AMERICAN JOURNAL OF PHARMTECH RESEARCH

**Journal home page:** http://www.ajptr.com/

# Biochemical Studies on Antioxidant Potential of Green Beans in Fresh and Processed Conditions

**Savita Chaurasia*[1], Rimsi Saxena[1]**

*1.Department of Biotechnology, IMS Engineering College, Ghaziabad. Uttar Pradesh- 201009, India.*

## ABSTRACT

The present study was undertaken to evaluate the total antioxidant potential of four different varieties of green beans namely *Phaseolus vulgaris* (french beans), *Vicia faba* (broad beans), *Cyamopsis tetragonoloba* (cluster beans) and *Vigna unguiculata* (cowpea beans) in both fresh and processed condition (microwave assisted). The total phenolic and flavonoid contents were determined by Folin Coilteaus and $AlCl_3$ assay respectively. The antioxidant activity was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, hydrogen peroxide($H_2O_2$) decomposition and reducing power assay. Efforts were also made to study the interaction of beans with metal ions. Total phenolic content ranged from 105.00 ± 0.021 to 300.00 ± 0.009 µg GAE·$g^{-1}$ (Gallic acid equivalent) of extract and flavonoid content ranged from 55.00 ± 0.017 to 550.00 ± 0.050 µg QE·$g^{-1}$ (Quercitin equivalent) of extract. All the extracts scavenged DPPH radical and decomposed $H_2O_2$. The reducing potential was found to be highest in *Cyamopsis tetragonoloba* followed by *Vigna unguiculata*, *Vicia faba* and *Phaseolus vulgaris*. Good correlation of phenolic content was observed with reducing power ability ( $r^2$ = 0.743) and DPPH radical scavenging property ($r^2$ = 0.694). The extracts chelated both the ionic forms of iron $Fe^{2+}$ and $Fe^{3+}$ but the affinity towards $Fe^{3+}$ was higher. Green beans can be considered as a potential source of antioxidants. Fresh extracts were found to have higher antioxidant potential as compared to processed extracts.

**Keywords:** Antioxidant, *Cyamopsis tetragonoloba*, *Vigna unguiculata*, *Vicia faba*, *Phaseolus vulgaris*, Green beans

*Corresponding Author Email: drsav16@yahoo.com

Received 09 October 2012, Accepted 15 October 2012

Please cite this article in press as: Chaurasia S *et al.*, Biochemical Studies on Antioxidant Potential of Green Beans in Fresh and Processed Conditions. American Journal of PharmTech Research 2012.

## INTRODUCTION

The very concept of food is changing from a past emphasis on health maintenance to the promising use of foods to promote better health to prevent chronic illnesses. Because dietary habits are specific to populations and vary widely, it is necessary to study the disease-preventive potential of functional micronutrients in the regional diet[1]. Current research is focused on the activity of the natural antioxidants present in fruits and vegetables, because potentially these components may reduce the level of oxidative stress. Oxidative stress has been defined as a disturbance in the equilibrium status of pro-oxidant/antioxidant systems in intact cells resulting in oxidative damage to lipids, proteins, carbohydrates, and nucleic acids, contributing to pathological dysfunction eventually resulting in diseased states[2]. Antioxidant compounds obtained from natural sources are receiving considerable attention due to the carcinogenicity of the synthetic ones[3]. In addition to vitamins and pro vitamins in fruits and vegetables, the presence of bioactive plant components, often called phyto chemicals, has been considered of crucial nutritional importance in the prevention of chronic diseases, such as cancer, cardiovascular disease and diabetes[4]. Because prevention is a more effective strategy than treatment for chronic diseases, a constant supply of phyto chemical-containing plants with desirable health benefits beyond basic nutrition is essential to furnish the defensive mechanism to reduce the risk of chronic diseases in humans.

Beans are a powerhouse of superior nutrition, and the most nutrient-dense carbohydrate source. 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 fiber, which lowers cholesterol levels[5]. Eating beans, peas, or lentils at least twice a week has been found to decrease colon cancer risk by 50%[6,7]. Legume intake also provides significant protection against oral, larynx, pharynx, stomach, and kidney cancers[8]. Recently we have reported antibacterial activity of green bean[9]. The search for new products with antioxidative properties is a very active domain of research. Therefore, present study was carried out to evaluate total phenol content, flavonoid content, total reducing power, radical scavenging activity and metal chelation properties of four varieties of green beans in fresh and processed condition.

