International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(1): 1663-1668

Received: 01-11-2018
Accepted: 04-12-2018

T Kamalaja
Scientist - FN, AICRP-H.Sc, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana State, India

K Rajeswari
Scientist - FN, AICRP-H.Sc, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana State, India

M Prashanthi
Scientist - FN, AICRP-H.Sc, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana State, India

Correspondence
T Kamalaja
Scientist - FN, AICRP-H.Sc, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana State, India

Analysis of bioactive compounds in leafy vegetables

T Kamalaja, K Rajeswari and M Prashanthi

Abstract
The leafy vegetables like Amaranth, Coriander, Gogu, and Mint, were chosen to analyze the bioactive compounds in different processing methods. Standard procedures were followed for extraction and analysis of bioactive compounds. The results revealed that the percent change in bioactive compounds in leafy vegetables was ranged from 2.79 to 48.9 for total phenolics, 0.8 to -17.4 for flavonoid content upon open cooking. The DPPH radical scavenging activity and FRAPS was 4.73 to 50.4, and 2.93 to 14. respectively2. The percent change in total phenolic content in leafy vegetables upon pressure cooking was ranged between 9.98 to 78.6, total flavonoids -25.8 to 53.3, DPPH radical scavenging activity -2.7 to 102.5, and FRAP ranged between 332 to 667.

Keywords: Leafy vegetables, bioactive compounds, open cooking and pressure cooking, FRAP, AOA, DPPH, phenols, flavonoids, antioxidants

Introduction

Leafy vegetables represent a valuable source of minerals, fiber, and vitamin C, as well as vegetable pigments (carotenoids, chlorophylls) and polyphenolic compounds, with high biological activity. Most of these compounds are known to act as strong antioxidants that protect human body against harmful effects of free radicals, such as heart diseases, tumors and aging processes (Panczenko-Kresowska B., 1997, Rietjens I.M.C.M *et al.,* 2002,, Wong S.P. *et al.,* 2006 and Bawa S., 2004) [1, 2, 3, 4]

Generally, vegetables are widely designated as "protective foods" in human diet due to their varied health benefits attributable to the richness in vitamins, essential fatty acids, minerals, amino acids and dietary fiber (Shukla P., 2016) [5] and various essential bioactive compounds (Da Silva Dias João Carlos, Imai S., 2017) [6]. These include health-promoting plant secondary metabolites composed of antioxidants and phenolic compounds. It is well acknowledged that to meet recommended daily allowance of nutrition, the World Health Organization (WHO) recommended at least 400g of fruit and non-starchy vegetables (WHO, 2013) consumption per day per person.

The antioxidants vitamins a, c, and e are considered to be responsible for, and contribute to, protective effects against oxidation due to singlet oxygen, superoxide, peroxyl, hydroxyl, and peroxynitrite radicals (pokorny *et al.,* 2001) [7]. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, inflammation, and neurodegenerative disorders such as parkinson's and alzheimer's diseases (Getoff n., 2007; Kaur C. and Kapoor H.C., 2002) [8, 13]. Antioxidants in plants may play a role in protecting human health.

Consumption of vegetables may help to reduce the risks of many age-related degenerative diseases. Green leafy vegetables and vegetable oils are sources of antioxidant components (Tang, S.Y. *et al.,* 2004, Wong C., *et al.,* 2006) [10, 3, 4]. In addition to antioxidants and vitamins, vegetables contain phenolic compounds that may, in part and provide specific protective effects against oxidative stress which can lead to coronary heart disease and cancer (Katalinic, V. M., *et al.*, 2006, Kaur C. and Kapoor H.C., 2002) [12, 13].

