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PHYTOCHEMICAL ANTIOXIDANT PROPERTIES IN GREEN AND PURPLE VARIETIES OF CABBAGE

Bunga.Thirupathi*, Shankar. Gangadhar Rao, Janapatla. Sravanthi, Ramavath. Amrutha kalyani

Department of Botany, University College of Science, Osmaina University.
Hyderabad 500007, Telangana State, INDIA.

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***Correspondence for Author**
Bunga. Thirupathi
Department of Botany, University College of Science, Osmaina University.
Hyderabad 500007, Telangana State, INDIA.

ABSTRACT

Phytochemical antioxidant properties in green and purple varieties of ***Brassica oleracia var capitata*** (cabbage) collected from local market of Hyderabad city were estimated. Concentrations of secondary metabolites such as proanthocyanidins, flavonoids, flavonols, phenols, anthocyanins were recorded maximum in purple variety as compared to green. Peroxidise activity showed maximum in purple variety. Polyphenoloxidase, catalase, glutathionereductase, showed maximum in green variety. ABTS, DPPH and FRAP antioxident activities were recorded maximum in green variety.

KEYWORDS: Antioxidants, secondary metabolites, antioxidant assays, phytochemicals, of *Brssica oleracia* varieties.

INTRODUCTION

Cabbage (*Brassica oleracea* or variants) is a most popular leafy green vegetable or purple biennial plant, grown as an annual crop in the world for its dense-leaved heads. The whole plant can be consumed raw as salad or cooked. There are three major types of cabbage: green, red, and Savoy Cabbage Heads generally range from (0.5 to 4 kilograms). The colour of green cabbage ranges from pale to dark green. Both green and red cabbages have smooth-textured leaves. Red cabbage has leaves that are either crimson or purple with white veins running through it.

Cabbage grows best in a relatively cool and humid climate. [1], 18 °C to 20 °C, with soil pH from 5.5 to 6.0, well drained loamy soils well supplied with organic matter.

Cabbage is widely used as a vegetable throughout the world because of its high nutritional value and diseases prevention properties. It is used in cancer prevention. [2] and as an, anti-inflammatory and antioxidant plant.[3] Cabbage juice is used for peptic ulcers. [4] and cholesterol reduction, Important phytochemicals which impart health benefits are present in cabbage. It is a rich source for vitamin C.[5] β carotene, poyphenols, anthocyanins, glucosinolates, glutamine, minerals such as manganese, calcium, zinc, potassium, phosphorus, vitamins like thiamine, riboflavin, pantothenic acid and folate.

Cabbage as become Internationally most popular vegetable throughout the world because of its adaptability to wide range of climatic conditions and soil, its rich phytochemicals ,high nutrient values, ease of production and storage.

MATERIALS AND METHODS

Chemicals required: TPTZ, NADP, ABTS, (2, 2'-azinobis3-ethylbenzthiazoline-sulphonicacid), DPPH (2, 2-Diphenyl -2-picryl hydrazine) obtained from Sigma –Aldrich and E Merck only.

The green and purple varieties of *Brassica oleracia* plants were collected from local market of Hyderabad city and washed with water, sun dried for 7days, pulverized in mill and sieved and stored in an airtight container for further use. Ten grams of dried leaf powder were extracted by maceration in 100ml of methanol at 30^0 C overnight, followed by extracting and stirring with 100ml of distilled water overnight, centrifuged at 5000 rpm for 15 to20 min and the supernatant were pooled and made up to 100ml.

Phenolic contents in the extracts were determined by the modified Folin ciacalteau method. [6] 2ml of the leaf extract was mixed with 5ml Folin ciacalteau reagent (diluted with water 1:10v/v) and 4ml (75g/l) of sodium carbonate .The tubes were vortexed for 15sec and allowed to stand for 30min at 40^0 C for colour development .Absorbance was then measured at 765nm using Shimadzu 160A UV-VIS spectrophotometer . Total phenolic contents were expressed as mg/g Dry Wt gallic acid equivalent.

