Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 1, 2013

Research Article

# EVALUATION OF ANTIOXIDANT POTENTIAL IN SELECTED LEAFY VEGETABLES OF ODISHA, INDIA

**RASMIRANI ROUTRAY[1], MANORANJAN KAR[2] AND RAJANI KANTA SAHU[1]**

**[1]Department of Botany, B.J.B (A) College, Bhubaneswar-751014, Odisha, India, [2]P.G. Department of Botany, Utkal University, Vanivihar, Bhubaneswar-751004, Odisha, India.**

*Received: 21 Oct 2012, Revised and Accepted: 05 Dec 2012*

**ABSTRACT**

Generally vegetables represent a class of under exploited plants that are stipulated to be rich source of natural antioxidant. Seven edible widely used leafy vegetables of Odisha have been analyzed for their DPPH radical scavenging activity, namely *Amaranthus tricolor, Amaranthus viridis, Brassica oleracea, Brassica campestris, Basella alba, Cucurbita maxima, Cicer arietinum* using methanol, ethanol, petroleum ether as solvent. Their total phenolic content was measured by Folin- ciocalteu reagent. The plant extracts were found to have different levels of antioxidant properties in the system tested. Correlation analysis established a positive Correlation between the phenolic contents and the in vitro free radical scavenging activity of the plant extracts. In all the species methanolic and ethanolic extract gave maximum yield of crude extract, phenol content as well as antioxidant activity. Highest antioxidant activity was demonstrated in *Brassica campestris* followed by *Amaranthus tricolor* and *Cucurbita maxima*. Accordingly minimum $IC_{50}$ values were obtained in the concentration of maximum antioxidant activity. These values are comparable with ascorbic acid as standard. The conclusions drawn from the study suggest that the rich phytochemical contents especially phenolics of the leafy vegetables and good antioxidant activity may be responsible for its wide and popular use in any balanced diet.

**Keywords**: Leafy vegetables, Antioxidant activity, Phenols, DPPH.

## INTRODUCTION

Providing modern healthcare to rural people in India is still a far-reaching goal due to economic constraints [1]. Hence, people mainly depend on the locally available plant materials to cure various health disorders [2,3]. Plants possess components, which render beneficial properties [4]. Hence, currently attention is being drawn towards exploring plant sources for substances that provide nutritional and pharmaceutical advantages to humans. Green leafy vegetables (GLVs) are a good source of minerals and vitamins. The ethno-botanical reports offer information on medicinal properties of GLVs like anti-diabetic [5], anti-histaminic [6], anti-carcinogenic [7], hypolipidemic [8] and anti-bacterial activity [9]. In most studies, crude extracts of GLVs were used to demonstrate their health beneficial potency.

Generation of oxygen radicals, such as superoxide radical ($O_2^{\cdot-}$), hydroxyl radial (·OH) and non free radical species such as $H_2O_2$ and singlet Oxygen ($^1O_2$) is associated with cellular and metabolic injury and accelerating aging cancer cardiovascular diseases, neurodegenerative diseases and inflammation [10,11,12]. Previous epidemiological studies have consistently shown that consumption of fruits and vegetables has been associated with reduced risk of chronic diseases, such as cardiovascular diseases and cancers [13,14,15] and neurodegenerative diseases including Parkinson's and Alzheimer's diseses [16] as well as inflammation and problems caused by cell and cutaneous aging [17]. Fruits and vegetables contain different antioxidant compounds such as Vitamin C, vitamin E, and carotenoids, whose activities have been established in recent years. Flavonoids, tannins and other phenolic constituents present in food of plant origin are also potential antioxidants [18,19]. Therefore the objectives of this present study were (1) To determine the antioxidant, phenolic content of commonly consumed leafy vegetables in Odisha in Methanol, ethanol and petroleum ether extracts with in vitro antioxidant assay such as DPPH radical scavenging assay. (2) To determine the level of correlation between the phenolic contents and antioxidant activity.

## MATERIALS AND METHODS

### Plant Materials

Leafy vegetables were collected from Bhubaneswar and its periphery of Odisha. The plant specimens were further authenticated at the Regional plant Resource Center located at Bhubaneswar. The leaves were washed, shade dried and then milled into coarse powder by wind meter and pestle. Seven leafy vegetables which were tested for their antioxidant activity were 1.*Cicer arietinum* L., 2.*Brassica oleracea*, 3.*Brassica campestris* L., 4.*Amaranthus tricolor* L, 5.*Amranthus viridis* L., 6.*Basella alba* L., 7.*Cucurbita maxima* L. Botanical information, Plant parts used and their medicinal importance were mentioned in Table-1.