## MATERIALS AND METHODS

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.

**Plant Material**

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

**Preparation of extract**

Beans were washed with distilled water and dried using blotting paper. The extensions and the tips of the fruit were trimmed and it was cut into small pieces. The pieces were crushed using a grinder.

- **Fresh extract** 10 gm of the crushed beans were weighed and passed through muslin cloth to obtain the crude extract. The filtrate was centrifuged at 3000 rpm for 10 minutes to remove any debris. The supernatant was collected and used for further experiment.
- **Processed extract** 10 gm of the crushed beans were weighed and passed through muslin cloth to obtain the crude extract. The filtrate was collected and microwaved at full power for 20 seconds. Next they were allowed to stand for 5 minutes and then centrifuged at 3000 rpm for 10 minutes to remove any debris. The supernatant was used for further experiment.

**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 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 ($0.1 mg \cdot ml^{-1}$) in methanol: water (50:50, v/v). Total phenolic content was expressed as of µg GAE $g^{-1}$ fresh weight of beans.

**Determination of flavonoid content**

Aluminium chloride method was used for flavonoid determination[11]. Briefly, 0.1 ml of each extract was mixed with 1.9 ml distilled water, then 0.1 ml 10% aluminum 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 ($0.2mg \cdot ml^{-1}$) was used as a standard. Total flavonoid content was expressed as µg $QE \cdot g^{-1}$ fresh weight of beans.

**Reducing power assay**

The reducing power was assayed as described by Yildirim et al.[12] with some modifications. 0.1 ml each extract was mixed with 0.9 ml distilled water, 2.5 ml phosphate buffer (50mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at $50^0C$ for 20 minutes. After incubation 2.5 ml 10 % TCA was added to the mixture and centrifuged at 3000 rpm for 10 minutes. 1.25 ml of 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.

**$H_2O_2$ Decomposition**

Hydrogen peroxide decomposition activity was determined by the method of Aebi H[13]. Hydrogen peroxide solution ($2mM \cdot L^{-1}$) was prepared with standard phosphate buffer (pH 7.4). 0.025 ml extract in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 240 nm after 15 min against a blank solution containing phosphate buffer. % $H_2O_2$ Decomposition was calculated by Eq.(1).

$$\% \ H_2O_2 \text{ Decomposition} = \left[\frac{A0-A15}{A0} \times 100\right] \quad (1)$$

Where,

A15 is the absorbance after 15min, $A_0$ is the absorbance at 0min

**DPPH scavenging assay**

The method of Mensor et al.[14] was used. 1ml of 0.3mM DPPH in methanol was added to 2 ml of each sample solutions (0.1 ml of extract and 1.9 ml methanol) 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. These were converted to % AA (antioxidant activity) using the Eq. (2).

$$\% \ AA = \left\{100 - \left\{\frac{[(As - Ab) \times 100]}{Ac}\right\}\right\} \quad (2)$$

Where, As was the absorbance sample, Ab was the absorbance of the blank and Ac was the absorbance of the control.

**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 100µl of extracts. 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 thiocyanate and gives an intensely red colour compound which remains in true solution. In the spectrometric determination a large excess of thiocynate should be used, since this increases the intensity and also the stability of the colour[15]. 0.01g of $FeCl_3$ was incubated with 100 µl 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.

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

Where,

Ac is the absorbance of the control, As is the absorbance of the sample

**Statistical Analysis**

Results are presented as mean ± SD of four independent experiments. Differences are estimated by ANOVA (Q1 Macros SPC software) and student's t test. The values of $p < 0.05$ were considered significant. Correlation analysis of reducing and DPPH scavenging activity versus the total phenolics was carried out using the Pearson Correlation in the Microsoft Excel program.

## RESULTS AND DISCUSSION

**Total Phenolic Content**

Phenolic compounds are a class of antioxidant compounds which act as free radical terminators[16].