Aluminium chloride colorimetric method was used for the determination of flavonoids. [7] 0.5ml of leaf extract in methanol were separately mixed with 1.5ml of methanol ,0.1ml of 10% aluminium chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. Kept at room temperature for 30 minutes. The absorbance of the reaction mixture was recorded at

415nm with a Shimadzu 160 A UV/Visible spectrometer .The calibration curve was prepared by preparing quercetin concentration 12.5 to 100µg/ml.

Total flavonols in the plant extracts were estimated using the method of. [8] To 2.0ml of the sample, 2.0ml of 2%AICl, ethanol and 3.0ml(50g/l) sodium acetate solution were added .The absorption was read at 440nm by Shimadzu 160A UV-VIS spectrophotometer after 2.5h at 20^0 C. The total flavonols content was calculated as quercetin (mg/g) using the following equation based on the calibration curve.

$Y=0.0255x$, $R^2=0.9812$, where x is the absorbance and is quercetin equivalent.
Determination of proanthocyanidins was based on the procedure reported by. [9] A volume of 0.5 ml of the extract solution was mixed with 3ml of 4% vanillin-methanol solution and 1.5 ml of HCL ,the mixture was allowed to stand for 15min , the absorbance was measured with Shimadzu 160A UV-VIS Spectrophotometer at 500nm .Total proanthocyanidin content were expressed as catechin equivalent(mg/g).

The total anthocyanins content was determined according to pH differential method by. [10] The leaf extract was dissolved in potassium chloride –hydrochloride acid buffer solution pH1.0 and sodium acetate trihydrate ($CH_3COONa.3H_2O$) buffer solution pH4.5.Methanolic extract with 3.6ml of the corresponding buffers and read against water as a blank at 510 and 700nm.Absorbance was calculated using the formula A=[(a510-A700)-(A510-A700)].Results are expressed in mg/g Dry wt.

Carotenoids were estimated by the method of. [11] One gram fresh sample was crushed with methanol and centrifuged. Residue was discarded and supernatant was concentrated to dryness. The dried extract was dissolved in 10ml of ether, 5ml of 10% methonolic KOH and the mixture was kept for one hour at room temperature in dark. The ether layer was washed with 1ml of 3% NaCl(in distilled water)for 3 times to remove alkaline methanol and dried over sodium sulphate for one hour .The absorbance of ether extract was measured at 450nm by using UV-Vis 160A double beam spectrometer ,and expressed as mg/g Fr wt.

Extraction of β-carotene**:**10g sample (leaf) were rinsed with distilled water to remove sand, cut into pieces and lyophilized to remove the moisture content .Resulting dried samples were powdered using blender. These ground samples were extracted twice with a total volume of 100ml of 70% aqueous methanol .The mixture was shaken on an orbital shaker for 75min at

2500rpm and then filtered through Whatman No1filter paper. The combined methanolic extract was then evaporated at 55^0 C using water bath and dried to powder in a lyopholyzer.
β Carotene was determined according to the method of. [12] The dried methanolic extract (100mg) was vigorously shaken with 10ml of acetone and hexane mixture (4:6) for 1 min. The absorbance of the filtrate was measured at λ=453,505,645 and 663nm by Shimadzu 116 A UV_VIS Spectrometer. Contents of β-Carotene were calculated according to the following equations:

-Carotene mg/g Fr wt =$0.216xA_{663}$ _$0.304xA_{505}$ +$0.452xA_{453}$.
Where A=absorbance recorded at specific wavelengths.

Extraction of enzymes: One gram of fresh plant materials was taken and placed in a pre cooled mortar and ground with 10ml of cold 0.005M Tri–HCl buffer (pH 7.0).The extract was passed through cheese cloth and centrifuged at 1000rpm for 20mins .the supernatant was used as crude enzyme for the activities of catalase , peroxidase, and polyphenol oxidase.