**Table 1: Botanical information and medicinal uses of leafy vegetables used in the study**

| Botanical Name | Family | Common Name | Local Name | Parts used | Medicinal Uses |
|---|---|---|---|---|---|
| *Amaranthus viridis L.* | Amarantheceae | Green Amaranth | Khada Saga | Leaf | Used to cure Piles & Stomach eches |
| *Amaranthus tricolor l.* | Amarantheceae | Red Amaranth | Khada Saga | Leaf | Enhance eye sight<br>Antioxidant |
| *Cucurbita maxima l.* | Cucurbitaceae | Pumpkin | Kakharu | Leaf | Anti helminthic |
| *Brassica oleraceae L.* | Brassicaceae | Broccoli | Phula Kobi | Leaf | Anti cancer |
| *Brassica campestris l.* | Brassicaceae | Mustard | Sorisa | Leaf | Anti cancer<br>Anti hemorrhage |
| *Cicer arietinum l.* | Fabaceae | Chick pea | Buta | Leaf & small stem | Anti inflammatory |
| *Basella alba l.* | Bassellaceae | Indian spinach | Poi | Leaf | Anti mutagenic |

### Preparation of the plant extract

The powdered plant material was weighed and extracted with solvents like Methanol, Ethanol and Hexane using soxhlet apparatus for 48 hours. The solvent was then removed under reduced pressure by using rotary evaporator, which obtained a greenish-black coloured sticky plant material. The remaining residue was stored in dessicators for further use.

**Chemicals Required**

Chemicals such as DPPH(1, 1, Diphenyl-2-Picrylhydrazyl), Methanol, Folin - Ciocalteu reagent, sodium carbonate, Catechol were used. All reagents were of analytical grade.

**DPPH radical Scavenging assay**

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Brand-Williams [20] with slight modification. The following concentrations of extracts were prepared 50µg/ml, 100 µg/ml, 200 µg/ml and 250 µg/ml. All the solutions were prepared with methanol 1 ml of each prepared concentration was mixed with 1 ml of 100 µm DPPH solution in methanol. Experiment was done in triplicate. The test tubes were incubated for 30 min. at room temperature in dark and the absorbance was measured at 517nm. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid was used as a standard and the same concentration were prepared as to test solutions. The difference in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as percentage scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

Scavenging effect (%) = Ac-As/Ac×100

Where, Ac is the absorbance of the control

As is the absorbance of the sample

**Determination of total phenol content**

Phenol was determined by folin-Ciocalteau reagent in alkaline medium and was expressed in terms of catechol used as standard [21] in mg/g dry sample.

**Statistical Analysis**

Experimental results were expressed as mean±standard deviation. All measurements were replicated three times. The datas were correlated using Karl Pearson's correlation coefficient at P<0.05. The pc software SPSS 20.0 was used for the calculations.

**RESULTS AND DISCUSSION**

DPPH radical scavenging activity is one of the most widely used methods for screening the antioxidant activity of plant extracts. The extents of DPPH radical scavenging at different concentrations (50-250 µg/ml) of the extracts were measured with ascorbic acid as standard. The radical scavenging effects were found to increase with increasing concentration and quite close to the standard(96.43% at 250µg/ml)(fig:1) indicating their strong antioxidant activity. The results obtained were clearly indicate the potential of the plant extracts in scavenging free radicals as high percentage inhibition in the range of 80%-99% (Table-2, 3&4) were observed at 250 mg/ml for the methanolic extracts of the seven plant species. Maximum activity have been observed in *Brassica campestris* (99.54%) followed by *Amaranthus tricolor* (94.21%), *Cucurbita maxima* (93.45%), *Brassica oleracea* (90.59%), *Amaranthus viridis (88.95), Basella alba(84.5), Cicer arientinum* (80.57%), with $IC_{50}$ values of 26.93 µg/ml, 27.63 µg/ml, 31.7 µg/ml and 32.46 µg/ml, 35.09 µg/ml, 37.03 µg/ml, 38.63 µg/ml (Table-5, Fig:1) and 27.4µg/ml for ascorbic acid(reference standard). The result of the present investigation shows that *Amaranthus tricolor* and *Brassica campestris* are rich in phenols and antioxidant activity in comparison to *Cucurbita maxima, Amaranthus viridis, Basella alba, Cicer arietinum, and* accordingly lowest $IC_{50}$ value was found in *Brassica campestris* and highest was found in *Cicer arietinum* which has least antioxidant activity. While comparing the two species of *Amaranthus tricolor* (red amaranth) which has red stem and *Amaranthus viridis* which has green stem, it was found that the red stem has more phenolic content and high antioxidant capacity than the green stem.It may be due to the presence of red anthocyanin pigment in *Amaranthus tricolor*.