**Table 1: Total phenolic content for Fresh and processed extracts**

| Sample | Fresh Extracts [µg GAE·$g^{-1}$ of extract] | Processed Extract [µg GAE·$g^{-1}$ of extract] |
|---|---|---|
| *P. vulgaris* | 105.5 ± 0.021[a] | 037.5 ± 0.011[a] |
| *V. unguiculata* | 200.6 ± 0.026[b] | 100.2 ± 0.015[b] |
| *C. tetragonoloba* | 300.3 ± 0.009[c] | 107.5 ± 0.013[c] |
| *V. faba* | 140.6 ± 0.024[d] | 052.5 ± 0.019[d] |

Each value is expressed as mean ± standard deviation (n=4); Means within same columns with different letter superscripts are significantly different ($P < 0.05$)

The fresh and processed extracts of four varieties of green beans were studied for their contents of total phenols. Table 1 shows the 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 *Cyamopsis tetragonoloba* and lowest was observed in *Phaseolus vulgaris*. The same pattern was found for the processed extracts. Results indicate that thermal processing of extracts affected the total phenolic contents. In all the extracts under study total phenolic contents decreased by 50 - 65% upon processing in microwave at full power for 20 seconds.

**Total Flavonoid Content**

The mechanisms of action of flavonoids are through scavenging or chelating process[17,18]. The flavonoid content of extracts was calculated as quercetin equivalent(QE) using aluminium chloride method as shown in Table 2. *Cyamopsis tetragonoloba* contained highest flavonoid content for both fresh and processed condition while the lowest was found in *Vicia faba* in both conditions. Decrease in flavonoid content was also observed on thermal processing.

**Table 2 :Total flavonoid content for Fresh and Processed extracts**

| Sample | Fresh Extracts [µg QE·g$^{-1}$ of extract] | Processed Extracts [µg QE·g$^{-1}$ of extract] |
|---|---|---|
| *P. vulgaris* | 125.5 ± 0.034$^{a}$ | 55.3 ± 0.004$^{a}$ |
| *V. unguiculata* | 75.2 ± 0.011$^{b}$ | 65.5 ± 0.010$^{b}$ |
| *C. tetragonoloba* | 550.4 ± 0.050$^{c}$ | 400.2 ± 0.035$^{c}$ |
| *V. faba* | 55.2 ± 0.017$^{d}$ | 40.1 ± 0.007$^{d}$ |

Each value is expressed as mean ± standard deviation (n=4); Means within same columns with different letter superscripts are significantly different ($P < 0.05$)

**Reducing Power Assay**

Results in Table 3 show the reducing power of bean extracts in fresh and processed conditions. In this assay, the yellow color of the mixture changes to various shades of green depending on the reducing power of each extract.

**Table 3: Reducing power ability of fresh and processed extracts**

| Extract | Absorbance (700nm) | |
|---|---|---|
| | Fresh extracts | Processed extracts |
| *P. vulgaris* | 0.237 ± 0.019$^{a}$ | 0.120 ± 0.008$^{a}$ |
| *V. unguiculata* | 0.447 ± 0.023$^{b}$ | 0.274 ± 0.023$^{b}$ |
| *C .tetragonoloba* | 0.540 ± 0.014$^{c}$ | 0.297 ± 0.021$^{c}$ |
| *V. faba* | 0.436 ± 0.019$^{d}$ | 0.171 ± 0.018$^{d}$ |

Each value is expressed as mean ± standard deviation (n=4); Means within same columns with different letter superscripts are significantly different ($P < 0.05$)

The conversion of the $Fe^{3+}$/ferricyanide complex used in this method to the ferrous form is due to the presence of reducers. By measuring the formation of Perl's Prussian blue at 700 nm, the reducing power of different extracts was analysed. Higher absorbance is indicative of high reducing power. *Cyamopsis tetragonoloba* had the highest reducing power followed by *Vigna unguiculata*, *Vicia faba* and *Phaseolus vulgaris* in both fresh and processed condition. Effect of thermal processing was also observed on reducing property. 35 to 60% decrease in reducing property was observed with processed extracts.