Polyphenol oxidase and peroxidase was estimated as per the method of. [13] the assay mixture contained, 2ml, of 0.1M (pH7.0) Tri-HCl buffer, 1ml of pyrogallol (0.01M) and 1ml of enzyme extract. The assay mixture was incubated for 5 minutes at 25^0 C. The reaction was stopped by adding 1ml of 2.5NH_2SO_4. The absorbance at 425nm was recorded using Shimadzu 160 A UV-visible double beam spectrophotometer, enzyme activity was expressed as units/g Fr wt.

Peroxidase activity: The assay mixture consisted of 2ml of 0.1M (pH-7.0) Tri— HCl buffer, 1ml of H_2O_2 (0.05M) and 1ml of enzyme .The reaction mixture was incubated at25^0C for 5 minutes. The reaction was stopped by adding 1 ml of 2.5N H_2SO_4. The absorbance was recorded at 425nm in Shimadzu 160A UV-Visible double beam spectrophotometer. The activity was expressed as change in absorbance.

Catalase activity was estimated as per the method of. [14] The reaction mixture consists of 1ml of enzyme, 2ml of hydrogen peroxide and 3ml of 0.05M Tri-HCl buffer (pH 7.0). The reaction was stopped by 1ml of 2.5 N H_2SO_4 .After 5 minutes of incubation at 20^0 C, the residual H_2O_2 was titrated with 0.05M,$KMNO_4$. A blank was prepared by adding 1 ml of 2.5 N H_2SO_4 initially to the reaction mixture at zero time .Catalase enzyme activity was expressed as units/g Fr wt.

The enzyme units were calculated by using the following formula; Catalase activity =25/2 x 0.85x V/W

Where, V=difference in the titre value between control and treatment, W=Fresh weight of the sample in grams, 0.85mg of H_2O_2 =1ml of (0.05M). $KMnO_4$

Glutathione reductase enzyme extraction: The leaf material was weighed separately and ground in water at a concentration of 1g/5ml.The extraction were centrifuged at 1000rpm for 10minutes and the supernatants were kept under refrigerated conditions and used for enzyme estimations.

Glutathione reductase activity was determined according to the method of [15].0.2ml of sample, 1.5ml of 0.3 M phosphate buffer, pH6.8, 0.5ml of 25mM EDTA, 0.2ml of 12.5mM oxidised glutathione and 0.1 ml of 3mM NADPH was added .Decrease in absorbance was measured against that of blank at 340nm .The enzyme activity is calculated and expressed a Units/mg Fr wt.

Ferric Reducing Antioxidant Power (FRAP) assay: Soxhlet extraction method was employed for the preparation of 50%alcoholic extracts of the leaf powdered, sample was extracted for 6 hours. The collected solvent extract was evaporated, dried and stored at 4^0 C.

The FRAP reagent was prepared from 300mM of sodium acetate buffer , (pH3.6),10mM of TPTZ solution , 40mM Hull as a solvent , 20mM (Fe^{+3}) chloride solution in a volume of ratio of 10:1:1, respectively. [16] The FRAP reagent was prepared freshly and warmed to 37^0C in a water bath before use .100µL of the diluted sample was added to 3ml of the FRAP reagent, The absorbance of the reaction mixture was recorded at 593nm by using Shimdzu 160 A UV-VIS double beam spectrophotometer, after 4 min in room temperature .The standard curve was constructed by using $FeSO_4$ solution and the results were expressed in percentage.

Extraction for ABTS and DPPH Assays: The leaf materials were collected dehydrated in a chamber below 40^0 C for 48h, powdered with a mechanical grinder and stored in an air-tight container.

The methanolic leaf extracts were prepared by adding 1g of dry powders the leaf materials in 100ml, methanol, further stirring at 150rpm at ambient temperature for 3 hours. Insoluble residues from the solutions were removed by centrifugation at 8,000g for 10min (cooling

centrifuge) and the clear supernatants were used for analysis. The extracts were stored at 4^0C in plastic vials, till further use. All the estimations were performed in triplicates.