**Table 2: DPPH scavenging activities of leafy vegetables in Methanolic extracts (values represent means±.S.D, n=3)**

| Conc.of extracts (µg/ml) | AG | AT | BO | BC | CM | BA | CA |
|---|---|---|---|---|---|---|---|
| 50 | 71.23±.19 | 90.47± 19 | 77±.19 | 92.83±.277 | 78.85±.19 | 67.5±.11 | 64.7±.11 |
| 100 | 77.01±.11 | 91.80±.195 | 79.42±.19 | 94.66±.19 | 82.34±.11 | 71.23±.19 | 67.73±.11 |
| 150 | 82.21±.29 | 92.88±.11 | 83.16±.11 | 97.45±.11 | 85.33±.19 | 75.23±.19 | 72.57±.19 |
| 200 | 86.09±.19 | 93.45±.11 | 87.16±.11 | 98.34±.11 | 90.09±.19 | 78.66±.19 | 77.2±.11 |
| 250 | 88.95±.19 | 94.21±.11 | 90.59±.11 | 99.54±.11 | 93.45±.11 | 84.5±.478 | 80.57±.19 |

**Table 3: DPPH scavenging activities of leafy vegetables in Ethanolic extracts(**values represent means±SD, n=3)

| Conc. of Extracts (µg/ml) | AG | AT | BO | BC | CM | BA | CA |
|---|---|---|---|---|---|---|---|
| 50 | 66.47±.19 | 73.26±.11 | 71.54±.11 | 88.38 ±.19 | 75.42 ±.19 | 63.73±.11 | 58.85±.19 |
| 100 | 73.39±.11 | 77.01±.11 | 75.73±.11 | 91.99±.195 | 78.34 ±.219 | 67.42±.19 | 62.66±.19 |
| 150 | 78.53±.11 | 82.21 ±.29 | 79.93±.1154 | 93.77 ±.11 | 81.33± 19 | 71.48 ±.11 | 67.42 ±.19 |
| 200 | 82.47±.19 | 86.09 ±.19 | 83.42±.19 | 95.93 ±.1154 | 85.20 ±.11 | 74.09 ±.19 | 71.54±.11 |
| 250 | 85.83 ±.11 | 91.61 ±.19 | 86.66 ±.19 | 97.52 ±.19 | 88.96 ±.205 | 75.23 ±.19 | 78.97 ±.11 |

**Table 4: DPPH scavenging activities of leafy vegetables in Petroleum Ether extract(**values represent means±SD, n=3)

| Conc. of Extracts (µg/ml) | AG | AT | BO | BC | CM | BA | CA |
|---|---|---|---|---|---|---|---|
| 50 | 63.48±.11 | 64.95±.19 | 65.2±.11 | 86.38±.19 | 64.41±.241 | 60.12±.11 | 50.28±.19 |
| 100 | 67.23±.19 | 71.1± 29 | 69.26±.11 | 90.34±.11 | 68.76±.19 | 62.34±.11 | 54.47±.19 |
| 150 | 69.9±.19 | 74.85±.19 | 72.63±.219 | 91.99±.195 | 73.33±.19 | 65.07±.11 | 58.78±.11 |
| 200 | 74.09±.19 | 78.66±.19 | 76.76±.19 | 93.77±.11 | 77.33±.19 | 67.99±.195 | 62.91±.11 |
| 250 | 78.66±.19 | 83.61±.19 | 80.69±.11 | 95.93±.1154 | 82.85±.19 | 72.19±.19 | 67.67±.11 |

Comparisons between the concentrations are Significant at 1percent level of significance.