**$H_2O_2$ Decomposition**

All the fresh and processed extracts under study showed $H_2O_2$ Decomposition activity. Among the fresh extracts *Phaseolus vulgaris* showed the highest decomposition while it was highest for *Vicia faba* among the processed extracts as shown in Table 4. Fresh extracts significantly decomposed $H_2O_2$ but processed extracts showed marginal decomposition. On processing significant change in the decomposition activity was observed for *Phaseolus vulgaris* while there was negligible difference for *Vigna unguiculata.*

**Table 4: $H_2O_2$ Decomposition activity for fresh and processed extracts**

| Extract | [%] $H_2O_2$ Decomposition | |
|---|---|---|
| | Fresh extracts | Processed extracts |
| *P. vulgaris* | 48.84 ± 0.016[a] | 01.46 ± 0.002[a] |
| *V. unguiculata* | 03.89 ± 0.016[b] | 02.01 ± 0.003[b] |
| *C. tetragonoloba* | 21.38 ± 0.004[c] | 04.48 ± 0.003[c] |
| *V. faba* | 24.24 ± 0.015[d] | 17.14 ± 0.003[d] |

Each value is expressed as mean ± standard deviation (n=4); Means within same columns with different letter superscripts are significantly different ($P < 0.05$)

**DPPH scavenging assay**

The antioxidants react with the stable free radical DPPH (deep violet color) and convert it to 1,1-diphenyl-2-picryl hydrazine resulting in decoloration. The scavenging effect of extracts were expressed as percentage activity (% AA) given in Table 5.

**Table 5: DPPH scavenging activity for fresh and processed extracts**

| Extract, 100[µl] | % Antioxidant Activity (AA) | |
|---|---|---|
| | Fresh extracts | Processed extracts |
| *P. vulgaris* | 32.80 ± 0.121[a] | 09.86 ± 0.095[a] |
| *V. unguiculata* | 88.96 ± 0.043[b] | 86.21 ± 0.043[b] |
| *C. tetragonoloba* | 85.77 ± 0.032[c] | 82.87 ± 0.063[c] |
| *V. faba* | 28.73 ± 0.032[d] | 04.35 ± 0.016[d] |

Each value is expressed as mean ± standard deviation (n=4); Means within same columns with different letter superscripts are significantly different ($P < 0.05$)

*Vigna unguiculata* showed highest scavenging activity both in fresh and processed followed by *Cyamopsis tetragonoloba.* In both the cases, there was no significant effect of thermal processing on DDPH radical scavenging activity. Under similar experimental condition , upon thermal processing 84% and 70% decrease in radical scavenging was observed with *Vicia faba* and *Phaseolus vulgaris* respectively

**Metal chelation activity**

The main strategy to avoid free radical (ROS; Reactive Oxygen Species) generation that is associated with redox active metal catalysis involves chelating of the metal ions. $Fe^{3+}$ with thiocyanate and $Fe^{2+}$ with bipyridal forms complex giving red and pink color respectively. In the presence of chelating agents, the complex formation is disrupted such that the color of the complex is decreased. Measurement of reduction in color given by the decrease in absorbance therefore, allows the estimation of the chelating activity of the coexisting chelator. Both fresh and processed extracts showed chelation towards the two forms of iron. Figure 1,2 shows that affinity of all the extracts towards $Fe^{3+}$ was higher. Upon microwave processing, in all the extracts, chelation activity for $Fe^{2+}$ decreased by 25 to 50% while $Fe^{3+}$ decreased by 10 to 35%.

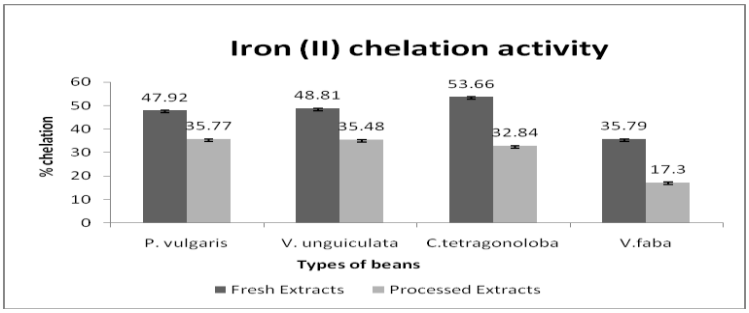


**Figure 1: Metal chelating activity for Fresh and processed extracts of $Fe^{2+}$**

Mean deviation/error bars in the graph represents the mean± standard deviation from the quadruplet samples that were tested; $P > 0.05$