The ABTS cation radical scavenging activity of the extracts was determined according to the modified method of [17] A stock solution of ABTS was produced by mixing 7mM aqueous solution of ABTS with potassium per sulphate (2.45mM) in the dark at ambient temperature for 12-16h before use .The radical cation solution was further diluted until the initial absorbance value of 0.7±0.005 at 734 nm was reached .For assaying test samples, 0.98mL ABTS solution was mixed with 0.02mL of the plant extracts. The decrease in absorbance was recorded at 0 min and after 6 min. Scavenging ability relative to the reaction control (without plant extracts as 100%) was calculated by using the formula:

ABTS* radical Scavenging activity (%) = [(Initial reading –final reading)/Initial reading]x100, where initial reading is absorbance at 0 min. And final reading absorbance for 6min.
The DPPH radical scavenging activity was estimated by measuring the decrease in the absorbance of methanolic solution of DPPH by. [18] In brief, to 5mL of DPPH solution (3.3mg of DPPH in 100mL methanol),1mL of each plant extracts were added, incubated for 30min in the dark and the absorbance (A^1) was read at 517 nm. The absorbance (A^0) of a reaction control (methanol instead of plant extract) was also recorded at the same wavelength. Ascorbic acid (5-50µg/mill was used as a standard. Scavenging ability (%) was calculated by using the formula: DPPH radical scavenging activity (%) = $[(A^0-A^1)/A^0]$ x100, where A^0 was the absorbance of reaction control and A1 was the absorbance of extracts or standards.

STATISTICAL AND DISCUSSION

All results are expressed as mean ±standard deviation .All results are means of three replicates .The data were correlation coefficient at $p<0.05$.SPSS 15 Version was used for the statistical analysis.

RESULT AND DISCUSSION

High concentrations of flavonoids(144.55) ,flavonols (390.45),anthocyanins(215.89), proanthocyanidins(967.11), phenols(108.5) and ascorbic acid(40.26)(mcg/ g fr .wt) was recorded in purple variety when compared to green variety (19.98), (75.26), (20.40), (115.26),(79.05),(2017). β carotene and total carotenoids showed higher concentrations (30.47), (20.12) (mcg/g fr.wt) in green as compared to 23.45 and 15.57 mcg/g fr .wt.in purple variety respectively. (Table 1, figure1).

Table 1: Showing the content of antioxidant secondary metabolites (µg/g dry wt) in green and purple varieties of *Brssica oleracia* except total carotenoids *and* β carotene are expressed as µg/g fr.wt.

Secondary metabolites	Green mean ±sd	Purple mean ±sd
β Carotene	30.47±15.22	23.45±11.71
Total Carotenoids	20.12±9.95	15.57±7.69
Flavonoids	19.98±9.94	144.55±71.94
Flavonols	75.26±37.45	390.45±194.34
Proanthocyanidins	115.26±57.56	967.11±482.37
Phenols	79.05±39.44	108.5±54.03
Ascorbic acid	20.17±9.99	40.26±19.99
Anthocyanins	20.40±10.06	215.89±107.71

Peroxidsae showed maximum activity (77.76 units) in purple variety compared to green variety (17.43 units). Polyphenoloxidase showed maximum activity (122 units) in green variety when compared to purple variety (17 units). Catalase and glutathione reductase showed maximum activity in green varieties with 7.43 and 4.44 units as compared to 5.46 and 3.15 units in purple varieties respectively
(table 2, figure 2).

Table 2: Showing antioxidant enzyme activities (Units/g Fr wt) in green and purple varieties of *Brassica oleracea.*

Enzymes	Green mean ±*sd*	Purple mean ±*sd*
Peroxidase	17.43±8.69	77.76±38.78
Polyphenol oxidase	122±60.99	17±8.43
Catalase	7.43±1.33	5.46±2.13
Glutathione reductase	4.44±1.97	3.15±1.42

ABTS DPPH and FRAP inhibition % was recorded maximum 99.28, 93.78, 56.41 % activity in green ,when compared to 99.11, 91.33, and 31.05 % activity in purple variety respectively. Similar work has been reported in three varieties of plants by. [19] (Table 3, figure 3.)