Consumption of a diet high in vegetables can increase the plasma antioxidants ascorbic acid, α- and β-carotene, lutein, and zeaxanthin (Hunter, K.J. and J.M. Fletcher. 2002, Sun, J., Y.F. *et al.*, 2002) [14, 15] Free radicals are harmful to living organisms because they can accelerate the aging process, sometimes causing cellular destruction, or, if the DNA is affected, irreversible malfunctions (Lim, P. *et al.*, 2004, Zheng, W. and S.Y. Wang. 2001) [16, 17]. Phenolic compounds found in vegetables have the potential to inhibit free radicals (Katalinic, V., *et al.*, 2006, Young, I.S. and J.V. Woodside. 2001 and Zhang, D. and Y. Hamauzu 2004) [12, 19, 20]. Antioxidants for use in foods, cosmetics, or medicinal materials are being examined for their potential role in prevention of human diseases to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (Cai, Y., *et al.,* 2004 and Sasaki, Y.F., *et al.,* 2002) [21, 22].

Green leafy vegetables (GLV) are rich sources of many nutrients and form a major category of vegetable groups that have been designated as 'nature's anti-aging wonders'. Gupta S., *et al.* 2005 [23] have reported that several GLV are rich sources of antioxidant vitamins. Brahmi (*Centella asiatica*), curry leaf (*Murraya koenigii*), fenugreek (*Trigonella foenumgraecum*) and keerae (*Amaranthus* sp.) are used in Indian culinary and are also known for their medicinal value. Experimental and epidemiological evidence suggests a significant role of diet in the prevention of degenerative diseases (P. J. Harris and L. R. Ferguson, 1993) [24]

Plant derived antioxidants, such as flavonoids and related phenolic compounds, have multiple biological effects, including antioxidant activity. Phytochemicals present in plant foods exert health beneficial effects, as they combat oxidative stress in the body by maintaining a balance between oxidants and antioxidants (A. Scalbert, *et al.,* 2005) [25]

Among plant foods, green leafy vegetables and grains are a rich source of antioxidants apart from energy, protein, and selected micronutrients in Indian diets (S. Rochfort and J. Panozzo, 2007) [26]. Traditionally grains and GLVs have played a major role in providing nutrition particularly in the Indian Subcontinent and in other developing countries (D. Sreeramulu, C. V. K. Reddy, and M. Raghunath, 2009) [27]. Since plant foods are often consumed in one or the other cooked forms, polyphenol and AOA intakes calculated on the basis of their content in raw foods are likely to be inaccurate. Therefore it was considered pertinent to study the effect of domestic processing, on the natural antioxidant activity and phenolic, flavonoid content of commonly consumed plant foods i.e. green leafy vegetables rich in these activities. Hence this study was initiated.

Materials and Methods

Four most commonly consumed leafy vegetables in Telangana State, i.e. Amaranth, Coriander, Gogu, and Mint was collected from the local market in fresh form. The procured leafy vegetables were open cooked and pressure cooked using normal tap water. Bioactive compounds were analyzed in raw (fresh) leafy vegetables as well as cooked leafy vegetables (both pressure and open cooked).

Reagents used were methanol, 6 N hydrochloric acid, Whatman No. 1 filter paper, distilled water, Gallic acid (GA), Folin-Ciocalteu reagent, sodium carbonate (7.5%), Rutin standard solution (10%), sodium nitrite (5 gm%), aluminium chloride (10 g%), sodium hydroxide (1 N), Trolox standard solution (10 mg%):, acetate buffer (0.2 M) (pH 3.6), hydrochloric acid (400 mM), TPTZ (2, 4, 6-tris (2-pyridyl)-s-triazine) (10 mM), ferric chloride (hexahydrate) (20 mM) (freshly prepared), FRAP working reagent (freshly prepared), Trolox standard solution (10 mg%), DPPH solution, and methanol.

Extraction of raw and cooked samples was done using 80% methanol acidified to pH 2.0 with 6N hydrochloric acid. Total phenolic content, flavonoids, and antioxidant activity was estimated in the extracts. Standard analysis procedures were followed for analysis for bioactive compounds in leafy vegetables, i.e. total phenolic content was analysed by Singleton *et al*. 1999 [28] method, total flavonoids by Zhishen *et al*., 1999 method. DPPH Radical-Scavenging Activity by Tadhani *et al*. 2007 [30] method and Ferric reducing antioxidant power (FRAP) was determined according to Benzie and Strain 1999 [31] methods.