AG- *Amaranthus viridis*, AT- *Amaranthus tricolor*, BO-*Brassica oleracea*, BC-*Brassica Campesteris*, CM-*Cururbita maxima*, BA- *Basella alba,* CA- *Cicer arietinum*

The antioxidant activities of seven leafy vegetables of Odisha were measured in DPPH radical scavenging assay at different concentrations ranging from 50 to 250 µg/ml in 3 different solvent like Ethanol, Methanol and petroleum ether(Table- 2, 3 &4). The percentage inhibition for each concentration and $IC_{50}$ values of the extracts were calculated(Table-5)(Fig:1). An $IC_{50}$ value is the concentration of the sample required to scavenge 50% of the free radicals present in the system. $IC_{50}$ value is inversely related to the activity. The extracts were found to have different levels of antioxidant activity in different solvents.

Crude extracts were obtained using Methanol, Ethanol, and Petroleum ether as solvent. Despite variation in the extraction yields, the antioxidant contents found were very good indicating that extraction was efficient. The yields of extracts using Methanol, Ethanol and Petroleum ether were varied may be due to the polarity of solvents used in extraction process.

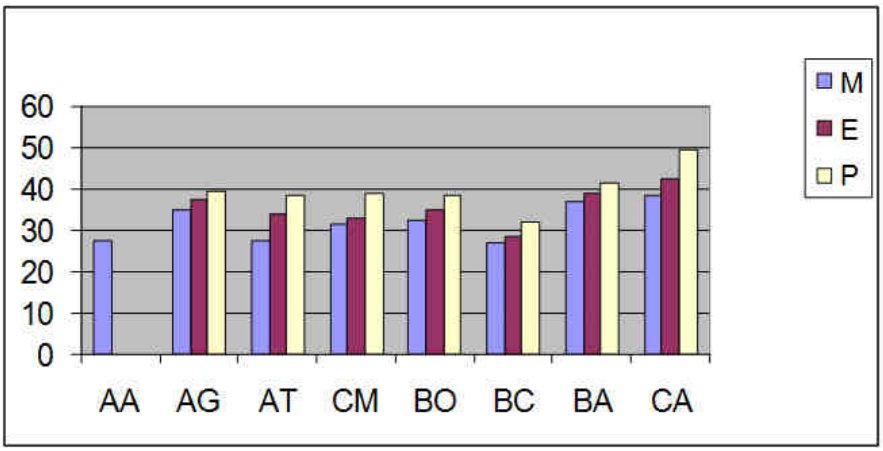


**Fig. 1: It Shows $IC_{50}$ values of seven leafy vegetables in different extracts**

**Table 5: Yield of crude extract, Phenol content and $IC_{50}$ values of leafy vegetables in different solvents.**

| Plants Name | Solvent Used | Yield of crude extract (gm/100gm) | Phenol content (mg/gm) | $IC_{50}$ Values (µg/ml) |
|---|---|---|---|---|
| *Amaranthus gigantea* | Methanol | 13.18 | .1 | 35.09 |
| | Ethanol | 14.5 | .02 | 37.61 |
| | Petroleum ether | 3.5 | .42 | 39.4 |
| *Amaranthus tricolor* | Methanol | 1 | 1.29 | 27.63 |
| | Ethanol | 2 | 1 | 34.12 |
| | Petroleum ether | 0.6 | 1.12 | 38.49 |
| *Cucurbita maxima* | Methanol | 5.87 | 1.23 | 31.70 |
| | Ethanol | 1.3 | .4 | 33.14 |
| | Petroleum ether | 1 | 0.7 | 38.83 |
| *Brassica oleracea* | Methanol | 9 | 1.19 | 32.46 |
| | Ethanol | 7.2 | 0.43 | 34.94 |
| | Petroleum ether | 1.3 | 0.82 | 38.34 |
| *Brassica campestris* | Methanol | 2 | 1.38 | 26.93 |
| | Ethanol | 1.2 | 0.2 | 28.3 |
| | Petroleum ether | 1 | 0.29 | 31.78 |
| *Cicer arietinum* | Methanol | 7 | 0.38 | 38.63 |
| | Ethanol | 8.9 | 0.67 | 42.48 |
| | Petroleum ether | 2 | 0.7 | 49.72 |
| *Basella alba* | Methanol | 2 | 0.43 | 37.03 |
| | Ethanol | 6 | 0.27 | 39.22 |
| | Petroleum ether | 1 | 0.34 | 41.58 |