Table 3: Showing the antioxidant assays activity (inhibition %) in green and purple varieties of *Brassica oleracea.*

Parameters	Green mean ±*sd*	Purple mean ±*sd*
ABTS assay	99.28 ±49.63	99.11±49.54
DPPH assay	93.78 ±46.84	91.33 ±45.60
FRAP assay	56.41 ±27.78	31.05 ±14.85

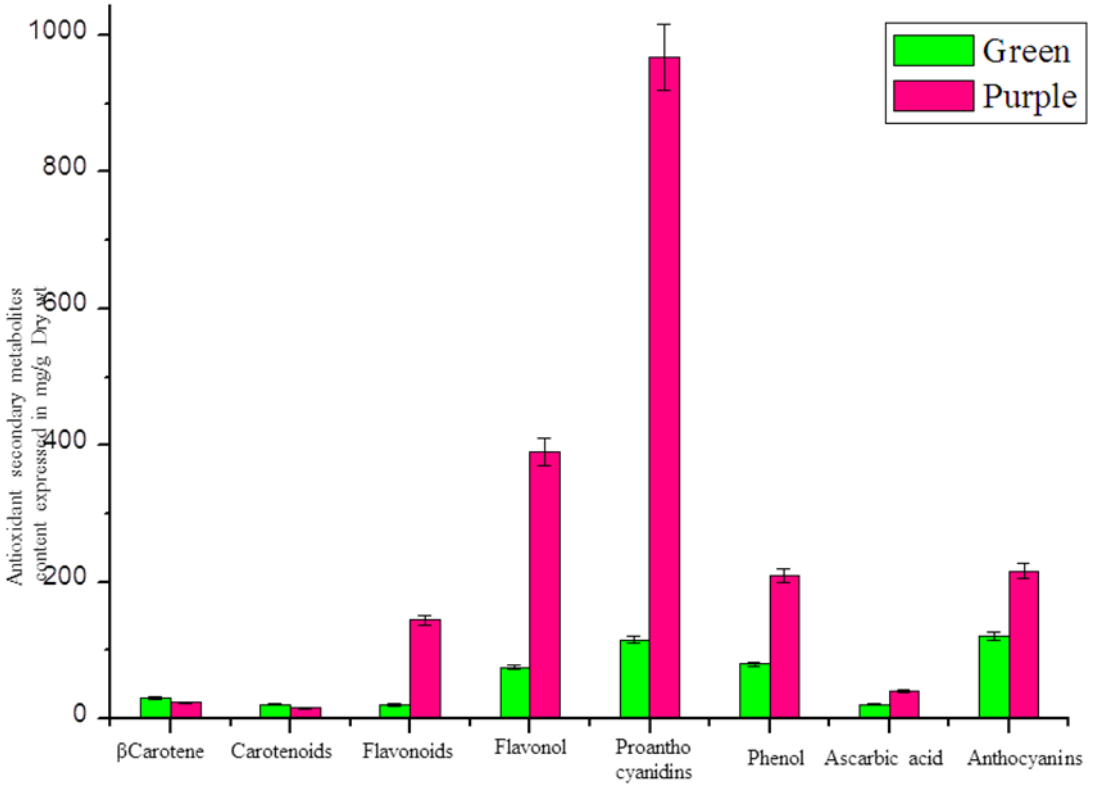

Figure 1: Showing the antioxidant secondary metabolites contents expressed in mg/g Dry wt, except total carotenoids and β carotene expressed as µg/g Fr Wt) in green and purple varieties of *Brassica oleracea.*