Results and discussion

Total phenols in leafy vegetables

Leafy vegetables proved to be the most abundant in phenolic content amongst all vegetables. TPC of leafy vegetables ranged from 197.4 to 446.32 mg GAE/100g in Control extracts (raw form). The ranking of leafy vegetables when extracted conventionally (Control i.e raw form): Mint > Amaranth > Coriander >Gogu. The TPC of leafy vegetables enhanced after thermal treatment. The TPC was increased 2.79 to 72.92 upon open cooking, whereas in pressure cooking the increase in TPC was 9.98 to 92.1 per cent.

The total phenolic content of leafy vegetables were compared with in the group (total leafy vegetables) and between the groups i.e. raw, open and pressure cooking showed significance difference at 1% level. Details are presented in table 1 and Fig 1.

Table 1: Total Phenol Content of Leafy vegetables and Per cent Change on Cooking as Compared to Raw (mg GAE/100g)

S. No	Foods	Raw (Control)	Open cooked (T_1)	% Change	Pressure cooked (T_2)	% Change	F-value
1	Amaranth	259.05 (±1.89)	277.85(±1.22)	7.25	358.23(±2.21)	38.28	2503.9**
2	Coriander	239.46 (±3.07)	414.08(±1.8)	72.92	460.24(±2.78)	92.19	5940.4**
3	Gogu	197.4(±2.8)	202.92(±1.6)	2.79	217.11(±2.0)	9.98	64.5**
4	Mint	446.32 (±3.82)	664.93(±1.9)	48.98	797.26(±1.8)	78.62	13184**

Values are mean ±standard deviation of three observations
** Significant at 1% level

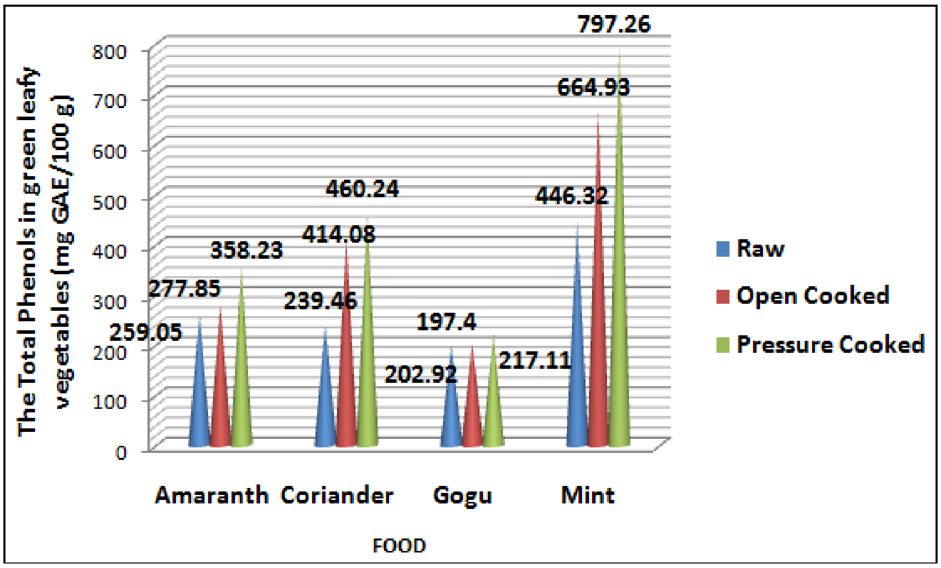

Fig 1: Effect cooking on total phenol content in leafy vegetables

Total Flavonoids in Leafy Vegetables

Leafy vegetables contained 99.62 to 186.8 mg RE/100g of flavonoids. The sequence of leafy vegetables according to their flavonoid content in the Control extracts (raw form) is: Mint >Gogu> Coriander leaves> Amaranth.