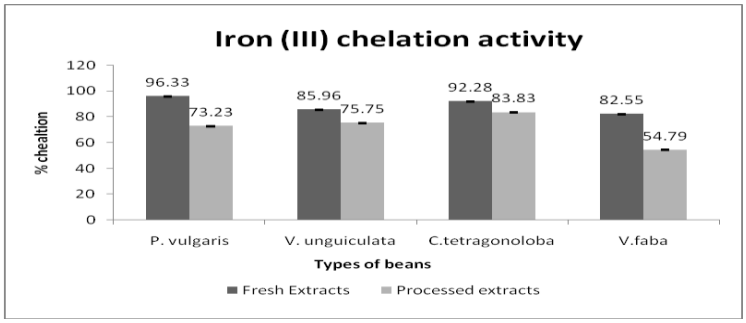


**Figure 2 :Metal chelating activity for Fresh and processed extracts of $Fe^{3+}$**

Mean deviation/error bars in the graph represents the mean± standard deviation from the quadruplet samples that were tested ; $P < 0.05$

**Correlation**

Total phenolic content showed good correlation with reducing power ability of fresh extracts of all the species of green beans studied with $r^2 = 0.743$(Figure. 3).

A good linear correlation ($r^2 = 0.694$,) between the total phenolic content and the scavenging of DPPH radical was also found (Figure. 4). These results indicated that the radical scavenging capacity of each extract might be mostly related to their concentration of phenolic hydroxyl group. 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[19,20].

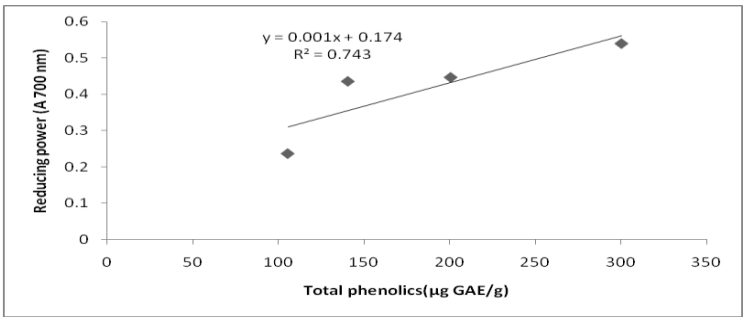


**Figure 3 :Correlation between total phenolics and reducing power ability, ($r^2 = 0.743$)**

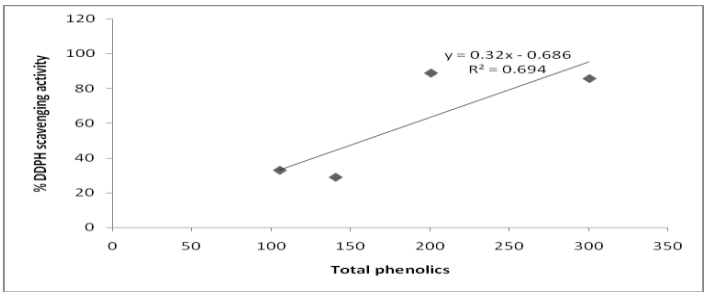


**Figure 4: Correlation between total phenolics and DPPH scavenging activity,($r^2 = 0.694$)**

## CONCLUSION

Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defence mechanisms[21].The four varieties of the green beans can be considered as good sources of antioxidant as shown by their total phenolic and flavonoid contents. *C.tetragonoloba* was found to be most potent antioxidant with highest total phenolic content and good reducing power. A significant correlation was obtained between antioxidant activity and phenolic content indicating that phenolic compounds contribute significantly to antioxidant activity of the investigated beans. Iron chelation activity was shown by all the species under study. All the four varieties of

beans showed chelation of both $Fe^{2+}$ and $Fe^{3+}$, but the affinity towards $Fe^{3+}$ was high. The extracts also significantly decomposed $H_2O_2$ and scavenged DPPH. The antioxidant activity of fresh extracts was found to be higher than the processed extracts. It may be due to loss in total phenolics and flavonoid content on thermal processing. So, it can be concluded that thermal processing affects the antioxidant potential of the extracts. Green beans can be seen as a potential source of dietary antioxidants. They may help in combating oxidative stress. Further study to identify the active compounds and their mechanism of action is in progress in our lab.