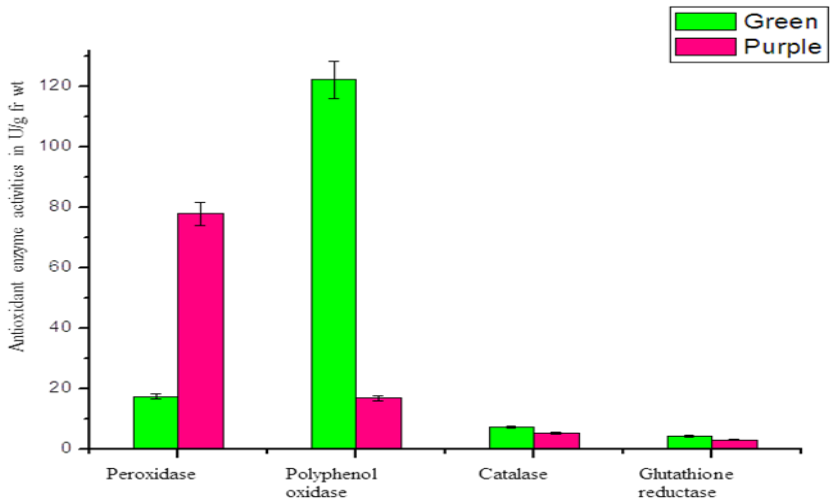

Figure 2: Showing antioxidant enzyme activities (Units /Fr wt)) in green and purple varieties of *Brassica oleracea.*

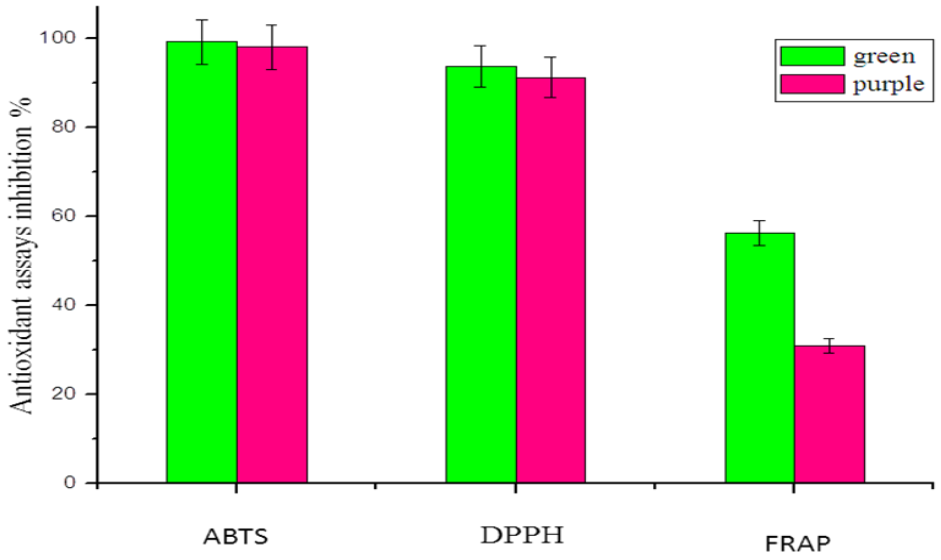

Figure 3: Showing Antioxidant assays (inhibition %) in green and purple varieties of *Brassica oleracea.*

Phytochemical and antioxidant properties were reported in basella alba. [20]

CONCLUSION

Comparison of the results gave indications that proanthocyanidins, flavonoids, flavonols, phenols, anthocyanins were recorded maximum in purple when compared to green variety Out of three antioxidant assays. Enzyme peroxidase showed higher activity in purple, polyphenol oxidase showed higher activity in green variety, catalase and glutathione reductase showed maximum activity in green varieties respectively. ABTS and DPPH gave high inhibition percentage than FRAP.

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REFERENCES

1. Production guidelines for cabbage. Agriculture, forestry & fisheries, republic of South Africa.
2. Ambrosone CB, Tang L. Cruciferous vegetable intake and cancer prevention: role of nutrigenetics. Cancer Prev Res (Phila Pa), Apr 2009; 2(4): 298-300.

