonoloba* and lowest for *V.unguiculata*. Results were compared with EDTA as standard (IC_{50} 3.50 mg/ml) (table 6).

Table 6: Metal Chelation activity of EDTA

Conc.(µg/ml)	EDTA
00.370	18.59 ± 1.11
00.925	21.94 ± 0.64
01.850	23.65 ± 0.06
02.775	41.01 ± 1.22
03.700	59.40 ± 1.06
07.400	70.06 ± 3.50
11.100	90.15 ± 1.92
IC_{50}	3.50 ± 0.32

Each value is expressed as mean ± standard deviation (n=6); P< 0.05

H_2O_2 Decomposition

H_2O_2 has ability to generate free radicals, in particular the hydroxyl radicals ($^{\cdot}OH$). Therefore, effect of green beans was studied for H_2O_2 decomposition at 240nm. At a dose of 0.1mg/ml, all the extracts slightly decomposed H_2O_2 in a time dependent manner (Table 7). The decomposition was highest with *V.unguiculata* (8.18%) followed by *V.faba* (5.95%), *P.vulgaris* (4.47%) and *C.tetragonoloba* (4.41%). The result was also compared to standard, ascorbic acid.

Table 7: H_2O_2 decomposition activity of ethanolic extracts and ascorbic acid

Time	% H_2O_2 decomposition				
	P.vulgaris	***V.unguiculata***	***C.tetragonoloba***	***V.faba***	**Ascorbic acid**
1 min	1.20±0.006*	1.48±0.006*	1.82±0.003*	1.12±0.003*	10.57±0.005**
5 min	2.40±0.004*	4.28±0.006*	3.19±0.003*	3.16±0.004*	30.91±0.007**
10 min	3.44±0.005*	6.69±0.005*	3.95±0.003*	4.64±0.004*	43.63±0.007**
15 min	4.47±0.005*	8.18±0.004*	4.41±0.003*	5.95±0.003*	54.38±0.009**

***Each value is expressed as mean ± standard deviation (n=6); P< 0.05**

****P<0.05 when compared with ascorbic acid**

Correlation

Total phenolic content showed good correlation with antioxidant activity in terms of % scavenging of DPPH radical for all the species of green beans studied with $r^2 = 0.902$ (Figure 2). The antiradical activity of phenolic compounds depends on their molecular structure, on the

availability of phenolic hydrogens and on the possibility for stabilization of the resulting phenoxyl radicals formed by hydrogen donation[16,17].

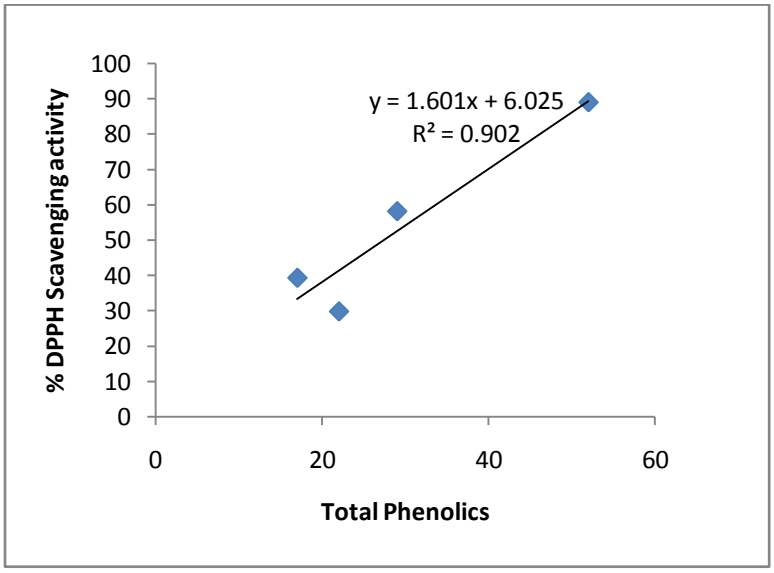

Figure2: Correlation between total phenolics and DPPH scavenging activity, (r^2 = 0.902)

CONCLUSION

Research has demonstrated that nutrition plays a crucial role in the prevention of chronic diseases, as most of them can be related to diet. Functional food enters the concept of considering food not only necessary for living but also as a source of mental and physical well-being, contributing to the prevention and reduction of risk factors for several diseases or enhancing certain physiological functions[18].

Thus present study was carried out with four varieties of green beans in search for effective, nontoxic natural compounds with antioxidative activity. The antioxidant potential, total phenolic and flavonoid contents of green beans commonly consumed in human diet were studied. The findings showed that all the four varieties of beans under study are a good source of dietary phytochemicals as shown by their total phenolic and flavonoid contents. They also possessed slight metal chelation property. Green beans can be seen as a potential source of neutraceuticals. They may help in combating oxidative stress.

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