This flavonoid content further improved on cooking of leafy vegetables. 0.81 to 66.7% more flavonoid was recovered on open cooking (T_1) except in gogu it was decreased by -17.4% in open cooking. Further, pressure cooked extraction (T_2) increased the flavonoid content by 6.02 to 53.3% except gogu, it was decreased by -25.8% in pressure cooking.

The total flavonoid content of leafy vegetables were compared with in the group (total leafy vegetables) and between the groups i.e. raw, open and pressure cooking showed significance difference at 1% level in amaranth, gogu and mint whereas coriander showed no significant difference. Details are presented in Table 2 and Fig 2.

Table 2: Total Flavonoids Content of leafy vegetables and Per cent Change on Cooking as Compared to Raw (mg RE/100 g)

S. No	Foods	Raw (Control)	Open cooked (T_1)	%Change	Pressure cooked (T_2)	%Change	F-value
1	Amaranth	99.62±1.56	100.43±2.44	0.81646	105.62±1.15	6.026431	9.81**
2	Coriander	121.5±1.96	202.6±1.12	66.75543	186.3±2.36	53.30393	1548NS
3	Gogu	149.3±2.26	123.2±1.51	-17.4806	110.73±1.89	-25.8571	317.7**
4	Mint	186.8±1.19	223.24±1.86	19.52846	202.56±2.25	8.454249	302.1**

Values are mean ±standard deviation of three observations
** Significant at 1% level

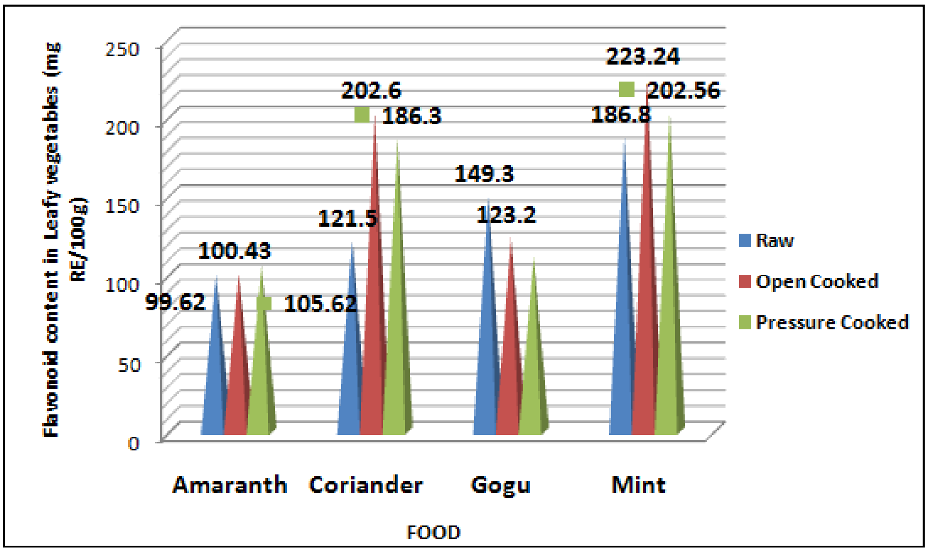

Fig 2: Effect cooking on flavonoid content in leafy vegetables

Antioxidant Activity

FRAP (Ferric Reducing Antioxidant Power)

FRAP of leafy vegetables ranged from 629 to 427 mg TE/100g on raw extraction (C). Mint leaves > Amaranth > Coriander >Gogu was the order of FRAP in fresh extract of leafy vegetables. However, on open cooking (T_1), 2.93 to 14.2% increase of AOA was observed in different leafy vegetables (Table 3 and Fig3). While on pressure cooking 4.15 to 6.4% increase of AOA was observed in leafy vegetables. The total AOA content of leafy vegetables were compared with in the group (total leafy vegetables) and between the groups i.e. raw, open and pressure cooking showed significance difference at 1% level.