3. Kusznierewicz B, Bartoszek A, Wolska, L et al. Partial characterization of white cabbages (Brassica oleracea var. capitata F. alba) from different regions by glucosinolates, bioactive compounds, total antioxidant activities, and proteins. LWT Food Science and Technology, 2008; 41, 1-9.
4. Miron A, Hancianu M, Aprotosoaie AC et al. [Contributions to chemical study of the raw polysaccharide isolated from the fresh pressed juice of white cabbage leaves]. Rev Med Chir Soc Med Nat Iasi, Oct-Dec 2006; 110(4): 1020-6.
5. Kurilich AC, Tsau GJ, Brown A, et al. Carotene, tocopherol, and ascorbate contents in subspecies of Brassica oleracea. J Agric Food Chem, Apr 1999; 47(4): 1576-81. 1999. PMID: 13300.
6. Wolfe, K. Antioxidant activity of apple. J. Agric. Food Chem, 2003; 51: 609-614.
7. Chang, Yang, M., Wen. Chern. Estimation of total flavonoids content in propolis by two Complementary colorimetric methods. J.food Drug Analysis, 2002; 10:178-182.
8. Kumran. Karunakaran.R.J. *In vitro* antioxidant ac tivities of methanol extracts of *Phyllanthus* species from India. Volume 40.Lebens-Wiss technologie, 2007; 344-352.
9. Sun JS, Tsuang Y W, Chen I J, Huang W, Hang Y S, Lu. An Ulltra a weak chemiluminiscence study on oxidative stress in rabbits following acute thermal injury, Burns1, 1998; 24: 225-231.
10. Giusti M M & Wrolstad R E. Unit F1.2.1-13.Anthocyanins.Characterization and measurement with uv-visiblespectroscopy. In R.E.Wrolstad (Ed.), Current protocols in Food Analytical Chemistry, New York, Wiley, 2001.
11. Jensen, A. Chlorophyll and carotenoids. In: Hellebust, A. and Crargei, J.S. (eds) Handbook of phytological methods. Cambridge, Cambridge university press, London. 1978; 5-7.
12. Nagata, M and Yamashita, Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit: Nippon shokuhin kogya gakkaish, 1992; 39(10): 925-928.
13. Kar and Mishra. Inorganic pyrophosphatase activity during rice leaf senescence. Can.j.bot, 1975; 53: 503-510.
14. Barber J M. Catalase and peroxidases in primary leaves during development and senescence. Z. pflanzen physio, 1980; 97:5: 135-144.
15. Beutler; Red Cell Metabolism: A Manual of Biochemical Methods (3rd ed.) Grune & Stratton, New York, 1984; 74–76. 22.

16. Sharique Ahmed .Ascorbic acid, carotenoids, total phenolic content and antioxidant activity of various genotypes of o*leracea encephala*. Journal of Medicinal and Biological Siences, 2009; 3: 1-7.
17. Roberta Re, Nicoletta Pellegrini, Anna Proteggente, Ananth Pannala, Min Yang, and Catherine Rice-Evans antioxidant activity applying an improved ABTS radical cation decolorization assay .Free Radical Biology & Medicine, 1999; 26(9/10): 1231–1237.
18. Brand-Williamson. Dietary intake and bioavailability of polyphenols. Source J Nutr. 2000:130(8S Suppl), 2000; 1258-1268.
19. Mrudula CM, Ashish A Prabhu, Ritu Raval. Phytochemical quantification and antioxidant capabilities of *Moringa olifera*, *Basella alba* and *Centella asiatica* leaf source. IJRSET, Feb-2014; 3(2).
20. B Thirupathi, S Gangadhar rao, Comparative phytochemical quantification in green and purple varieties of basella alba for preventive management of nutritional deficiency and stress related diseases.World Journal of Pharmacy and Pharmaceutical Sciences, 2014; 3: 1061-1071.