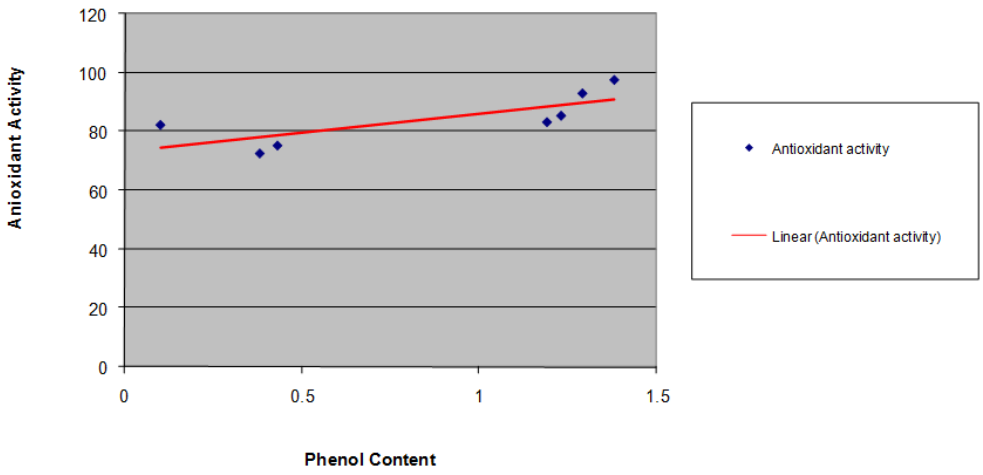


**Fig. 2: It Shows Correlation between antioxidant activity and total phenol content in leaves of seven leafy vegetables, r=0.77**

In the quantitative analysis of antioxidative components the total phenolic contents in methanol, ethanol and petroleum ether extracts of seven leafy vegetables given in the table-4. Phenols are known to possess a wide range of therapeutic uses, such as antioxidant, antimutagenic, antitumour activities [22]. Recent studies have shown that many poly phenols contribute significantly to the antioxidant activity [23,24] and act as highly effective free radical scavengers due to their redox properties that allow them to get as reducing agents [25]. Several comprehensive works have been done on the effects of phenolic compounds and antioxidant activity (A positive correlation) of *Amaranthus tricolor* [26] *Basella alba [27] Brassica oleracea* [28]. This same trend was also obtained in our study. A significant correlation was observed between phenolic contents and the scavenging of DPPH radical (methanolic extracts) in all the leafy vegetables($r=0.77$, $p<0.05$) (Fig:2). When comparing the data *Brassica campestris* had the highest phenolic content (1.38mg/g) followed by *Amaranthus tricolor, Cucurbita maxima, Brassica oleracea, , Amaranthus viridis, Basella alba, Cicer arietinum.* These results (table-5) indicated that the radical scavenging capacity of each extract might be related to their concentration of phenolic hydroxyl group.

## CONCLUSION

From this study it is concluded that the antioxidant capacities, total phenolic content of the seven leafy vegetables commonly consumed in Odisha (India) are considered as good sources of antioxidants as observed in DPPH scavenging assay. However among all *Brassica campestris* has the highest antioxidant activity. The results of the three leafy vegetables *Amaranthus tricolor, Brassica oleracea, cucurbita maxima* are equally potent like Brassica proving its wide use as food with nutritional and medicinal value.

## ACKNOWLEDGMENTS

The authors are thankful to KIIT School of Biotechnology (KIIT University) for providing necessary facilities for carrying out the experimental work.