Table 3: Antioxidant activity in leafy vegetables (by FRAP) and Per cent Change on Cooking as Compared to Raw (mg TE/100g)

S. No	Foods	Raw (Control)	Open cooked (T_1)	%Change	Pressure cooked (T_2)	%Change	F-value
1	Amaranth	427.08(±1.56)	439.62(±0.86)	2.93	450.2(±0.9)	5.413506	302.5**
2	Coriander	346.01(±0.99)	377.11(±2.61)	8.98	360.4±1.18)	4.158839	236.5**
3	Gogu	312.26(±1.86)	356.8(±1.46)	14.26	332.4(±2.21)	6.449753	426.7**
4	Mint	629.8(±1.7)	658.4(±3.01)	4.54	667.8(±2.9)	6.033661	170.4**
Values are mean ±standard deviation of three observations ** Significant at 1% level							

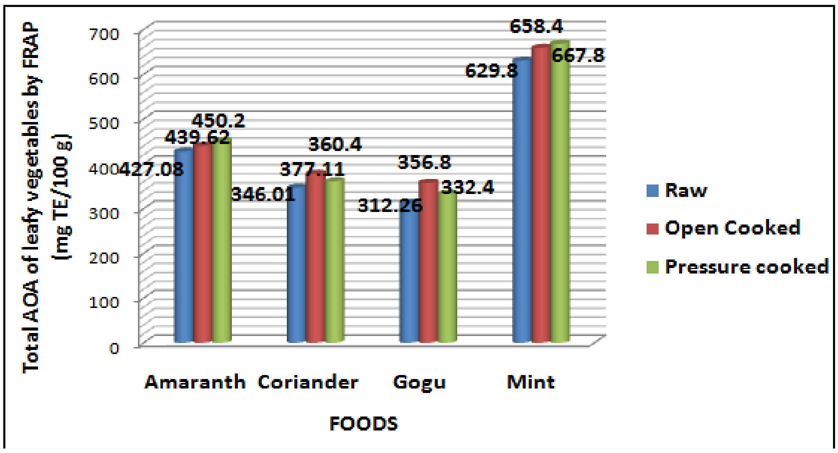

Fig 3: Effect cooking on flavonoid content in leafy vegetables

DPPH Radical Scavenging Ability

Leafy vegetables scored DPPHRSA ranging from 1366.45 to 345.81 mg TE/100g. Table 3 presents the results of DPPHRSA and the difference between the Control and other extracts of leafy vegetables. They ranked as follows when extracted conventionally i.e. raw form: Mint leaves > Coriander leaves > Amaranth>Gogu. Open Cooking (T_1), showed a rise of 4.73 to 50.47% radical scavenging ability and pressure cooking showed a rise of28.3 to 102.3% radical scavenging ability except gogu it showed reduce in AOA by -2.72%. The increase was observed due to release of bound phenolic compounds as well as carotenoids.

The total AOA content of leafy vegetables were compared with in the group (total leafy vegetables) and between the groups i.e. raw, open and pressure cooking showed significance difference at 1% level except gogu it showed no significant difference (Table 4 and Fig 4).

Table 4: Antioxidant activity in leafy vegetables (by DDPH) and Per cent Change on Cooking as Compared to Raw (mg TE/100g)

S. No	Foods	Raw (Control)	Open cooked (T_1)	%Change	Pressure cooked (T_2)	%Change	F-value
1	Amaranth	410.9(±2.55)	521.4(±0.56)	26.89219	527.59(±1.34)	28.39864	4507.68**
2	Coriander	469.81(±1.71)	887.34(±2.29)	88.8721	951.83(±1.62)	102.5989	56885.7**
3	Gogu	345.81(±2.43)	362.2(±1.44)	4.739597	336.37(±2.42)	-2.72982	111.17NS
4	Mint	1366.45(±2.34)	2056.1(±2.89)	50.4702	1856.5(±2.69)	35.863	53970.5**
Values are mean ±standard deviation of three observations ** Significant at 1% level							