## ACKNOWLEDGEMENT

Authors are thankful to Dr. D.K. Awasthy, Reader, Department of Botany, M.M.(P.G) College, affiliated to CCS University, Meerut, India for identification of plant material.

## REFERENCES

1. Devasagayam TPA, Tilak JC, Boloor KK, Sane K, Ghaskadbi S, Lele RD. Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. J Assoc Phys India 2004; 52:794-804.
2. Galli F, Piroddi M, Annetti C, Aisa C, Floridi E, Floridi A. Oxidative stress and reactive oxygen species. Contrib Nephrol 2005; 149: 240-260.
3. Ito N, Hirose M, Fukushima S, Tsuda H, Shirai T, Tatematsu M. Studies on antioxidants: their carcinogenic and modifying effects on chemical carcinogenesis. Food Chem Toxicol 1986; 24(10-11):1071-82.
4. Aruoma, Okezie I. Methodological considerations for characterizing potential antioxidant actions and bioactive component in plant foods. Mutat Res 2003; 523-524:9-20.
5. Bazzano LA, Thompso AM, Tees MT, Nguyen CH, Winham DM. Non-soy legume consumption lowers cholesterol levels: A meta-analysis of randomized controlled trials. Nutr Metab Cardiovasc Dis 2011; 21: 94-103.
6. O'Keefe SJ, Ou J, Aufreiter S, O'Connor D, Sharma S, Sepulveda J, Fukuwatari T, Shibata K, Mawhinney T. Products of the colonic microbiota mediate the effects of diet on colon cancer risk. J Nutr 2009; 139:2044-8.
7. Singh PN, Fraser GE. Dietary risk factors for colon cancer in a low-risk population. Am J Epidemiol 1998; 148:761-764.
8. Aune D, De Stefani E, Ronco A, Boffetta P, Deneo-Pellegrini H, Acosta G, Mendilaharsu M. Legume intake and the risk of cancer: a multisite case-control study in Uruguay. Cancer Causes Control 2009; 20:1605-15.

9. Chaurasia S, Saxena R. Antibacterial Activity of Four Different Varieties of Green Beans. Res J Pharm Biol Chem Sci 2012; 3(3):70-74.
10. Singelton VR, Orthifer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 1999; 299:152-178.
11. Quettier DC, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx MC, Cayin JC, Bailleul F, Trotin F. Phenolic Compounds And Antioxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour. J Ethnopharmacol 2000; 72: 35-42.
12. Yildirim A, Mavi A, Kara A. Determination of antioxidant and antimicrobial activities of Rumex crispus L. extracts. J Agric Food Chem 2001; 49:4083–4089.
13. Aebi H. Catalase in vitro. Methods Enzymol 1984; 105:121-126.
14. Mensor LL, Menezes FS, Leitao GG, Reis AS, Santos TC, Coube CS. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res 2001; 15:127-130.
15. Tripathi YB, Chaurasia S. Interaction of S. nux-vomica products and iron: with reference to lipid peroxidation. Phytomedicine 2000; 7:523-528.
16. Shahidi F, Wanasundara PKJPD. Phenolic antioxidants. Crit Rev Food Sci Nutr 1992; 32:67-103.
17. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem 2002; 13(10):572-584.
18. Kessler M, Ubeaud G, Jung L. Anti- and pro-oxidant activity ofrutin and quercetin derivatives. J Pharm.Pharmacol 2003; 55:131-142.
19. Catherine A., Nicholas JM, Rice-Evans, George P. Structure antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biol Med 1996; 20:933 – 956.
20. Ramarathnam N, Ochi H, Takeuchi M. Antioxidant defense system in vegetable extracts. In: Shahidi F(ed). Natural Antioxidants; chemistry, health effects and applications. AOCS Press, Champaign, IL; 1997; 76 – 87.
21. Young IS, Woodside JV. Antioxidants in health and disease. J Clin Pathol 2001; 54(3):176-86.