## REFERENCES

1. Grover JK, Yadav S, & Vats V.Medicinal plants of India with antidiabetic potential. J of Ethnopharmacology 2002;81:81-100.
2. Chopra RN, Nayar SL, & Chopra IC. Glossary of Indian medicinal plants. New Delhi: CSIR. 1956.
3. Grover JK & Vats V. Shifting paradigm" from conventional to alternate medicine", an introduction on traditional Indian medicine. Asia-Pacific Biotechnology news 2001;5:28-32.
4. Tanabe H, Yoshida M & Tomita N. Comparison of the antioxidant activities of 22 commonly used herbs and spices on the lipid oxidation of pork meat. Animal science J 2002 ;73: 389-393.
5. Kesari AN, Gupta RK & Watal G. Hypoglycemic effects of *Murraya koengii* on normal and alloxan -diabetic rabbits. J of Ethnopharmacology 2005;97:247-251.
6. Yamamura S, Ozawa K, Ohtani, K, Kasai R & Yamasaki K.Antihistaminic flavonoids and aliphatic glycosides from *Mentha spicata*. Phytochemistry 1998;48:131-136.
7. Rajesh Kumar NV, Joy KL, Kuttan G, Ramsewak RS, Nair MG & Kuttan, R. Antitumour and anticarcinogenic activity of *Phyllanthus amarus* extract.J of Ethnopharmacology 2002;81:17-22.
8. Khanna AK, Rizvi F & Chander R. Lipid lowering activity of *Phyllanthus niruri* in hyperlipemic rats. J of Ethnopharmacology 2002; 82:19-22.
9. Kubo I, Fijita K, Kubo A, Nehei K, Nehei K & Gura T Antibacterial activity of coriander volatile compounds against *Salmonella choleraesuis*. J of Agricultural and Food Chemistry 2004;52:3329-3332.
10. Ames BN Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. Science 1983;221:1256-1264.
11. Stadtman ER Protein oxidation and aging. Science. 1992; 257:1220-1224.
12. Sun Y Free radicals, antioxidant enzymes and carcinogenesis. Free Radical Biology and Medicine. 1990;8:583-599.
13. Gerber M, Boutron-Ruault MC, Hercberg S, Riboli E, Scalbert A & Siess MH Food & cancer: state of the art about the protective effect of fruits and vegetables. Bulletin du Cancer. 2002; 89(3):293-312.
14. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, et al.Bioactive compounds in food, their role in the prevention of cardiovascular diseases and cancer.American J of Medicine 2002; 113(9B):71S-88S.
15. Serafini M, Belleco R, Wolk A and Ekstorm AM. Total antioxidant potential of fruit & vegetables and risk of gastric cancer.Gastroenterology 2002;123(4):985-991.
16. Di Matteo V & Esposito E. Biochemical and theraupeutic effects of antioxidant in the treatment of Alzeimer's disease, Parkinson's disease and amyotrophic lateral sclerosis.Current Drug Target CNS and neurological Disorder 2003; 2:95-107.
17. Ames BN, Shigenaga MK, and Hagen, TM. Oxidants, antioxidants and the degenerative diseases of aging. Proceedings National Academy of Science USA 1993;90;7915-7922.
18. Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP and Rice-Evans Cl.Polyphenolic flavanols as scavfngers of aqueous phase radicals and as chain breaking antioxidants.Archives of Biochemistry and Biophysics 1995;322(2):339-346.
19. Van Acker SABE, Van den Vijgh WJF and Bast F.Structural aspects of antioxidant activity of flavonoids.Free radical Biology and Medicine 1996; 20(3): 331-342.
20. Brand-williams W, Cuvelier ME and Berset C.Use of free radical method to evaluate antioxidant activity.Lebensmittel Wissenschaft and Technologie 1995;28(1):25-30.
21. Sadasivam S, Manikam A Biochemical Methods.India. Wiley Eastern Limited 1992.
22. Othman A, Ismail A, Ghani NA and Adenan I.Antioxidant capacity and phenolic content of cocoa beans.Food chemistry 2007;100:1523-1530.
23. Demla M, Verma H.Invitro antioxidant activity, total phenolic and total flavonoid content of different extracts of *Solanum xanthocarpum* Berries.Int J pharm pharm sci 2012;4:154-157.
24. Adithya ES, Sasikumar JM, Krishnakumar KA, Lakshmi MS, Christabel H.Invitro antioxidant activity, mineral content and HPLC analysis of *Talinum portulacifolium*(forssk.) asch.ex schweinf, leaf & stem.Int J Pharm Pharm sci 2012;4:423-429.
25. Luo XD, Basile MJ, Kennelly EJ.Polyphenolic antioxidants from the fruits of *Chrysophyllum cainito* L.(star apple).J of Agricultural and Food Chemistry 2002; 50: 1379-1382.
26. Khandaker L, Ali B, Md. Oba S. Total polyphenol and Antioxidant Activity of Red Amaranth (*Amaranthus tricolor* L.) as affected by different Sunlight level. Japanese Society for Horticultural Science 2008;77(4):395-401.
27. Olajine AA, Azeez L. Total antioxidant activity, phenolic, flavonoid and ascorbic acid content of Nigerian vegetables. African J of Food Science and Technology 2011; 2:022-029.
28. Koksal E, Gulcin I. Anioxidant activity of Cauliflower (*Brassica oleracea* L.). Food Chemistry 2008;32:65-78.