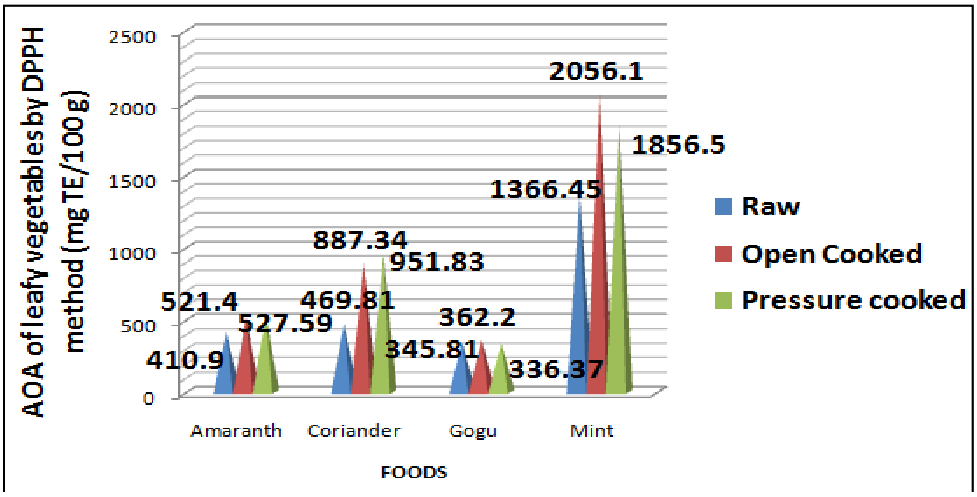

Fig 4: Effect cooking on AOA (by DPPH) in leafy vegetables

Summary and Conclusion
The commonly consumed leafy vegetables (Amaranth, Coriander, Gogu and Mint) in Telangana state were collected from the local market. They were subjected to most common cooking methods i.e. open cooking and pressure cooking. Bioactive compounds were analyzed in the leafy vegetables in raw as well as cooked form. The results revealed that, leafy vegetables contained significant amounts of phenolics, flavonoids, and antioxidant capacity. Among the leafy vegetables studied, mint showed high amounts of phenols, followed by amaranth, coriander, and gogu. High flavonoid content was found in mint, followed by gogu, coriander, and Amaranth. Antioxidant activity (FRAP) was noted to be high in mint followed by amaranth, coriander and gogu. DPPHRSA was found to be high in mint followed by coriander, amaranth and gogu. Results were observed that both open and pressure cooking processes, increased the total phenol content in all leafy vegetables. Increase in flavonoid content was observed upon cooking in all leafy vegetables, except coriander with no significant difference. Ferric reducing antioxidant power was increased upon both open and pressure cooking. DDPHRSA was also found to be increased upon cooking in all leafy vegetables (open and pressure cooking) except gogu, it showed no significant difference.
The study concludes that, both the open cooking and pressure cooking methods can be recommended for utilization of leafy vegetables. In turn they may help to reduce the risks of many age-related degenerative diseases.

Acknowledgements
This work has been supported by the Indian council of Agricultural Research (ICAR), Central Institute for Women in Agriculture (CIWA) and AICRP-Home Science, PJTSAU.

Reference
1. Panczenko-Kresowska B, Wolnerodniki a żywienie. Wiadomościzielarskie. 1997; 10:17-18.
2. Rietjens IMCM, Boersma MG, de Haan L, Spenkelink B, Awad HM, Cnubben NHP *et al*. The pro-oxidant chemistryof the natural antioxidants vitamin C, vitamin E, carotenoidsand flavonoids. Environmental Toxicology and Pharmacology. 2002; 11(3-4):321-333.
3. Wong SP, Leong LP, Koh W, Hoe J. Antioxidant activitiesof aqueous extracts of selected plants. Food Chemistry. 2006. 99(4):775-783.
4. Bawa S. Rolaflawonoidów W. zapobieganiu powstawania chorób cywilizacyjnych. [W:] Brzozowska A., Gutkowska K (red.) Wybraneproblemynauki o żywieniuczłowieka uprogu XXI wieku. Warszawa: Wyd. SGGW, 2004, 117-122.
5. Shukla P, Kumar R, Raib AK. Detection of minerals in green leafy vegetables using laser induced breakdown spectroscopy. Journal of Applied Spectroscopy. 2016; 83(5):872-877.
6. Da Silva Dias João Carlos, Imai S. Vegetables consumption and its benefits on diabetes. Journal of Nutritional Therapeutics. 2017; 6(1):1-10.
7. Pokorny J, Yanishlieva N, Gordon MH. Antioxidants in food: Practical applications. Woodhead Publishing Ltd., Cambridge, U.K, 2001.
8. Getoff N. Anti-aging and aging factors in life: The role of free radicals. Radiat. Phys. Chem. 2007; 76:1577-1586.
9. Kaur C, Kapoor HC. Antioxidant activity and total phenolic content of some Asian vegetables. Intl. J. Food Sci. Technol. 2002; 37:153-161.
10. Tang SY, Whiteman M, Peng ZF, Jenner A, Yong EL, Halliwell B. Characterization of antioxidant and antiglycation properties and isolation of active ingredients from traditional Chinese medicine. Free Radical Biol. Med. 2004; 36:1575-1587
11. Wong C, Li H, Cheng K, Chen F. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem. 2006; 97:705-711.
12. Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem. 2006; 94:550-557.
13. Kaur C, Kapoor HC. Antioxidant activity and total phenolic content of some Asian vegetables. Intl. J Food Sci. Technol. 2002; 37:153-161.
14. Hunter KJ, Fletcher JM. The antioxidant activity and composition of fresh, frozen, jarred and canned vegetables. Innovat. Food Sci. Emerg. Technol. 2002; 3:399-406.
15. Sun J, Chu YF, Wu X, Liu RH. Antioxidant and antiproliferative activities of common fruits. J Agr. Food Chem. 2002; 50:7449-7454.
16. Lim P, Wuenschell GE, Holland V, Lee D, Pfeifer GP, Rodriguez H, Termini J. Peroxyl radical mediated oxidative DNA base damage: Implications for lipid peroxidation induced mutagenesis. Biochemistry. 2004; 43:15339-15348.
17. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agr. Food Chem. 2001; 49:5165-5170.
18. Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem. 2006; 94:550-557.
19. Young IS, Woodside JV. Antioxidants in health and disease. J. Clin. Pathol. 2001; 54:176-186.
20. Zhang D, Hamauzu Y. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chem. 2004; 88:503-509.
21. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 Chinese medicinal plants associated with anticancer. Life Sci. 2004; 74:2157-2184.
22. Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Imams K. The comet assay with 8 mouse organs: Results with 39 currently used food additives. Mutat. Res./Genet. Toxicol. Environ. Mutagen. 2002; 519:103-109.
23. Gupta S, Lakshmi JA, Manjunath MN, Prakash J. Analysis of nutrient and antinutrient content of underutilized green leafy vegetables. LWT Food Sci Technol. 2005; 38:339-345
24. Harris PJ, Ferguson LR. Dietary fibre: its composition and role in protection against colorectal cancer, Mutation Research. 1993; 290(1):97-110.
25. Scalbert A, Manach C, Morand C, em´esy CR, Jim´enez L. Dietary polyphenols and the prevention of diseases, Critical Reviews in Food Science and Nutrition. 2005; 45(4):287-306.
26. Rochfort S, Panozzo J. Phytochemicals for health, the role of pulses, Journal of Agricultural and Food Chemistry. 2007; 55(20):7981-7994.
27. Sreeramulu D, Reddy CVK, Raghunath M. Antioxidant activity of commonly consumed cereals, millets, pulses and legumes in India, Indian Journal of Biochemistry and Biophysics. 2009; 46(1):112-115.

28. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin- Ciocalteu reagent. Methods of Enzymology. 1999; 299:152-158.
29. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999; 64(4):555-559.
30. Tadhani MB, Patel VH, Subhash R. In vitro antioxidant activities of Stevia rebaudiana leaves and callus. Journal of Food Composition and Analysis. 2007; 20(3):323-329.
31. Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology. 1999; 299:15-27.